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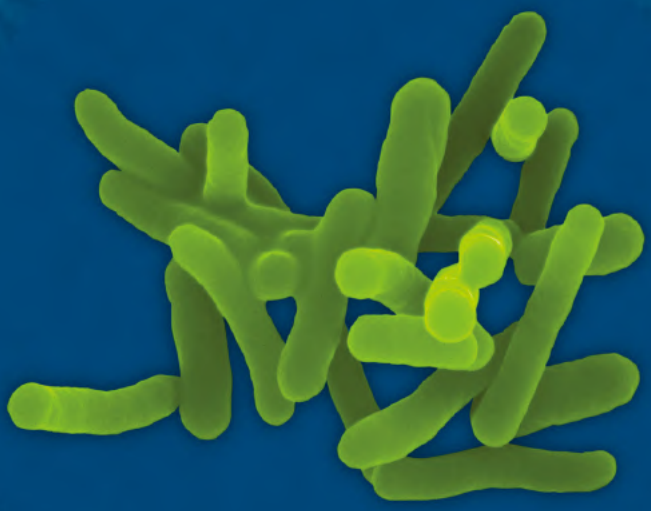
Risk assessment of *Listeria monocytogenes* in foods Part 1: Formal models

Meeting report



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MICROBIOLOGICAL RISK ASSESSMENT SERIES

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Food and Agriculture Organization of the United Nations
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Abbreviations

AFFI	American Frozen Food Institute
CC	clonal complex
CFU	colony-forming unit
CI	confidence interval
DR	dose-response
EFSA	European Food Safety Agency
EGR_x	exponential growth rate at x °C
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
FCS	food contact surface
FFLLoRA	Frozen Food *Listeria* Lot Risk Assessment
FSA	Food Standards Agency of the United Kingdom of Great Britain and Northern Ireland
FSAI	Food Safety Authority of Ireland
GWAS	genome wide association studies
HOG	heads-on, gutted
JEMRA	Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment
LAB	lactic acid bacteria
LD_{50}	median lethal doses
LM	*Listeria monocytogenes*
MAP	modified atmospheric packaging
MPN	most probable number
MRA	microbiological risk assessment

NFCS	non-food contact surface
PROFEL	European Association of Fruit and Vegetable Processors
QMRA	quantitative microbial risk assessment
QRA	quantitative risk assessment
RTE	ready-to-eat
ST	sequence type
WGS	whole genome sequencing
WHO	World Health Organization

Declaration of interests

All participants completed a Declaration of Interests form in advance of the meeting. The Interests declared were not considered by FAO and WHO to present any conflict in light of 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.

Executive summary

Scope and objectives

The Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) convened a meeting in Rome, Italy, from 24 to 28 October 2022, with the objective of developing formal full risk assessment models for *Listeria monocytogenes* in selected foods. In the light of the available data and the current risk assessment approaches, the expert group aimed to collectively ascertain the stages from primary production to consumption to be represented in the model, including approaches that accommodate the testing of scenarios, interventions and sampling schemes that could reduce the risk of listeriosis. The expert group also suggested possible future revisions of the three annexes of the Codex *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) based on the available evidence (Annex 1).

The expert group agreed that:

- Within each food group, the following food commodities were considered in the models: cut and packaged leafy greens (ready-to-eat lettuce), whole leafy greens (whole lettuce), ready-to-eat (RTE) diced cantaloupe, whole cantaloupe, smoked fish and gravad fish (RTE seafood) and non-RTE blanched frozen vegetables (frozen vegetables).
- A full primary production to consumption risk assessment model should be applied to the food commodities utilizing a modular approach, ideally flexible to be reused between them and for other similar food commodities (e.g. frozen beans, fresh herbs). The different modules included in the risk assessment models for the four food groups proposed by the expert group will be implemented with open-source software.
- The primary production (pre-harvest) module should enable the assessment of the introduction of the pathogen in the raw materials, taking into account, if possible, the effects of season, agrifood practices, and climate change.
- The latent possibility of cross-contamination from primary production to consumption should be considered.
- The dose-response (DR) model will be adapted from existing models that consider variability in pathogen virulence and consumer susceptibility and are common to all food commodities.

- Inclusion of whole genome sequencing (WGS) and other -omics data on *L. monocytogenes* may inform the risk assessment.
- The different (sub)modules of the models that describe the relevant stages of a food chain can be used to evaluate the impact of the stages on the risk in "what-if" scenarios.
- An uncertainty and sensitivity analysis should be performed to identify which model inputs and assumptions have the greatest impact on the model outputs (such as dose and risk for the consumer), which will help identify the relevant "what-if" scenarios and data needs.
- There is value in gathering more data on *L. monocytogenes* in the food chain from different sampling and testing schemes to inform risk assessment.

Conclusions

The expert consultation concluded the following:

Leafy greens

- The assessment of the risk of listeriosis due to the consumption of leafy greens should be undertaken through a full primary production to consumption approach. Existing data can support a modular approach.
- Production activities including irrigation, fertilization and other on-farm management practices have an impact on the occurrence of *L. monocytogenes* on farm and could be modelled in a primary production (pre-harvest) module.
- Season has been recognized as an important factor driving the microbial kinetics in soil and on leaves, and as such, it should be introduced into the model. Seasonal environmental conditions as an input may be altered in "what-if" scenarios created to assess the potential impacts of climate change.
- RTE and whole leafy greens (non-RTE) should be addressed in the model and should share a common primary production module. Lettuce was proposed as a representative commodity for the leafy greens model.
- Relevant stages to be represented in the risk assessment were identified, including: growth of leafy greens (field, controlled environment, hydroponics), harvesting, cooling, washing, sanitizing, cutting, packaging, multiple transportation steps, display at retail, and consumer practices.
- The model should be structured to measure the effectiveness of the prevention of contamination by soil/irrigation water, the efficacy of washing

with or without sanitizers, the prevention of cross-contamination events along the production chain, the application of good hygiene practices during processing, the efficacy of sampling schemes, and the impact of different consumer practices related to handling and storage.

- The risk assessment should consider the possibility of cross-contamination and/or recontamination; e.g. cross-contamination at processing facilities and/or at home.
- The model should have the flexibility to assess climate change impact; e.g. scenarios that include extreme weather events and seasonal effects on the occurrence of *L. monocytogenes* in the environment.

Frozen vegetables

- The model should pertain to the risk of *L. monocytogenes* in non-RTE frozen vegetables.
- The assessment of the risk of listeriosis should span processing to consumption, as the main factors driving the risk have been identified in the stages of processing and consumer handling. *L. monocytogenes* can contaminate vegetables post-blanching; the model should consider the possibility of contamination events post-blanching.
- Relevant stages to be represented in the risk assessment were identified, including: cleaning, washing, blanching, freezing, packaging, display at retail, consumer practices such as defrosting and cooking, as well as common practices relating to non-intended use.
- The risk assessment should be structured to measure the effectiveness of blanching or other inactivation steps pre- or post-packaging, the prevention of contamination post-blanching, and the efficacy of sampling schemes at the end of processing.
- The risk assessment should evaluate the impact of different consumer practices related to storage and cooking, and the impact of shifting consumer practices to increase compliance with cooking of non-RTE frozen foods (i.e. through better labelling and targeted messaging for more susceptible groups on how to cook frozen vegetables).

Cantaloupe

- The model should pertain to the risk of *L. monocytogenes* in whole and RTE diced cantaloupe.
- The assessment of the risk of listeriosis due to the consumption of cantaloupe

should be undertaken through a full primary production to consumption approach. Existing data can support a modular approach.

- Relevant stages to be represented in the risk assessment were identified, including: cantaloupe growing in fields, harvesting, cooling, washing, sanitizing, dicing, display at retail, consumer practices and multiple transportation steps.
- Flexibility to account for the impact of farming practices, extreme weather events, climate change, as well as for diverse market practices should be included in the risk assessment.
- The risk assessment should consider cross-contamination events at pre-harvest (e.g. irrigation water, soil, fertilizer), at processing (e.g. pooling of fruits from other producers, food contact surfaces/equipment and/or dicing), retail (e.g. market practices), and at consumer level (e.g. contamination from rind to flesh during slicing/dicing).
- The risk assessment should be structured to measure the effectiveness of the prevention of contamination during pre-harvest (e.g. soil, irrigation), the application of on-farm preventive measures (e.g. cantaloupes grown on soil barriers), the effect of processing stages, such as cleaning/washing, sanitization and removal of bruised parts, and the impact of consumers practices.
- The effect of the time/temperature profiles throughout the food supply chain (e.g. processing, transport, retail and consumer homes) should be included in the risk assessment.

RTE seafood

- A full primary production (harvest and farming) to consumption risk assessment for the risk of *L. monocytogenes* should be developed for RTE seafood. Hot- and cold-smoked fish and gravad fish were proposed as a representative food category for the RTE seafood model.
- The risk assessment should be flexible to accommodate other RTE fish products in the future (e.g. sashimi and ceviche).
- Relevant stages to be represented in the risk assessment were identified, including: growth of fish (open sea, aquaculture), harvesting, evisceration and head cutting, filleting, different smoking steps, gravad fish steps, freezing, slicing, packaging, multiple transportation steps, retail and consumer handling.
- Cross-contamination should be considered in the model because RTE fish

products are produced in several steps, sometimes within one facility, in other cases in different facilities in different countries.

- The effect of added lactic acid bacteria cultures for biocontrol of *L. monocytogenes* should be evaluated in the risk assessment.
- Whole genome sequencing/strain typing data, when available, could be used to assess dominant strains at different production stages in order to inform cross-contamination modules.
- The risk assessment should consider raw materials coming in with different levels of contamination. It is important to include the effect of the time/temperature profiles throughout the food supply chain (e.g. processing, transport, retail and consumer homes) in the risk assessment.

Dose-response

- Based on a review of the published dose-response models for *L. monocytogenes*, the expert group proposed the use of existing models based on susceptible populations with underlying conditions that increase the risk of listeriosis (or different risk of illness in different age-gender groups). The proposed dose-response model considers consumer susceptibility and virulence characterization based on the genomic data that are currently available.
- An updated set of parameters for this model that account for three classes of virulence of *L. monocytogenes* should be put forward, incorporating current data on specific virulence profiles associated with sequence types (ST) and/or clonal complexes (CCs).
- There is a need for additional 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 ST/CCs of *L. monocytogenes* can be performed.

WGS

- The global prevalence of *L. monocytogenes* in the exposure assessment part can be replaced by the specific prevalence for groups of ST/CCs when WGS data have demonstrated that these groups of ST/CC are overrepresented in a specific commodity.
- The three classes of virulence proposed in MRA38 should be used in the model. Scientific literature should be used to compile a list of ST/CCs belonging to different virulence groups.
- At this time, the genetic biomarkers for enhanced robustness or increased

fitness are not sufficiently conclusive for subgrouping *L. monocytogenes* with respect to differences in behaviour to be incorporated in the exposure assessment part.

- The uncertainty between phenotypic and genotypic profiles of *L. monocytogenes* should be considered and carefully examined before being used in risk assessment.

The expert group highlighted several paragraphs in the three annexes in the *Guidelines on the application of general principles of food hygiene to the control of Listeria monocytogenes in foods* (CAC/GL 61-2007) that could benefit from an update (Annex 1).

In conclusion, the expert group elaborated formal models for the risk assessment of *L. monocytogenes* for lettuce, cantaloupe, frozen vegetables and RTE fish. As a next step, these models should be programmed, tested and reviewed. The review should verify that the models are sufficiently flexible to run different scenarios and incorporate new data to account for the national, regional and international context. Following the review, the models should be made publicly available. These novel models will advance the state of knowledge on *L. monocytogenes* risks by incorporating advances in next generation technologies and by spanning primary production to consumption.

1

Introduction

In response to a request from Codex for scientific advice, FAO and WHO have undertaken a series of meetings to review the previous and current state of knowledge on *Listeria monocytogenes* in various foods worldwide and the risk assessment models for this important pathogen. During a virtual meeting of experts in 2020, the existing data on the attribution, characterization and monitoring of *L. monocytogenes* was reviewed. Based on this work, it was recommended that the risk assessment models for *L. monocytogenes* be reviewed, modified, updated, or newly developed to reflect the most current data and knowledge that has been accumulated over the last two decades for this pathogen. Specifically, the expert group recommended extending future risk assessment to diverse commodity sub-groups and to consider all steps from production to consumption.

The 2004 FAO/WHO risk assessment on *L. monocytogenes* (FAO/WHO, 2004a; 2004b) provided scientific insight into the risk of listeriosis for susceptible populations. The risk assessment models were limited to a cross-section of ready-to-eat (RTE) foods known to cause human listeriosis, such as pasteurized milk, ice cream, cold-smoked fish, and fermented meats. These models addressed risk from the point of distribution to consumption. 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 the original risk assessment considered.

A JEMRA report, MRA38 published in 2022, emphasized that deaths due to *L. monocytogenes* continue to occur across the globe, with more countries reporting listeriosis and including previously non-identified RTE vehicles (FAO/WHO, 2022). The 2022 report also highlighted that, although the main problem remains the colonization of the processing environment by *L. monocytogenes* due to poor

cleaning and sanitation and lack of hygienic design, the sources of contamination from the primary production (e.g. agricultural and fishery sources) are often overlooked and may be an important contributor to the challenge of controlling *L. monocytogenes* in the food chain (MRA38). To provide a more comprehensive picture regarding *L. monocytogenes* contamination, it was recommended that future risk assessments be extended to diverse commodity sub-groups and that the entire production to consumption chain be considered.

To facilitate this new work, a JEMRA meeting was convened in Rome, Italy, from 24 to 28 October 2022, with the objective of developing formal risk assessment models for *L. monocytogenes* in four selected foods, namely; leafy greens, frozen vegetables, cantaloupe and RTE seafood, spanning from the stages of production/capture to consumption (FAO/WHO, 2022). This report focuses on the approach that the expert group used in the deliberation of the risk assessment models (Topping *et al.*, 2022), the relevant literature that was reviewed to help identify the components of the exposure model, and the aim and the scope for the four food models (Section 4). The corresponding equations, descriptions, state variables and parameter values are described in Annex 3.

An additional task identified by the previous expert group was to consider ways in which genomic markers of *L. monocytogenes* strains could be included in risk assessment models, both in exposure assessment and hazard characterization steps (FAO/WHO, 2022). Sections 5 and 6 of the report discuss the current state of knowledge and literature used in the deliberation for the inclusion of genomic data in risk assessments for *L. monocytogenes*.

Finally, the next steps and potential uses for the proposed models are also discussed (Sections 7 and 8).

2

Background

Since the last FAO/WHO risk assessment models for *L. monocytogenes* in foods were developed, in 2004, many changes have occurred. Further study of *L. monocytogenes* and technological advancements have led to greater understanding of the properties and distribution of *L. monocytogenes* in the food chain. At the same time, food preparation and consumption trends have also changed, with increased emphasis on consuming less processed foods and eating more fresh fruits and vegetables, consumer trends shifting towards convenient foods, leading to the development of various ready-to-eat (RTE) foods and new food trends, as well as consumers using foods differently from their intended uses (e.g. frozen fruits and vegetables used in drinks and smoothies). For fresh produce and many RTE foods, abnormal fluctuations of temperature, inappropriate holding temperatures and temperature abuse throughout handling, processing, distribution chain, at retail and/or food service establishments, have been reported as the main drivers for increasing the risk of listeriosis (Franz *et al.,* 2010; Omac *et al.*, 2017). Cross-contamination during handling and processing is also an important factor for RTE foods, since these foods have direct contact with various surfaces (e.g. conveyors, handling equipment, sorting tables, containers, etc.) and typically do not receive additional processing or a "kill step" prior to consumption. For RTE foods that are prepared and/or packaged at retail and consumed at home, there is also a concern due to extensive handling of these foods during preparation and potential for cross-contamination from the retail environment, since the presence of *L. monocytogenes* in these environments is not uncommon (Sauders *et al.*, 2009). Taken collectively, all of these factors call for the development of new risk assessment models for listeriosis that can be used to control the spread and growth of this foodborne pathogen in the production chain, with particular emphasis on foods that have emerged as new vehicles of listeriosis, such as fresh and frozen produce, and RTE seafood products.

2.1 REVIEW OF QUANTITATIVE RISK ASSESSMENT MODELS FOR LISTERIOSIS ATTRIBUTABLE TO PRODUCE

Based on a review of the existing listeriosis quantitative risk assessment (QRA) models published between January 2000 and May 2022, a total of 11 models were identified as a source of listeriosis illnesses. The models addressed one or more steps in the supply chain. Six of the 11 models investigated four non-RTE products, namely: lettuce (n=2), baby spinach (n=1), leafy greens served in salad bars (n=2) and frozen vegetables (n=1). The remaining five focused on RTE products, namely lettuce salad, fresh-cut lettuce, fresh-cut cantaloupe, leafy vegetables and fruits/vegetables (Annex 2, Table A 2.1).

Despite the broad understanding that the microbial contamination of produce can occur at the pre-harvest stage, none of the QRA models identified included pre-harvest factors as sources of *L. monocytogenes* on-farm. Three QRA models simulated processing (Carrasco *et al.*, 2010; Guzel, 2015; Omac *et al.*, 2017), and assessed the effects of a range of processing stages or interventions, namely: ionizing radiation, cold atmospheric plasma, sanitization and modified atmosphere packaging (MAP) (Annex 2, Table A 2.2).

In the processing environment, cross-contamination can typically occur from human carriers, from harbourage sites, and from food surfaces. From the pool of existing produce QRA models, cross-contamination events were evaluated in five QRA models. In all cases, cross-contamination was modelled by transfer coefficients, which constitute a simple empirical approach that depends heavily on the source, recipients and number of contacts (Hoelzer *et al.*, 2012a). Transfer coefficients during processing stages were defined for packaging and handling steps (Guzel, 2015), and for handling mistakes, conveyor belts, and packing equipment (Omac *et al.*, 2017), whereas those related to handling at home were modelled for contaminated boards, hands and knives (Domenech *et al.*, 2014), and for unwashed cutting boards or countertops, unwashed kitchen tools, and unwashed hands (Ding *et al.*, 2013) (Annex 2, Table A 2.1).

These assessments highlighted the importance of accurately representing the transfer coefficients in the cross-contamination modules, since they have been shown to moderately drive the final risk estimate of listeriosis. Moreover, fresh and RTE minimally-processed products have a very short shelf-life. They are highly perishable due to their high moisture content, and, in particular, when wounding occurs during handling or processing, it leads to many physical and physiological changes that noticeably affect their quality (Miceli *et al.*, 2019). Despite these factors, some produce QRA models have assessed unrealistic

"what-if" scenarios for extended consumption times that are likely to highlight that home storage time determines the risk of listeriosis (Table 2). A realistic QRA model should also take into account not only the washing with sanitizer step according to the sanitizer label, but also its effectiveness to reduce *L. monocytogenes* depending upon time of exposure to the sanitiser, temperature, sanitizer concentration and the product itself.

One of the identified QRA models was employed for non-RTE frozen vegetables (Zoellner *et al.*, 2019), aiming to understand to what extent consumer preparation methods different from the packaging instructions affect the risk of listeriosis.

2.2 REVIEW OF QUANTITATIVE RISK ASSESSMENT MODELS FOR LISTERIOSIS ATTRIBUTABLE TO RTE SEAFOOD

Smoked and gravad fish are products of considerable public health implications of listeriosis since (1) they are not heat-treated; (2) have a long shelf-life; (3) are mostly vacuum-packed – which does not preclude *L. monocytogenes* growth; and (4) are generally eaten with no prior heating.

A review of the existing listeriosis QRA models published between January 2000 and May 2022 found that 11 models pertained to seafood. All of them focused on incomplete supply chains, starting at the end of processing or retail and ending at either retail or at consumer consumption. From these, only one QRA model assessed the growth of *L. monocytogenes* from the end of processing until consumption (Pouillot *et al.*, 2007; 2009) and two began the analysis from the point of retail (FDA-FSIS, 2003; Pérez-Rodríguez *et al.*, 2017). Seventy percent of the available QRA models only represented the consumption module, whereas only one QRA focused on a non-RTE product (traditional fish products; Bomfeh, 2011). None of the QRA models accounted for cross-contamination. Eight QRA models investigated cold/hot-smoked fish (salmon, trout or non-specified). The FDA-FSIS (2003) model included various seafood products, namely smoked seafood, raw seafood, preserved fish and cooked RTE crustacean, whereas gravad fish as a source of listeriosis was investigated earlier by Lindqvist *et al.* (2000), and, more recently, by Pérez-Rodríguez *et al.* (2017) (Annex 2, Table A 2.3).

Apart from the *L. monocytogenes* contamination in raw materials (fish) as a primary source (Skjerdal *et al.*, 2014), smoked fish can acquire the pathogen from food contact surfaces in the processing environment. Moreover, the fate of *L. monocytogenes* is variable along the processing steps of evisceration and filleting,

brining, smoking, slicing and vacuum-packaging. None of these sources or routes of contamination have been evaluated in the identified seafood QRA model. The QRA model with the broadest scope was that of Pouillot *et al.* (2007; 2009) which followed the cold smoked salmon supply chain from the end of processing until consumption. This model incorporated the assumptions of: (1) a Jameson effect to account for the inhibitory effect of the background microbiota on *L. monocytogenes* in the product within the vacuum-package atmosphere; and (2) the use of dynamic time-temperature profiles to represent realistic temperature oscillations between cold storage at the end of processing and home refrigeration.

The QRA model for listeriosis linked to cold smoked fish by FAO/WHO (2004b) also considered the effect of indigenous lactic acid bacteria, assuming that at high levels it can suppress the growth *of L. monocytogenes*. This model demonstrated the ability of cold smoked fish to support the growth of *L. monocytogenes*. It showed that even under the optimistic assumption that the growth rate inhibition of *L. monocytogenes* due to the growth of lactic acid bacteria is 95 percent, the listeriosis cases per thousand people would be around 70-fold more than if cold smoked fish did not support the growth of *L. monocytogenes* (Annex 2, Table A 2.4).

Vacuum-packaging is widely used in the smoked/gravad fish industry as it delays the proliferation of aerobic spoilage bacteria and minimizes oxidative reactions (Tocmo *et al.*, 2014). Nonetheless, microaerophilic or facultative anaerobic microorganisms, such as *L. monocytogenes*, may thrive under such conditions, and an extended shelf-life can give sufficient time for the pathogen to increase to infective levels. Within this context, many of the QRA models have tested "what-if" scenarios with shorter time for consumption. Researchers have obtained different estimates of the degree of reduction in a number of cases for smoked fish under various conditions.

The Pasonen *et al.* (2019) model was built on a Bayesian two-state Markov chain approach consisting of three modules: a module for occurrence data, a module for consumption data, and a predictive model for the total number of listeriosis cases in the population. As a Markov chain Monte Carlo simulation, bottom-up and top-down approaches are combined, and thus all unknown parameters can be jointly estimated from a single compact model. As a result, Pasonen *et al.* (2019) could estimate the uncertainty distribution of parameters, truly reflecting the information contained in the data. Even the r parameter of the exponential dose-response model was estimated from Finnish data by using the reported number of listeriosis cases to calibrate the dose-response function for the target populations.

3

Theoretical framework and modelling approach

Qualitative methods, owing to their inherent constraints, have proven their utility for risk ranking and addressing issues in risk assessment questions where few data are available. However, given the availability of data and models, QRA was considered as a more robust tool to effectively inform risk managers about the complexities posed by *L. monocytogenes* in RTE foods. These complexities include estimating the efficacy of sampling plans and the impact of control measures. Risk assessment quantitative models for *L. monocytogenes* spanning from production to consumption will be developed in selected food commodities, namely: cantaloupe (whole cantaloupe and RTE diced cantaloupe), leafy greens (whole lettuce and RTE pre-cut lettuce), frozen vegetables (non-RTE blanched frozen vegetables) and RTE seafood (cold-/hot-smoked fish and gravad fish). Stages from primary production to consumption – except for frozen vegetables whose scope will span from processing to consumption – will be represented in a Modular Process Risk Model framework, making use of the Monte Carlo simulation approach to propagate variability and uncertainty. Modules will be structured in a flexible manner so that they can be reused between the four food commodities and for other similar food commodities such as fresh herbs, frozen beans or sashimi.

Cross-contamination modules will be included for the primary production, processing and home preparation stages to represent the latent possibility of cross-contamination or recontamination with *L. monocytogenes* from food-contact surfaces. Whenever data allows between-batch variability will be modelled, a feature that will be exploited when assessing the performance of within-batch testing schemes as intervention strategies to control the risk. The impact of stages and "what-if" scenarios representing interventions to reduce the risk will be

ascertained from the models. In addition, the primary production modules will take into account the effects of season, agrifood practices and climate change, whenever possible. An uncertainty and sensitivity analysis will be performed for each model to identify which inputs and assumptions have the greatest impact on the model outputs (e.g. dose and risk for the consumer), which will help recognize relevant "what-if" scenarios and data needs. Decisions on the relevant stages to be modelled will be based on available data and "what-if" scenarios appropriate for the control of the risk.

The data required for the exposure assessment models have been identified, and they mainly come from published sources, mainly peer-reviewed scientific articles and reports. Extracted data are related to prevalence and counts of *L. monocytogenes*, cross-contamination transfer coefficients, growth and survival parameters of *L. monocytogenes* in the different food commodities and reduction factors of *L. monocytogenes* due to various processing interventions. Meta-analysis models can be adjusted prior to obtaining summarized parameters by integrating data from multiple microbial growth experiments and from microbial reduction/transfer factors. Data that can be considered as generic or transferable, such as temperatures along the cold chain and home fridge temperatures, will be obtained from published QRA models or industry sources. In cases where data are not available, reasonable assumptions and expert knowledge elicitation will be employed.

Validated primary and secondary microbiology models will also be used. The hazard characterization (dose-response) model will be adapted from existing models that consider variability in pathogen virulence and consumer susceptibility. It will be common to all food commodities. Inclusion of whole genome sequencing (WGS) and other omics data on *L. monocytogenes* may inform the risk assessment. The different modules included in the risk assessment models for the four food groups proposed by the expert group will be implemented with open-source software.

4

Exposure assessment

With the aim of improving the transparency of how the four models are to be developed, the expert group used the formal model approach (Topping *et al.*, 2022). The formal model approach aims to:

- define the objective and scope of the model;
- present the reviewed literature that helped to identify the components of the model;
- lay out the theoretical framework, modelling approaches and externalities;
- help to implement each processing step with equations, descriptions, state variables and parameter values; and
- present the model's strengths, weaknesses, exclusions, and position in the literature.

4.1 CANTALOUPE

4.1.1 Aims and purpose

Several large outbreaks of listeriosis have been associated with the consumption of cantaloupe (*Cucumis melo*) (McCollum *et al.*, 2013; NSW, 2018). Cantaloupe consumption may pose a risk of listeriosis because it grows in close contact with the ground, making it susceptible to contamination from soil and irrigation water. Furthermore, the complex netting structure of the rind allows pathogenic bacteria to attach to the fruit's surface (Ukuku and Fett, 2002; Mahmoud *et al.,* 2008). When the fruit is sliced, either during processing or at home, bacteria may be transferred into the flesh (Ukuku *et al.*, 2012), which is rich in sugars and low

in acidity (pH ~ 6.7), thus providing a favourable environment for the growth of *L. monocytogenes* and foodborne pathogens in general (Gil *et al.*, 2006). If contaminated cantaloupe is not refrigerated, *L. monocytogenes* can reach high levels before spoilage (Ukuku *et al.*, 2012, Huang *et al.*, 2015, Nyarko *et al.*, 2016a, 2016b, Salazar *et al.*, 2017, Danyluk *et al.*, 2014). Therefore, since contamination of cantaloupes by *L. monocytogenes* can occur at any step of the cantaloupe production chain, including primary production in the field, post-harvest processing, and storage as well as household handling and preparation, the expert group recommended the development of a full production-to-consumption risk assessment model (MRA38) aiming to represent the introduction and fate of *L. monocytogenes* on and in cantaloupe.

The model considers two products: RTE diced cantaloupe and whole cantaloupe as intact fruit. Risk factors for the introduction and subsequent growth of *L. monocytogenes* along the whole food chain for cantaloupe are included as model inputs. The pre-harvest module is common to these two products, whereas two post-harvest modules are modelled to allow the representation of the two processing chains: pre-cut and whole cantaloupe. This modular approach also enables the adaptation of the modelling framework to characterize certain practices, such as washing and/or sanitization, and checks for cross-contamination.

During the processing of fruits destined to produce RTE pre-cut cantaloupe, sanitization procedures for whole fruits as well as cross-contamination by slicing/dicing equipment and other food-contact surfaces are considered in the model. Contamination sources during transport, retail and in consumer homes, e.g. through food contact surfaces and utensils, are also proposed. The growth of *L. monocytogenes* throughout the whole production to consumption continuum is to be modelled using time-temperature data and includes a lag phase at the first stage where growth is assumed (i.e. transport to retail).

4.1.2 Framing the model

The production chain from primary production to consumption for RTE and whole cantaloupe is summarized in Figure 1. The cantaloupe risk assessment model proposed here includes seven modules. Module 1, "Primary production", details agricultural practices associated with *L. monocytogenes* risk or mitigation in the field. Module 2, "Harvest and transportation", covers the harvesting stage and the transportation to a packinghouse, while Modules 3 and 4 detail post-harvest cleaning, washing and processing. Module 5 involves the temperature chain. In the

case of RTE diced cantaloupe, this encompasses growth during storage, transport to retail, retail, transport to home, and home storage, whereas in the case of whole cantaloupe, the module includes both growth and survival along the same distribution chain. Module 6 considers risk factors associated with the handling of whole cantaloupe at retail in informal markets (e.g. traditional markets), while Module 7 addresses similar risk factors in the context of consumer handling. An assessment of the processes – cross-contamination, growth, survival, mixing, partitioning and removal of *L. monocytogenes* – is shown for each of the modules taken into account for the construction of the QRA model (Table 1).

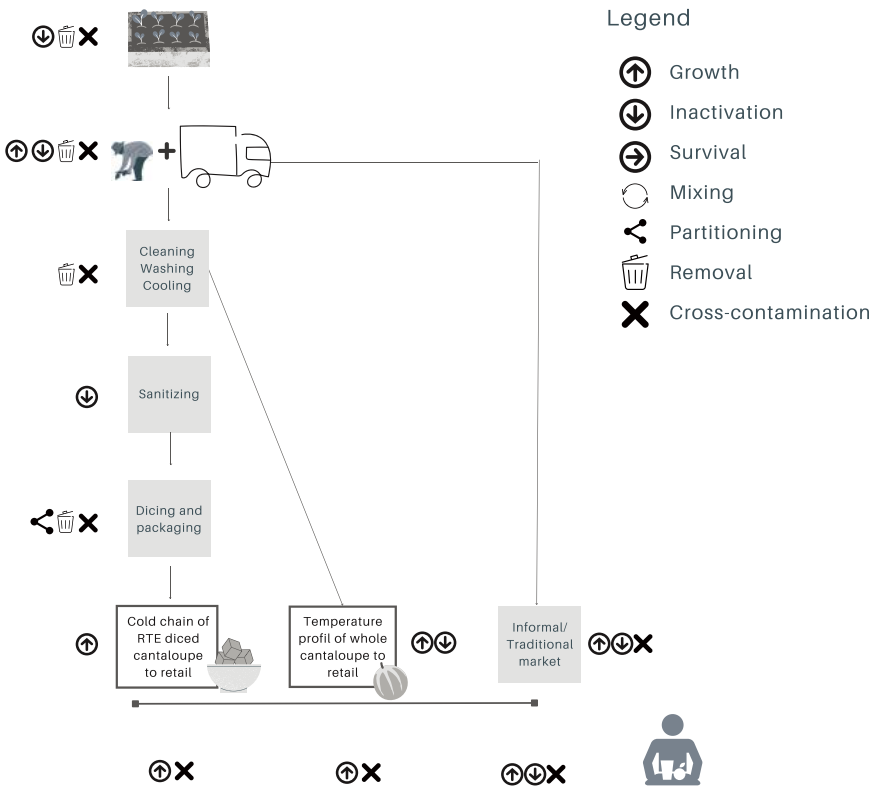

Figure 1: Stages of the production of RTE diced cantaloupe and whole cantaloupe, with an indication of the processes to be modelled

Table 1: Summary of the processes (growth, survival, mixing, partitioning, removal and cross-contamination) to be modelled for each of the modules of the QRA of *L. monocytogenes* (LM) in RTE diced cantaloupe and whole cantaloupe

Module[1]	Growth	Survival/Inactivation	Mixing	Partitioning	Removal	Cross-contamination
#1 Primary production (Pre-harvest)	-	Decline of LM on the rind is 1-2 log/cm^2 over 15 days	-	-	Growth of cantaloupe on soil barriers as a mitigation strategy	From soil, water, faeces; Impact of climate and extreme weather events; Adjacent land use: closeness to pastures and water sources
#2 Harvest and transportation (Begins with plucking and ends on arrival in the packinghouse)	If rind is injured, consider potential for later growth	If rind is intact, decline of LM	-	-	Removal of visible soil after harvest	Contamination from food contact surfaces (containers, equipment); Cross-contamination between individual fruits; Contamination from workers
#3a,b Cleaning, washing (relevant for both RTE diced and whole cantaloupe)	-	-	-	-	LM removal by surface brushing LM removal by washing with water	Water as a source of contamination and internalization into the fruit via stem scars; Cross-contamination from conveyor belts
#3a Sanitizing (only relevant for cut cantaloupe)	-	Decline of LM according to sanitizer, concentration and exposure time	-	-	-	-

#4a Processing -cutting and packaging (RTE only)	-	-	-	Dicing of whole cantaloupe	Cutting outvisibly damaged parts	Cross-contamination from dicer/ slicer and/or from surface to flesh
#5a Cold chain of RTE diced cantaloupe including retail	LM growth in cantaloupe dices	-	-	-	-	-
#5b Temperature profile of whole cantaloupe from packinghouse to home	If rind is injured, LM growth in flesh	If rind is intact, decline of LM	-	-	-	-
#6b Informal retail of whole cantaloupe, including street vendors of slices	If rind is injured, LM growth in flesh	If rind is intact, decline of LM	-	-	-	Cross-contamination from food contact surfaces and workers; Contamination from utensils and non-food contact surfaces in the case of street vendors slicing or dicing cantaloupe
#7a Consumer handling of RTE diced cantaloupe	LM growth in cantaloupe dices	-	-	-	-	Contamination from utensils and food contact surfaces
#7b Consumer handling of whole cantaloupe	LM growth in flesh if cantaloupe is cut and stored	If rind is intact, decline of LM				Contamination from rind to slices/dices; Contamination from utensils and food contact surfaces

[1] "a" is specific to RTE diced cantaloupe and "b" to whole cantaloupe

Module 1: Primary production

The Module, "Primary production" of cantaloupes, should consider agricultural practices and the potential contamination of the melons with *L. monocytogenes* from agricultural sources. Specifically, the model should take into account agricultural practices such as growing cantaloupes on soil barriers (e.g. mulch), and transfer of *L. monocytogenes* from soil, irrigation water, and, if possible, fecal contamination by wildlife and livestock. Cantaloupes are grown on the ground, making contamination from soil a likely event. The prevalence of *L. monocytogenes* in agricultural soils is variable, ranging from 0.0 to 17.0 percent, as reported in a number of studies (Dowe *et al.*, 1997; Locatelli *et al.*, 2013a; Linke *et al.*, 2014; Szymczak *et al.*, 2014; Weller *et al.*, 2015). Fields that are fertilized with animal manure are more likely to be contaminated with *L. monocytogenes* (Szymczak *et al.*, 2014), and inadequate composting can also contribute to the transmission of undesired microorganisms from manure to the soil (Miceli and Settanni, 2019).

The survival of *L. monocytogenes* in soil has been shown to be affected by soil pH, ambient temperature, season, irrigation method and soil texture (Girardin *et al.*, 2005; Nicholson *et al.*, 2005; Oliveira *et al.*, 2011; McLaughlin *et al.*, 2011; Locatelli *et al.*, 2013b). In contrast, *L. monocytogenes* survival in soil is not affected by the type of manure added to the soil (Nicholson *et al.*, 2005), and there is no difference in *L. monocytogenes* survival in soils contaminated through spiked compost compared to soils contaminated with irrigation water (Oliveira *et al.*, 2011). The transfer rate of *L. monocytogenes* by soil splash from contaminated soil to the surface of fresh produce can be approximated from the experiments performed by Girardin *et al.* (2005) and Oliveira *et al.* (2011).

The main irrigation water sources for crops are municipal water, rainwater, groundwater and surface water. The prevalence of *L. monocytogenes* in irrigation water appears to be variable, yet surface water is one of the riskiest water sources (Allende and Monaghan, 2015). Many data are available on the prevalence of *L. monocytogenes* in water environments (Table A 3.1 in Annex 3). The quality of the irrigation water (quantified by prevalence and levels of *L. monocytogenes*) is included in the model as an important parameter for the contamination of cantaloupes in the field with *L. monocytogenes* (EFSA, 2014).

Fields close to a pasture or a water source have been reported to have a threefold higher odds ratio of being contaminated with *L. monocytogenes* as determined by a geospatial model (Weller *et al.*, 2016). The location of the cultivation field, adjacent land use, as well as topography and climate (including local variability and extreme weather events related to the changing climate) were considered as factors that can influence the magnitude and frequency of transfer of *L.*

monocytogenes from environmental sources to growing crops (FAO/WHO, 2023a). Use of land for human settlement, animal rearing, industrial activities, open defecation, sewage and waste disposal as well as drift from adjacent agricultural activity introduces microbiological hazards that may persist in the growing environment (FAO/WHO, 2023a).

Inactivation of *L. monocytogenes* on cantaloupe rind during the pre-harvest period is considered in the model as several studies have shown a decline in *L. monocytogenes* on intact cantaloupe rind over time (Ukuku and Fett, 2002; Nyarko *et al.*, 2016a).

Module 2: Harvest and transportation

Module 2 considers the practices at the harvest stage, including the handling of fruit, personal hygiene of the field workers and, if possible, cross-contamination events between intact cantaloupes and mobile conveyor belts, containers, vehicles and other food contact surfaces.

Cantaloupes are typically harvested by hand and sometimes transferred to mobile conveyor belts to load the cantaloupes onto the transport containers or vehicles in the field. The loading from the conveyor belt to the vehicle can be fully automated or manually aided by a worker. At any stage of harvesting, rough handling of fruits by workers such as throwing or dropping fruits may cause injuries to the rind and lead to the internalization of *L. monocytogenes* from the surface into the flesh. The stem cut introduced by removing the cantaloupe from the vine may serve as a port of entry for *L. monocytogenes* that may then grow (Nyarko *et al.*, 2016a). Chemical treatments of the stem scar at harvest have been considered impractical on a large scale; and this intervention was therefore not incorporated in the model. A pathogen prevalence reduction step is possible at harvest if workers manually remove dirt or manure containing *L. monocytogenes* that is present on the rind surface. However, there were no quantitative data on the efficacy of this process, which led to its exclusion from the model.

The mechanisms for cross-contamination at harvest include the transfer of *L. monocytogenes* from surfaces in contact with the melons, such as packaging crates or parts of the vehicle surface. Cross-contamination between individual melons was not considered highly relevant and therefore not included in the model.

While insufficient personal hygiene of field workers was considered as a potential contributor to the contamination of cantaloupe with *L. monocytogenes* due to the manual handling of the fruit, it was not deemed relevant for the model. This decision was based on the experts' opinion that *L. monocytogenes* contamination on workers' hands and gloves would be rare and also due to the absence of data to populate this model.

After harvest, cantaloupes harvested in highly intense cultivation systems are typically transported to a packinghouse, where the fruits may be cleaned, washed and cooled. Given the short transport times, typically under a few hours, the potential for growth of *L. monocytogenes* during this time was not considered relevant. However, in less formal markets, the transport can be direct from the farm to the vending stalls, which can provide enough time for any internalized *L. monocytogenes* in injured fruits to grow significantly at ambient temperatures. In this scenario of longer transports, cross-contamination between melons, and with other food commodities if they are transported simultaneously, may be significant. In the packinghouse or producer stage, fruits from different farms can be mixed. However, no data were available to inform a transportation module. Further assumptions or data are needed to include growth and inactivation in the model.

Module 3a, b: Cleaning and washing (relevant for both RTE diced cantaloupe and whole cantaloupe)

Module 3 addresses hygiene issues associated with brushes used for dry cleaning, and the washing equipment for spray or immersion washing of freshly harvested cantaloupes. In the packinghouse or processing facility, cleaning of cantaloupes may include an optional dry-brushing step. Cross-contamination from unhygienic conveyor systems and brushes has been shown to be relevant (McCollum *et al.*, 2013), and is therefore included in the model.

Cantaloupes are intentionally put in contact with water that comprises spray or dunk washing as well as hydrocooling to remove field heat. The reduction of *L. monocytogenes* on cantaloupe surfaces after washing treatments is typically below 1.0 log colony-forming unit (CFU)/cm^2 (Ukuku and Fett, 2002; Ukuku *et al.*, 2005; Ukuku *et al.*, 2012; Guzel *et al.*, 2017).

Contamination and infiltration of fresh fruit with enteric pathogens from water during dunk washing and hydrocooling have been shown for tomatoes, mangoes, oranges, apples and avocados (Buchanan *et al.*, 1999; Eblen *et al.*, 2004; Penteado *et al.*, 2004; Bordini *et al.*, 2007; Bartz *et al.*, 2015; Chen *et al.*, 2016). Specifically, the internalization of *L. monocytogenes* through the stem scar of cantaloupes from water used for immersion of cantaloupes either during dunk washing or hydrocooling has been shown (Macarisin *et al.*, 2017). Growth of *L. monocytogenes* during the washing step was not considered relevant.

Module 3a: Sanitizing (relevant for RTE diced cantaloupe)

The main objective of washing is to remove visible physical dirt and/or organic matter, aided by brushes, so that the subsequent disinfection can be effective

against spoilage and pathogenic microorganisms. This step is crucial for melons used for cutting or slicing, but not mandatory in the case of melons that are consumed as a whole product. Therefore, the model will include the step of sanitizing as a strategy for prevention of microbial contamination by controlling the quality of wash water and/or for a limited reduction of *L. monocytogenes* on melon surfaces (FAO/WHO, 2023).

Module 4a: Processing – cutting and dicing (relevant for RTE diced cantaloupe)

Module 4 addresses risks associated with the production of RTE diced cantaloupe. The presence of *L. monocytogenes* on the cantaloupe surface was considered a relevant risk factor for the contamination of the final product. Although *L. monocytogenes* declined slowly on artificially inoculated cantaloupe rinds over time (Ukuku and Fett, 2002; Nyarko *et al.*, 2016a), their presence still represents a contamination risk to the flesh of melons during slicing and dicing. Previous studies mimicking procedures used in processing units (and home preparation) for fresh-cut cantaloupe showed that *L. monocytogenes* were readily transferred from the rind to the edible melon portions during slicing and cutting, with the extent of contamination on the rind determining the number of cells transferred (Beuchat, 1996; Selma *et al.*, 2008; Patil, 2017; Ukuku *et al.* 2012;). As few as 150 bacterial cells per cm^2 were necessary on the cantaloupe rind for contamination to be transferred to the edible portions of the fruit while cutting (Patil, 2017). Cross-contamination of cantaloupes with *L. monocytogenes* from slicing and dicing equipment and other food contact surfaces was quantified in Patil (2017). It is regarded as a relevant risk factor to be included in the model.

Partitioning, by cutting and dicing the whole cantaloupe in small parts, may affect the prevalence of *L. monocytogenes* during slicing, as one cantaloupe will not be equal to one portion of RTE diced product. Dicing and extensive mixing of cantaloupe pieces in larger batches is not relevant due to the delicate nature of the edible portions. Therefore, while some portions of RTE diced product will contain pieces stemming from more than one individual cantaloupe, extensive mixing is not expected, and as such was not considered relevant at this stage.

During processing, the removal of visibly damaged parts of individual cantaloupes may mitigate spoilage and reduce the load of *L. monocytogenes* potentially introduced into the finished RTE product. As there were no data identified to validate or quantify this potential intervention step, and because experiments with dye showed the real distribution of the dye throughout the whole fruit from the stem scar (Macarisin *et al.*, 2017), this process was not included as a reduction step in the model.

Module 5a: Cold chain (relevant for RTE diced cantaloupe)

Module 5a considers risks for *L. monocytogenes* growth in RTE pre-cut cantaloupe associated with time-temperature profiles during transport, storage, retail and in the consumer home. Adequate cooling before storage reduces build-up of condensation on the cantaloupe surface, thereby preventing growth of spoilage organisms, mould and pathogens on the fruit during cold storage. A controlled cold chain at producer and retail stages, and during transport between stages, is critical for limiting the growth of *L. monocytogenes*. Numerous experiments to quantify the growth rate of *L. monocytogenes* in cut cantaloupe have been conducted at different storage temperatures (Farber *et al.*, 1998; Fang *et al.*, 2013; Hong *et al.*, 2014; Ukuku and Fett, 2002; Danyluk *et al.*, 2014; Guzel *et al.*, 2017; Patil, 2017; Moreira, 2019) and, overall, they are in agreement. The growth rate ranged from 0.01 h^{-1} at 4 °C to 0.90 h^{-1} at 37 °C. No inhibitory effect of competitive microbiota on growth inhibition was taken into account as available data from literature on possible growth inhibition were not conclusive. As contamination is likely to have occurred in previous stages, a lag phase was not assumed.

Module 5b: Temperature profile (relevant for whole cantaloupe)

Module 5b considers the risk factors for *L. monocytogenes* growth on a whole cantaloupe associated with time-temperature profiles during transport, storage, retail and in the consumer home. As referenced previously, adequate cooling before storage prevents growth of spoilage organisms, mould and pathogens on the fruit during cold storage. Shelf-life of whole cantaloupes is maximized if they are transported at temperatures lower than 7 °C (Ding *et al.*, 2013; Sant'Ana *et al.*, 2014), and stored at ambient temperature (assumed to be 20–22 °C in the reference scenario) during retail and in the consumer home. Growth of *L. monocytogenes* on whole intact cantaloupes was considered irrelevant based on existing data (Marik *et al.*, 2020). However, the module should provide an option for the growth of *L. monocytogenes* after being internalized into cantaloupes either by bruising, disease on the surface or by access through the stem scar. Both modules 5a and 5b should account for a lag phase following the first introduction of *L. monocytogenes* into cantaloupes.

Module 6b: Informal retail (relevant for whole cantaloupe)

The module for the retail of intact cantaloupe considers events of cross-contamination from comingling with other produce and from food contact surfaces. The module should accommodate a variety of practices, such as merchandizing in unsanitary conditions on the ground or next to other contaminated foods or live animals. For growth of *L. monocytogenes* on whole products, refer to module 5b.

Module 7a: Consumer handling (relevant for RTE diced cantaloupe)

As in Module 5a, the temperature of cantaloupe during storage is an important variable affecting the growth of *L. monocytogenes* in cut cantaloupe. Cross-contamination rates from produce to food contact surfaces and vice versa have been estimated by Hoelzer *et al.* (2012a), Zilelidou *et al.* (2015) and Kuan *et al.* (2017b), and can be used in the model.

Module 7b: Consumer handling (relevant for whole cantaloupe)

Module 7b considers risk factors associated with consumer handling of intact cantaloupes. Essentially, the same cross-contamination risk factors from rind to flesh apply as modelled in Module 4 (cutting and processing cantaloupes). Cross-contamination transfer coefficients utilized in Module 2 can be taken from Hoelzer *et al.* (2012a). Given the available knowledge, the experts did not consider the possible effect of washing melons at the consumer's home.

4.1.3 Summary of assumption and data

Annex 3, Section 1 presents an extensive explanation of the data and the assumptions for building the model for *L. monocytogenes* in whole cantaloupe and RTE diced cantaloupe. Input variables, parameters, descriptions and equations are compiled in tables for each of the modules in Annex 3, Section 2.

4.2 ROMAINE LETTUCE

4.2.1 Aims and purpose

Leafy greens, a generic term including arugula, iceberg and romaine lettuce, endive, spinach, kale and others, can become contaminated with *L. monocytogenes* at any step of the production chain. During primary production, *L. monocytogenes* can contaminate growing leafy greens through irrigation water, fertilization, birds, domestic and wild animals (Gil *et al.*, 2015), or adverse weather events such as flooding (NSW, 2018). Moreover, season has been recognized as an important factor driving the microbial kinetics in soil and on the leaves of vegetables (Oliveira *et al.*, 2011). In addition, contamination of leafy greens by *L. monocytogene*s can occur during post-harvest handling and processing, storage, transportation and retail, as well as consumer handling and preparation. Therefore, risk assessment of listeriosis due to the consumption of leafy greens should be undertaken from primary production to consumption. Because effective decontamination steps are not readily available for fresh leafy greens to be consumed raw, prevention of contamination is critical, and modelling conditions and practices that can lead to contamination and cross-contamination along the production chain is important

in providing practical solutions for improved control measures (Possas and Perez-Rodriguez, 2022).

Lettuce was the leafy green commodity chosen as a reference by the expert group, as it is a widely available vegetable and is consumed in many parts of the world (Kim *et al.*, 2016) and most of the research data available for leafy greens have been generated for lettuce. The risk assessment model aims to represent the introduction and fate of *L. monocytogenes* in romaine lettuce throughout the production chain, i.e. focusing on activities and stages during pre-harvest, harvest, post-harvest handling and processing, transportation, retail and consumer handling. The model considers two products: RTE pre-cut lettuce and whole lettuce. The latter represents the most common form of consumption in many parts of the world. The pre-harvest and harvest modules are common to RTE pre-cut lettuce and whole lettuce, whereas two post-harvest/processing modules are modelled separately to allow the representation of the two processing chains: pre-cut and whole lettuce. Furthermore, it is proposed that, for whole lettuce, an additional pathway of commerce at informal retail level (i.e. markets, vendors, etc.) be taken into account.

The model's output considers the risk of listeriosis per portion, and should be useful in assessing the effectiveness of different strategies in preventing contamination during pre-harvest, harvest and processing (e.g. cooling, washing, sanitizing), and to prevent cross-contamination events along the value chain, including processing and consumer practices related to handling and storage. The model should also have the flexibility to assess climate change impact.

4.2.2 Framing the model

The model represents the full production chain of lettuce, including: cultivation (conventional, protected agriculture and hydroponics), harvesting, packinghouse/processing (cutting, washing, packaging and cooling), multiple transportation steps, display at retail and consumer practices (Figure 2).

The leafy greens model comprises eight modules that represent three food pathways: 1) RTE pre-cut lettuce sold in formal retail; 2) whole lettuce sold in formal retail; and 3) whole lettuce sold in informal markets and by street vendors. Module 1, "Primary production", considers cultivation of lettuce in open fields, protected agriculture and hydroponics. Module 2, "Harvest", includes the field cutting, removal of outer leaves, arrangement into crates or boxes, and sprinkling with cold water. This module is common to the three different food pathways. Module 3 assesses chilling, which includes cooling and cold storage, and is a module common to RTE pre-cut lettuce and whole lettuce sold in formal retail. Module 4 considers transportation for the three food pathways. Cold transportation is considered for RTE pre-cut lettuce and whole lettuce sold in formal retail, whereas transportation at ambient temperature (i.e. 20–22 °C) is considered for the lettuce sold in informal markets. Module 5 represents the

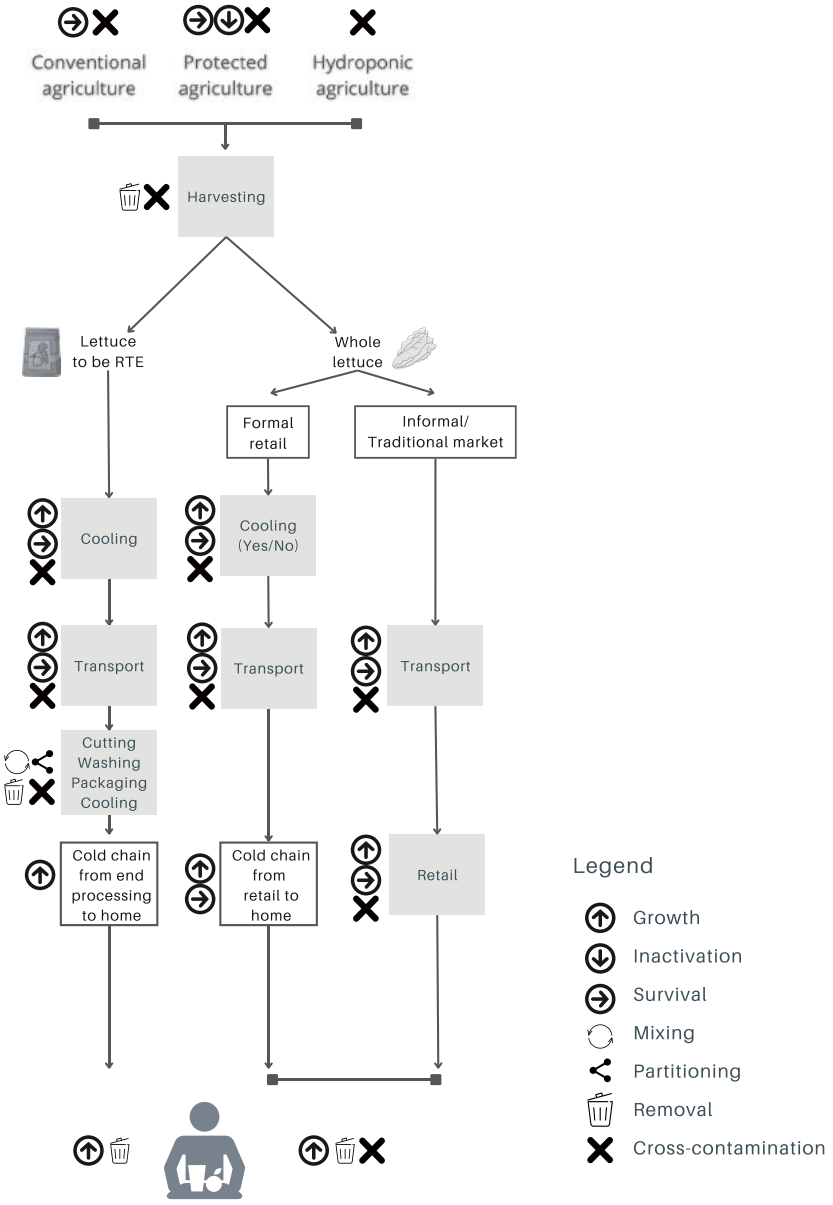

Figure 2: Stages in the production of RTE pre-cut lettuce and whole lettuce, with an indication of the processes to be modelled

processing operations of cutting, washing, packaging and cooling for processing of RTE pre-cut lettuce. Modules 6a and 6b, named as cold chain and temperature chain, pertain to the kinetics of *L. monocytogenes* in RTE pre-cut lettuce from end of processing to transport to homes, and in whole lettuce during retail and transport to homes, respectively. Module 7 focuses on *L. monocytogenes* growth and cross-contamination taking place at informal retail markets. Module 8a mimics the consumer handling of RTE pre-cut lettuce, whereas Module 8b, c represents the consumer handling of whole lettuce purchased at formal or informal retail level. Table 2 summarizes the modules, risks and mitigation strategies that were considered in the model at each step.

Module 1: Primary production

Module 1 includes the cultivation of lettuce in conventional production (open field), protected agriculture, and hydroponics conditions, as these cultivation systems can lead to different microbiological profiles and risks (FAO/WHO, 2023a). However, a distinction is to be made between cultivation systems, from a microbiological perspective. Although organic farming employs animal manure as soil fertilizer, there is no strong evidence in the literature that vegetables grown in such conditions pose a higher risk of listeriosis than conventionally produced vegetables.

Soil and irrigation water are known to be primary sources of hazardous microbiological contaminants in the production environment (FAO/WHO, 2023b). For lettuce cultivated in conventional open fields, sources of contamination and risk factors can be irrigation water, fertilized/amended soil (Oliveira *et al.*, 2011), recent application of manure, recent last irrigation (Strawn *et al.*, 2013b; Weller *et al.*, 2015), vicinity of surface water or pasture (Weller *et al.*, 2016), birds, domestic and wild animals, and flooding (Gil *et al.*, 2015; NSW, 2018). For lettuce grown in protected agriculture cultivation, sources of contamination have been linked to irrigation water and soil amendments, whereas in hydroponically grown lettuce, sources of contamination have been associated with nutrient solutions and water (Koseki *et al.*, 2011; Standing *et al.*, 2013; López-Galvez *et al.*, 2014).

In terms of *L. monocytogenes* viability on lettuce leaves, it is considered that, if the leaves get injured, there is potential for the subsequent growth of *L. monocytogenes* (Aruscavage *et al.*, 2008; Aruscavage *et al.*, 2010). Koseki *et al.* (2011) also demonstrated that foodborne pathogens inoculated onto cut leaves of spinach could survive and proliferate within one day at ambient temperature, whereas they decreased to undetectable levels when inoculated onto the leaves of growing plants. In the model, *L. monocytogenes* cells are assumed to decline if cells end up on intact leaves either from soil splash after irrigation (Girardin *et al.*, 2005) or from direct irrigation (Oliveira *et al.*, 2011). Internalization of *L. monocytogenes* through contaminated nutrient solutions is considered as a mechanism of spread in hydroponic cultivation, although it is acknowledged that it is not likely to contribute much to food safety due to the low levels of transfer.

Table 2: Summary of the processes (growth, survival, mixing, partitioning, removal and cross-contamination) to be modelled in each of the modules of the QRA of *L. monocytogenes* in RTE pre-cut lettuce and whole lettuce sold in formal retail and informal retail

Module[1]	Growth	Survival/Inactivation	Mixing	Partitioning	Removal	Cross-contamination
#1 Production – Conventional	-	Survival in population on the leaves; however, *L. monocytogenes* may have better survivability on injured leaves than on intact plants (Hoelzer *et al.*, 2012b)	-	-	-	Contamination can come from soil, soil amendments, irrigation water, birds, domestic and wild animals. Adjacent land uses: closeness to pastures and water sources. Use of soil barriers (mulch) as a mitigation strategy
#1 Production – Protected agriculture	-	Temperature fluctuations and desiccation may contribute to inactivation. However, *L. monocytogenes* may have better growth on injured leaves than on intact plants	-	-	-	Contamination can come from soil, soil amendments and irrigation water.
#1 Production - Hydroponics	-		-	-	-	Potential contamination from hydroponic nutrient solutions and/or water

#2 Harvest	Growth is not expected at this point (time too short between cutting and placing the product in crates/boxes)	-	-	-	Removal due to cleaning (cutting outer leaves), if performed	Cross-contamination may occur from soil (depending on the production system), from hands/gloves and tools, and between plants. Potential cross-contamination from the crates
#3a,b Chilling for processing RTE pre-cut lettuce (a), and for whole lettuce to be sold in formal retail (b)	Potential for growth for injured plants	Survival on intact plants	-	-	-	Potential cross-contamination from water/ice, condensation from cooling equipment.
#4a,b,c Transportation for processing RTE pre-cut lettuce (a), and for whole lettuce to be sold in formal retail (b) and informal retail (c)	Potential for growth for injured plants	Survival on intact plants	-	-	-	Potential cross-contamination between food commodities if they are transported in the same vehicle.
#5a Processing – cutting, sanitizing, packaging and cooling	-	-	Mixing of lettuces during cutting	Partition for packaging	Removal due to washing/sanitizing	Potential misuse of sanitizing solution. Cross-contamination may occur from equipment, utensils and other surfaces.

#6a Cold chain for RTE pre-cut lettuce (from end processing to home)	Growth depending on time-temperature profile and gas environmental conditions (e.g. MAP)	-	-	-	-	-
#6b Temperature chain of whole lettuce sold at formal retail (retail and transport to home)	If leaves are injured, consider *L. monocytogenes* growth	Survival if leaves are intact	-	-	-	-
#7c Informal retail of whole lettuce	If leaves are injured, consider *L. monocytogenes* growth	Survival if leaves are intact	-	-	-	Consider cross-contamination potential in traditional markets due to contact with contaminated food commodities, display on the ground, poor practices and vectors.
#8a Consumer handling of RTE pre-cut lettuce	Growth in RTE pre-cut lettuce during home storage	-	-	-	Potential washing of RTE lettuce with tap water	-
#8b,c Consumer handling of whole lettuce purchased in formal or informal retail	Growth during home storage	-	-	-	Removal due to washing with tap water and/ or removal of damaged/ spoiled parts	Cross-contamination from utensils such as knife and chopping board

[1] "a" is a module for the RTE pre-cut lettuce route, "b" for whole lettuce sold in formal retail, and "c" for whole lettuce sold in informal markets

Module 2: Harvest

Module 2 corresponds to the "Harvest" of lettuce. *L. monocytogenes* is not assumed to grow, if present on injured leaves, due to the short lapse of time between cutting the lettuce heads and their placement in boxes/crates. However, cross-contamination may occur from harvesting equipment, hands/gloves, soil from roots, and between plants. Differences in contamination transfer may occur depending on the production system, i.e. conventional, protected agriculture and hydroponics. While harvesting, the outer soil-contaminated leaves of lettuce may be removed. Lettuces with minimal soil contamination are placed in packing boxes or crates; and may be chilled by having water sprinkled on them.

Module 3a, b: Chilling (relevant for RTE pre-cut lettuce and whole lettuce sold at formal retail level)

Lettuce may be cooled using forced air, hydrocooling or vacuum cooling systems. During cooling, there is potential for cross-contamination from water/ice and condensation from cooling equipment (Gil *et al.*, 2015). After cooling (if performed), lettuce in crates, packaged or not, are transported to formal retail or to the processor of RTE produce. The chilling module is therefore common to the pathways of RTE pre-cut lettuce and whole lettuce to be sent to formal retail.

Module 4: Transportation

Module 4 assesses the "Transportation" of lettuce for three different pathways: production of RTE pre-cut lettuce, sale of lettuce at formal retail, and sale of whole lettuce at informal retail. At this step, *L. monocytogenes* growth is not expected on intact lettuce, although growth may occur in injured leaves (Marik *et al.*, 2020). The potential for growth will depend on the transportation conditions, such as time, temperature and humidity. Decline in the number of *L. monocytogenes* may also occur on intact leaves depending upon the environmental conditions (Marik *et al.*, 2020). In the case of transportation of whole lettuce, it is possible that cross-contamination may occur between plants as well as between other food commodities (if they are transported in the same vehicle), and contamination from the crates themselves.

Module 5a: Processing: cutting, washing and sanitizing, packaging and cooling (relevant for RTE pre-cut lettuce)

Module 5a represents the processing operations involved in the production of

RTE pre-cut lettuce. Washing is a common practice to remove soil and other large particles. Sanitizers may be added into washing water to minimize the risk of cross-contamination through the washing process. Growth of *L. monocytogenes* during cutting, sanitizing and packaging is not expected, although contamination of lettuce may occur from food contact surfaces when cutting, or when washing.

Cross-contamination is an important factor to consider during processing, since there are multiple surfaces such as conveyor belts, shredders, centrifuges, sorting tables, containers and packaging machine in contact with the produce. For instance, Johnston *et al.* (2006) determined that the population of *E. coli* increased by 0.16 log CFU/g during the packaging of cabbage, suggesting cross- contamination as the most likely explanation. Furthermore, as explained in Module 4, cross-contamination can take place during cooling through water, ice and condensation (Gil *et al.*, 2015).

Module 5a represents the risk factors and sources of contamination of whole lettuce when prepared as RTE lettuce or RTE mixed salads at retail (e.g. in deli bars at supermarkets). The environmental and handling conditions may differ in these scenarios. Cross-contamination sources in these cases could be similar (but not limited) to those described for consumer handling (Module 8). Repurposing of lettuce (i.e. lettuce originally intended to be sold as a whole vegetable, but then redirected to RTE processing due to the presence of damaged parts) is not considered in the model (for the sake of assessing the risk per batch, a batch of lettuce from one field is considered to be used for a single purpose). To extend the shelf-life of leafy green salads, MAP is used.

Module 6a: Cold chain (relevant for RTE pre-cut lettuce)

Module 6a models the growth of *L. monocytogenes* in MAP pre-cut lettuce from cold storage at the end of processing until home storage. Growth depends on the gas composition of the package and the time temperature profile assumed. Lag phase duration will be considered from the point when pre-cut lettuce packages are chilled (Sant'Ana *et al.*, 2012).

Module 6b: Temperature chain (relevant for whole lettuce sold at formal retail)

Module 6b pertains to the growth of *L. monocytogenes* in/on whole lettuce commercialized at formal retail establishments, covering the stages of display at

retail and transportation to homes. As presumed in the module for pre-harvest (Module 1), the pathogen on the leaves may either grow or decline if present on damaged or intact leaves, respectively (Li *et al.*, 2002; Koseki and Isobe, 2005; Girardin *et al.*, 2005; Oliveira *et al.*, 2011). Temperature abuse has been identified as the most important factor contributing to increasing the risk of *Listeria* contamination on leafy greens at retail (Franz *et al.*, 2010). The expert group agreed that cross-contamination at formal retail is considered unlikely to occur, since whole lettuces are very often displayed in the same boxes in which they were packed at the packinghouse or farm.

Module 7c: Informal retail of whole lettuce

A module representing sale at informal markets is considered in the model, in order to ascertain the impact of this type of commerce on the final risk of listeriosis. Module 7c considers the same *L. monocytogenes* kinetics in/on whole lettuce as in Module 6b. Furthermore, it assumes that cross-contamination may occur due to contact with other contaminated food commodities and/or contaminated surfaces, due to displaying on contaminated stands or crates, or due to poor handling practices.

Module 8a: Consumer handling (relevant for RTE pre-cut lettuce)

As in Module 6a, *L. monocytogenes* is assumed to grow on RTE pre-cut lettuce during home storage. Before it is served, the consumer may or may not wash the product with tap water. Furthermore, pre-cut lettuce is typically not consumed once it has visible signs of deterioration.

Module 8b, c: Consumer handling (relevant for whole lettuce)

Listeria monocytogenes can grow on/in whole lettuce during home storage (Li *et al.*, 2002; Koseki and Isobe, 2005). At this point in the chain, even if present on intact leaves, no decline in *L. monocytogenes* is assumed. It is also assumed that the whole lettuce is cut or shredded and washed with tap water. Poor hygiene practices at home – such as lack of cleaning of kitchen equipment and utensils or using the same chopping board to slice raw meat and raw vegetables – may lead to cross-contamination. The same sources of cross-contamination and growth of *L. monocytogenes* may occur in commercial food preparation establishments where lettuce or mixed salads are prepared.

4.2.3 Summary of assumption and data

Annex 4, Section 1 presents data and assumptions that were used in building the risk model for *L. monocytogenes* in whole lettuce and RTE pre-cut lettuce. These are based on the available literature and are explained in each module. Descriptions of variables/parameters, equations and sources are compiled in Annex 4, Section 2.

4.3 FROZEN CORN/PEAS

4.3.1 Aims and purpose

While *L. monocytogenes* cannot grow in frozen vegetables, these products should be handled appropriately to ensure food safety. Recent foodborne disease outbreaks linked to frozen vegetables demonstrate population-level risk of listeriosis from the consumption of uncooked frozen vegetables (i.e. consumed without heat treatment), in salads or as ingredients in other RTE products (e.g. smoothies) made for or by consumers (Zoellner *et al.*, 2019). In addition, if frozen vegetables are defrosted and stored at low temperatures, *L. monocytogenes* can grow and represent a risk (Kataoka *et al.*, 2017). Frozen vegetables are often marketed with validated cooking instructions and are not considered as RTE foods (Sampedro *et al.*, 2022; FSAI, 2022). However, frozen vegetables may also be consumed by adding them to smoothies or salads without following these instructions in their entirety (Sampedro *et al.*, 2022). Due to these consumer behaviours, the US FDA identified specific frozen vegetables, such as peas, kale, carrots and spinach, as products that are likely to be consumed without being cooked (FDA, 2017).

Two major risk assessments for listeriosis associated with frozen vegetables are available. Zoellner *et al.* (2019) published an MRA motived by a 2016 outbreak linked to various frozen vegetables, while EFSA (2020) MRA was developed following the 2018 outbreak implicating frozen corn. The European Food Safety Agency (EFSA)'s model only focused on the home preparation module, and was based on several controversial (i.e. overestimated) assumptions such as: a relatively high mean prevalence of *L. monocytogenes* in frozen vegetables (11.4 percent), absence of a lag phase, maximum storage time outside the fridge (96 h), and a high proportion of uncooked servings (23 percent). Even under these worst-case scenarios, the EFSA model identified blanched frozen vegetables as

having the lowest risk of listeriosis in comparison with sausages, hot-smoked fish, paté, gravad fish, cooked meat and cold-smoked fish.

On the other hand, the Frozen Food Listeria Lot Risk Assessment (FFLLoRA) tool, developed by Zoellner *et al.* (2019), considered features of the processing stage such as clustering of *L. monocytogenes* in packages, end-product testing and batch size. Although the FFLLoRA tool was modelled differently from the EFSA model, their results agreed that the number of illnesses in the susceptible population was low. Based on FSAI (2022) results, assuming a maximum proportion of uncooked servings of 10 percent, the EFSA model estimated annual cases of listeriosis to be between 0.1 and 1.2 in the European Union (EU) population. According to the FFLLoRA tool, assuming a probability of cooking of 0.5, their model predicted nil listeriosis cases per batch of frozen vegetables (95 percent confidence intervals (CIs): 0-6) (one batch is assumed to produce 1 million servings).

The risk assessment model described here aims to represent the introduction, inactivation, removal and potential growth of *L. monocytogenes* in non-RTE blanched vegetables such as corn and peas from processing to consumption. By assessing the change in the risk of listeriosis per serving, the model will measure and compare the effects of blanching (or any additional inactivation step, pre- or post-packaging), end-product sampling plans and increased environmental monitoring and control to reduce cross-contamination, thereby enhancing consumer practices to increase compliance with the cooking of non-RTE frozen foods (e.g. better labelling, better consumer education), and interactions between consumer behaviour and consumer risk group (e.g. targeted messaging for high-risk groups to cook frozen vegetables).

4.3.2 Framing the model

The model represents the production chain for non-RTE frozen peas/corn, covering processing (i.e. cleaning, washing, blanching, freezing, packaging and potential post-processing treatment) and consumer preparation (i.e. defrosting, cooking and non-intended uses) (Figure 3). Therefore, the frozen vegetables model includes two large modules: "Processing" and "Consumer preparation". The processing module includes the stages of blanching, freezing and packaging, considering the potential for recontamination after blanching, post-packaging treatment, and end-product testing. The consumer preparation module encompasses defrosting, cooking and non-intended uses (i.e. not cooking). Table 8 summarizes the modules, risks and mitigation strategies that were considered in the model at each level.

The expert group agreed not to include primary production in the model, as the most important risk factors appear from processing onwards, specifically post-blanching. There is limited information on *L. monocytogenes* occurrence in vegetables intended for quick-freezing in processing facilities. The different nature of such vegetables, e.g. fruits of a cereal crop (corn), seed pods of a pod fruit, root vegetables (carrot), and green beans, implies different agronomic practices and, therefore, contamination routes and factors, all of which complicate the assessment of the risk of primary production for frozen vegetables. Regardless, not including preharvest stages is a limitation in the scope of the model.

Module 1: Processing

While scarce data on *L. monocytogenes* occurrence exist at pre-harvest and harvest stage for vegetables intended to be frozen, a few studies have determined the prevalence and numbers of *Listeria* spp. and *L. monocytogenes* on raw produce arriving at processing facilities (Magdovitz *et al.*, 2021; Truchado *et al.*, 2022). In Magdovitz *et al.* (2021), out of a total of 290 samples of carrots, corn, peas and green beans, 33.1 percent and 5.9 percent samples were positive for *Listeria* spp. and *L. monocytogenes*, respectively. Of the 17 *L. monocytogenes* positive sample, 14 had greater than 100 MPN/g of *L. monocytogenes*, and three samples had counts between 10-100 MPN/g. In another study, *L. monocytogenes* was detected in corn (13.6 percent), peas (6.3 percent), and green beans (4.2 percent) arriving at processing facilities (Magdovitz *et al.*, 2021).

Preparation

Frozen vegetables are typically sold cleaned to the point of being only the edible portion of vegetable. Vegetables are, for example, cleaned and peeled. The model will start by representing the prevalence and numbers of *L. monocytogenes* after these steps just before blanching. To do so, it is suggested that the prevalence data for minimally processed vegetables be used as initial values.

Blanching

Blanching is a heat treatment during which most *L. monocytogenes* are eliminated from vegetables (Mazzotta, 2001, Ceylan *et al.*, 2017). There is existing literature on the effect of hot water (85 and 87.8 ^{o}C) and steam blanching (85 and 96.7 ^{o}C) on *L. monocytogenes* in peas, spinach, broccoli, potatoes and carrots (Ceylan *et al.*, 2017).

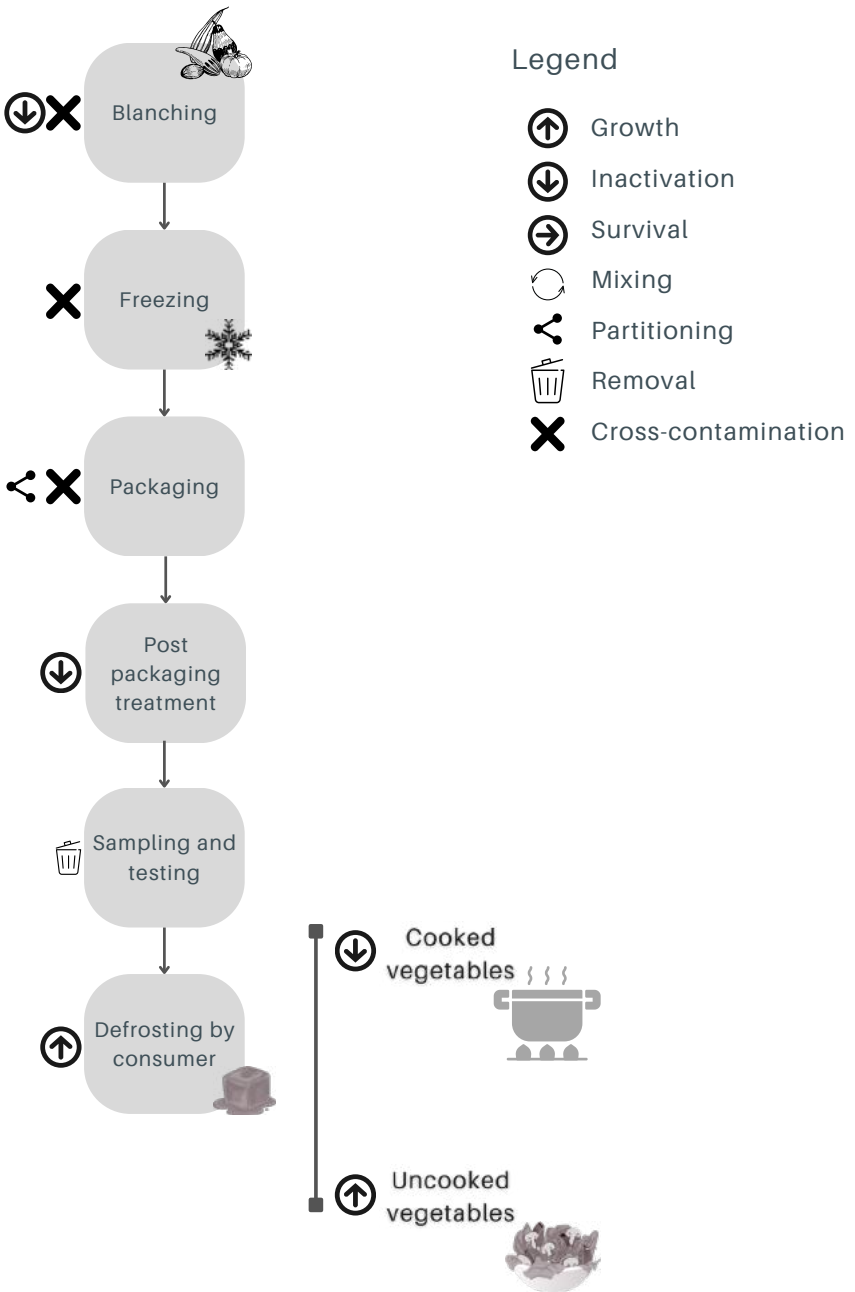

Figure 3: Stages of the production of non-RTE frozen vegetables, with an indication of the processes to be modelled

Table 3: Summary of the processes (growth, survival, mixing, partitioning, removal and cross-contamination) to be modelled for each of the modules of the QRA of *L. monocytogenes* in non-RTE frozen peas/corn

Module	Growth	Survival/Inactivation	Mixing	Partitioning	Removal	Cross-contamination
#1 Processing - Blanching	-	Some inactivation occurs	-	-	-	Stage of cooling using water after blanching may cause cross-contamination
#1 Processing - Freezing	-	-	-	-	-	Freezing equipment can be a source of *L. monocytogenes*
#1 Processing - Packaging	-	-	-	Bulk frozen vegetables put into packs	-	Cross-contamination from packaging machines
#1 Processing – Post-packaging treatment	-	A generic inactivation step after packaging such as heat treatment will reduce *L. monocytogenes* populations	-	-	-	-
#1 Processing – Product sampling and testing	-	-	-	-	Batch of food removed if not compliant	-
#2 Consumer preparation - Defrosting	Growth resumes after defrosting	-	-	-	-	-
#2 Consumer preparation – Cooked vegetables	-	Inactivation by heat	-	-	-	-
#2 Consumer preparation – Uncooked vegetables	Growth if prepared improperly (e.g. used raw while labelling indicates cooking needed)					

Hot water blanching accomplished a >5 $\log_{10}$ reduction of *L. monocytogenes* within 0.5 min at 85 and 87.8 °C. The effect of steam blanching was variable at two temperatures. For a >5 $\log_{10}$ reduction of *L. monocytogenes*, it took 2 min in carrots and spinach, but 3.5 min in broccoli and peas at 85 °C. At a higher steam temperature (96.7 °C), a >5 $\log_{10}$ reduction of *L. monocytogenes* was achieved within 1 min in all the vegetables studied (Ceylan *et al.*, 2017). In the EFSA risk assessment model (EFSA, 2020), the time and temperature combinations used for storage and processing (e.g. blanching, cooling) were indicated as the main factors affecting contamination and growth of *L. monocytogenes* in blanched frozen vegetables during processing.

Cross-contamination

A review of the literature suggests that there is a high risk of post-blanching cross-contamination, from equipment in contact with food (e.g. feeders, slicing machines, transporters, conveyor belts and freezing tunnels) as well as from non-food contact surfaces (e.g. floors, drains). For example, studies have shown frequent recovery of *L. monocytogenes* from environmental samples associated with freezing tunnels, conveyor belts, and other harbourage sites post-blanching (Fagerlund *et al.*, 2017; Magdovitz *et al.*, 2021; Truchado *et al.*, 2022). Therefore, a significant investment in preventive controls and environmental monitoring would be needed to rely on blanching as the sole kill step if the goal is to produce RTE products. EFSA (2020), the European Association of Food and Vegetable Processors (PROFEL, 2020) and the American Frozen Food Institute (AFFI) (AFFI, 2022) have published guidelines for improved environmental control and monitoring of vegetables after thermal treatment. EFSA (2020) has provided recommendations on processing environment monitoring and end-product monitoring for processors of blanched vegetables. PROFEL (2020) has published guidelines for preventing *L. monocytogenes* contamination in post-thermal treatment, and for assessing potential accumulation of *L. monocytogenes* in the broader processing environment. AFFI has published a *Listeria* control programme that includes both general good manufacturing practices and specific frozen food recommendations, such as recommendations for freezer management (AFFI, 2022).

Freezing

While freezing of the produce stops further pathogen growth, during the freezing step there is an opportunity for cross-contamination of produce with *L. monocytogenes*. It has been shown that there is no significant decrease in *Listeria* spp. in frozen produce, even if stored for 100 days (Pappelbaum *et al.*, 2008). Therefore, this step presents an opportunity to assess the effect of improved environmental controls to reduce cross-contamination. In environments that can

harbour *L. monocytogenes* and lead to frequent food cross-contamination, such as those of deli meats retail establishments, a QRA model (Pouillot *et al.*, 2015b) demonstrated that minimizing the contamination levels of *L. monocytogenes* in incoming food and the transfer of *L. monocytogenes* from the environment or niches to the food directly decreases the predicted risk of illness, and that more frequent environmental contamination events have more impact on risk than a greater number of *L. monocytogenes* cells per cross-contamination event.

Packaging

Packaging partitions the frozen bulk product into saleable units and, similar to a freezing step, presents an opportunity for cross-contamination. For the model, it would be acceptable to consider the overall risk of post-blanching cross-contamination as one process.

Post-freezing inactivation treatment

It has been reported that the implementation of alternative processing technology in current frozen vegetable production chains is challenging (van der Sman, 2020), with most of the literature focusing on produce quality rather than microbiological safety. While there is a need for further studies on this topic, the experts agreed that the model should include a generic inactivation step, should future advances in processing lead to a process that can achieve post-freezing inactivation treatments. The expert group suggested that the model should consider a generic inactivation step post-freezing (e.g. during packaging or post-packaging), by some unspecified process defined by the effect on contamination. This is meant to represent an alternative approach to reducing *L. monocytogenes* risk, potentially moving towards shipping an RTE product.

Product sampling schemes

To assess the effectiveness of microbiological testing, the model needs to consider batches in the processing module. Each simulation is considered to correspond to a batch. The frequency of sampling, the number of samples, and the microbiological criteria parameters are used to define the proportion of batches not delivered to consumers due to non-compliance. In principle, the processing-to-consumption risk assessment will provide the microbiological status of the batch (prevalence and contamination level) sent for testing. One way to validate that the model is predicting contamination reasonably well is to compare the model predictions at this stage to data for *L. monocytogenes* prevalence at the end of processing and retail. To model the risk of a very specific product, where survey data are available (for example, those presented in Tables A 5.1 and A 5.2 of the Annex 5), these data could be used as inputs to the risk assessment. The starting point

would no longer be contamination before blanching but contamination after the manufacturing process. For example, from contamination data acquired on packaged products, it is possible to predict the impact of product sampling and analysis, as well as home preparation, on the risk of listeriosis.

RTE possibility

The expert group suggested conducting a "what-if" scenario to evaluate whether a proportion of frozen product was produced as RTE. This would allow some consumers who intend to consume the product without further cooking to then choose to buy an RTE product, rather than the baseline assumption in these models that all products are non-RTE regardless of a consumer's intention. This scenario is similar to how the US frozen cookie dough industry now produces some RTE cookie dough after at least one major outbreak demonstrated consumers purchasing the product with no intention of cooking it (Neil *et al.*, 2012). We acknowledge that the frozen food industry would need flexibility to achieve RTE status differently for different food commodities and specific processes, which is why the model includes parameters that could represent changes for improved hygiene (i.e. reduction of cross-contamination), and pre- and post-freezing kill steps (e.g. blanching or as yet unidentified technologies).

Module 2: Consumer handling

It is very likely that counts of *L. monocytogenes* in raw produce are low (Jeyaletchumi *et al.*, 2011; Kuan *et al.*, 2017a). Typically, cross-contamination of food from processing environments will not result in high microbial levels, since the number of *L. monocytogenes* cells on produce surfaces is generally low (Zoellner *et al.*, 2019). In addition, frozen vegetables do not support the growth of *L. monocytogenes* during storage, and non-RTE frozen vegetables are not intended to be consumed without heat treatment. Therefore, the consumer handling phase becomes decisive, even if processors maintain the best manufacturing practices and processing environment monitoring activities.

Defrosting and storage after defrosting

The model considers the practices of consumers related to defrosting (e.g. in a refrigerator, microwave, or at room temperature). Growth is considered in the event of defrosting at room temperature and under refrigeration. The model of Zoellner (2019) can be used for modelling the growth of *L. monocytogenes* in defrosting (and thawed) vegetables.

Although *L. monocytogenes* cannot grow at freezing temperatures, they can still survive for extended periods of time while frozen (Pappelbaum *et al.*, 2008), and

resume growth when defrosted, needing a very short lag phase time at ambient temperature (Kataoka *et al.*, 2017). Consumers may thaw frozen vegetables on the counter-top at room temperature, in the refrigerator or using the microwave, *L. monocytogenes* growth will occur if thawed vegetables are kept long enough at temperatures supporting growth. There are additional data on the growth of *L. monocytogenes* during thawing and storage (Kataoka *et al.*, 2017; PROFEL, 2020).

Cooked or uncooked vegetables

Frozen vegetables can be consumed as cooked or raw foods. Consumption of uncooked non-RTE frozen vegetables has been reported in surveys conducted in England (Willis *et al.*, 2020) and Ireland (FSAI, 2022). From these studies, two points emerge. First, clear cooking and handling instructions are required on these products. Second, some consumers still do not cook these vegetables as instructed.

The model will need to account for the relative proportions of cooked and raw consumption according to the type of population (i.e. susceptible versus general), to accurately estimate risk. For example, blanched frozen vegetables were incriminated in a multi-country outbreak of *L. monocytogenes* ST6 in the EU (2015-2018), where the probability of illness per serving of uncooked blanched frozen vegetables was estimated to be up to 3 600 times greater than for cooked frozen vegetables for the elderly (65-74 years old) population (EFSA, 2020). The parameters describing the cooking practices for different consumer populations present the opportunity to address the impact of changing consumer practices related to cooking, and the interactions between consumer behaviour and risk groups. Specifically, the model should contain "what-if" scenarios that increase compliance with recommended cooking practices, and particularly increase compliance among the highest-risk consumer groups, by whatever increment seems realistic as an outcome of targeted consumer education campaigns. These scenarios would quantify the potential benefits from those risk management strategies, assuming such behaviour change is achievable.

For cooked vegetables, different heat treatment intensities can be applied by consumers, varying between very strong heat treatments (i.e. fully cooked) to lesser heat treatments (e.g. light heating with a microwave). The model will assess the effect of cooking in reducing *L. monocytogenes* levels. Growth in uncooked vegetables or lightly cooked vegetables will be modelled by the same parameters used for defrosting at room temperature, assuming there are data available for estimating realistic fractions of product being subjected to long holding times (such as in Kataoka *et al.*, 2017; PROFEL, 2020).

4.3.3 Summary of assumption and data

Data and assumptions that can be used for constructing the risk model for *L. monocytogenes* in non-RTE frozen vegetables have been taken from the literature and compiled in Annex 5, Section 1. Descriptions of variables/parameters, equations and sources are presented in Annex 5, Section 2.

4.4 READY-TO-EAT FISH

4.4.1 Aims and purpose

A risk assessment model for *L. monocytogenes* in smoked fish from the point of retail was developed by FAO/WHO (2004a,b). The expert group agreed that such a model should be revised to cover the entire food chain, due to the increased number of outbreaks related to seafood, the increased trend of consumption of smoked fish and other seafood products, and the new knowledge and data available on *L. monocytogenes* along the production to consumption chain. As discussed in Section 1, several international listeriosis outbreaks linked to seafood have been detected during the last decade. Whole genome sequencing (WGS) has shown a large degree of similarity between patient isolates with several months or even years between them, and between isolates from seafood and humans, even in different countries. Smokehouses receiving fish from many suppliers and selling to many countries have been instrumental in the spread of contaminated products. For example, from 2010 to 2021, smoked or gravad salmon products were identified as the most likely source of listeriosis in 22 independent outbreaks and 228 cases in Germany (Lachmann *et al.*, 2022).

It is difficult to determine specific causes as to why listeriosis illnesses related to seafood RTE products appear to be on the increase, but at least three plausible reasons have been hypothesized.

One hypothesis is that the actual number of cases has not increased, but that more cases that previously would have been considered sporadic are now investigated using WGS and linked to outbreaks. Genotypic relatedness between human illnesses has been found retrospectively from different years, and has launched a new way of conducting outbreak investigations, starting with the pathogen rather than with a specific food. To be able to mitigate the listeriosis risks related to RTE seafood products, it is essential to reduce the number and size of outbreaks. For this purpose, a new risk assessment model adapted to the current practices is to be developed.

A second hypothesis is the increased popularity of RTE seafood. In the past, smoked fish consumption was often linked to special occasions, but as it became less expensive to produce, it also became a more affordable and frequent part of the diet in many countries, including among elderly people (Business Research Insights, 2022). The increased consumption could be a reason why more listeriosis cases are related to RTE fish products than before (Business Research Insights, 2022).

A third hypothesis is that a higher fraction of the fish produced is used as raw material for RTE foods, leading to fewer opportunities to select the best quality raw materials for RTE foods and use the standard quality product for cooked, fried and baked dishes.

Data from recent studies have shown that fish used as raw material for smoked products can already be contaminated with *L. monocytogenes* in the sea, with contamination further exacerbated during the filleting that precedes smoking, marination or graving (Skjerdal *et al.*, 2014). Implementation of WGS has indicated that different genotypes dominate in the environment, processing and in different countries, both at sea and on land (Félix *et al.*, 2022). As processing of fish may occur within one facility or at several facilities in different countries, the products may be contaminated with multiple strains, with different virulence potential, along the farm/harvest to consumption continuum. The long value chain is accompanied by specialized processing where, for instance, one large producer of RTE fish products may receive unprocessed fish or fillets from many suppliers from different countries, then process the fish for various products, and export to several countries and continents. This production logistic makes cross-contamination and spread of *L. monocytogenes* possible in different stages of the value chain, as seen in an outbreak linked to a process facility in Estonia (ECDC/EFSA, 2019).

The risk assessment model described here aims to represent the introduction and fate of *L. monocytogene*s in hot- and cold-smoked fish and gravad fish, from primary production to consumption; although it should be flexible to accommodate other RTE fish products in the future, such as sashimi and ceviche. The model will be used to assess the contribution of the initial levels of contamination of the fish raw material from slaughter or harvest, the contribution of cross-contamination, the effect of time/temperature profiles throughout the food supply chain (i.e. processing, transport, retail and in consumer homes), and the effect of processing and preservation practices, including the effect of lactic acid bacteria cultures added for biocontrol of *L. monocytogenes*.

The previous RTE seafood model (FAO/WHO, 2004b) only covered smoked fish. However, since there is strong evidence that gravad fish is an emerging risky product (e.g. 9 out of 15 outbreaks due to seafood were linked to gravad

fish (MRA38 Annex 2, Section A2.3; Nakari *et al.*, 2014)), gravad fish (sugar/salt marinated) will also be included in the new model. Thus, the risk assessment will consider hot- and cold-smoked fish (e.g. salmon) and gravad fish (e.g. salmon/halibut). Sushi has been excluded because: (i) few outbreaks have been linked to this product; (ii) it represents a low risk according to an as yet unpublished risk model constructed by one expert on the panel; (iii) its association with low consumption among high-risk groups (i.e. the elderly and pregnant women); and (iv) a recent assessment indicated a low risk of listeriosis unless Listeria had reached high levels before the fish was prepared for sushi, which could occur in fish species with a long shelf life and/or storage at temperature abuse conditions (VKM, 2019). Products like sashimi, carpaccio and ceviche are to a large extent fish fillets sliced and marinated shortly before consumption, and therefore, the steps in the model before smoking are relevant to these products. Such products can easily be included in a module-based model by exchanging the smoking or graving modules. Some countries have traditional products like fermented fish; for instance, *rakfisk* in Norway, which is already known as a high risk product causing outbreaks (Axelsson *et al.*, 2020). Such products were not considered relevant in the present model, as the processing is different and the relevance in an international context is limited. Nevertheless, even local products can be implemented in a module-based model by taking only the fresh fish steps in the proposed model into consideration and combining them with other, specific modules for the products, such as, but not limited to, drying and salt-curing.

4.4.2 Framing the model

The RTE seafood model represents the production chain of cold-/hot- smoked fish and gravad fish, including: harvesting of fish from open sea and aquaculture, head cutting and evisceration, filleting, smoking or graving, freezing, slicing, packaging, multiple transportation steps, retail and consumer storage (Figure 4).

The model comprises three modules. Module 1, "Primary processing and transportation", represents the slaughter of fish and the first stages of processes such as filleting, chilling, and transportation to a secondary facility. Module 2, "Secondary processing", differentiating between smoked fish and gravad fish. For the production of smoked fish, the stages to be modelled are smoking, slicing and packaging, whereas for gravad fish, the stages are maceration, slicing and packaging. Module 3, "Cold chain", covering the growth of *L. monocytogenes* in both products during transport to retail, display at retail, transport from retail to home, and home storage prior to consumption. An assessment of the processes – cross-contamination, growth, survival, mixing, partitioning and removal of *L. monocytogenes* – is shown for each step within the modules (Table 4).

Module 1: Primary processing and transportation

Primary processing

Fish can be harvested or caught from sea water or fresh water all over the world. However, farmed species used for RTE fish are concentrated in some regions. The data for *L. monocytogenes* prevalence in live fish or immediately after capture/ slaughter are scarce; however, some data exist for aquaculture fish. *L. monocytogenes* can be present in fish feed, on feeding equipment, in pipelines, in dead fish in the cages, etc. (Hoel *et al*, 2021, Miettinen and Wirtanen 2006). After slaughtering, the fish is exsanguinated, eviscerated and cleaned. This is the first product of fish: heads-on, gutted (HOG). In other cases, the fish is beheaded. In any case, the fish is then transported, stored and sold in boxes, preferably chilled with ice or frozen. If the fish is used in the processing of RTE products, the concentrations of *L. monocytogenes* in the fish before the processing starts are included in the exposure assessment.

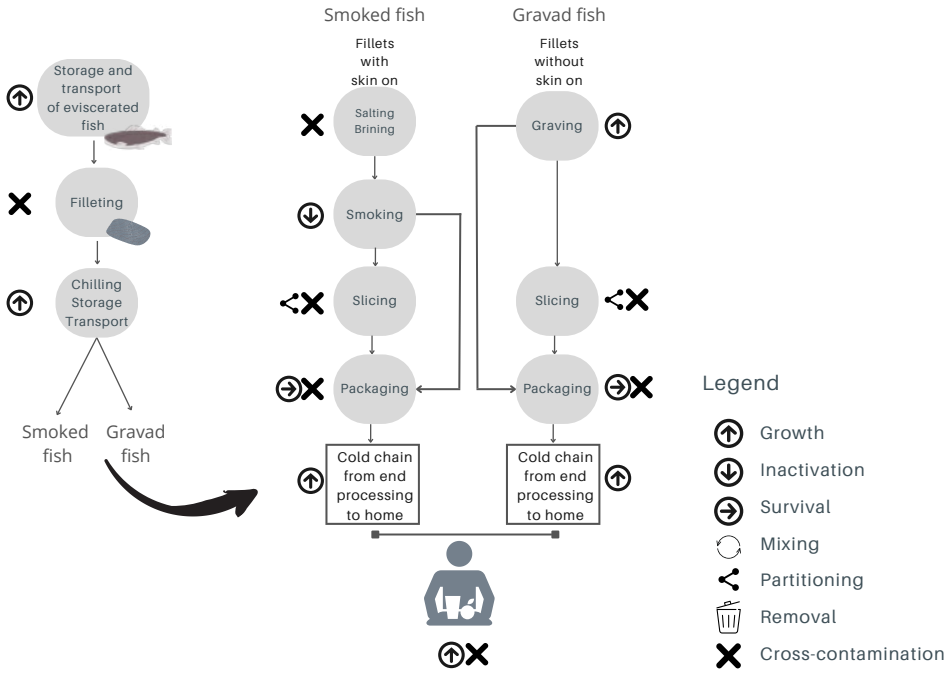

Figure 4: Stages of the production of smoked fish and gravad fish, with an indication of the processes to be modelled

Table 4: Summary of the processes (growth, survival, mixing, partitioning, removal and cross-contamination) to be modelled for each of the modules of the QRA of *L. monocytogenes* in smoked and gravad fish

Module[1]	Growth	Survival/Inactivation	Mixing	Partitioning	Removal	Cross-contamination
#1 Primary processing – Storage of eviscerated fish and transport to filleting	Growth can occur in fish during cold storage and transport If filleting is carried out in the same facility, there will not be any transport stage	-	-	-	-	-
#1 Primary processing – Filleting	-	-	-	-	-	Cross-contamination can occur during filleting.
#1 Primary processing and transportation – Chilling, cold storage and transport to secondary processing	Growth during cold storage. If the Dalgaard model is used, set phenol to zero and salt to the physiological concentration	-	-	-	-	-
#2a Secondary processing – Salting/ Brining	-	-	-	-	-	For brine injection: consider in depth cross- contamination via needles For dry salting, consider contamination on the surface
#2a Secondary processing – Smoking for smoked fish	-	Reduction depends upon the type of smoking, cold or hot	-	-	-	-
#2b Secondary processing – Graving for gravad fish	Growth is expected at the normal temperature used for maturation (6 °C)	-	-	-	-	-
#2a,b Secondary processing - Slicing	-	-	-	-	Partitioning into slices	Potential cross-contamination from slicing machine
#3a,b Cold chain	Growth from storage at processing until end of home storage	-	-	-	-	-

[1]"a" is a module for the production of RTE smoked salmon, and "b" for the production of gravad fish

Deboning and filleting is the next step in the processing of the fish. In the production facilities, the separation between unclean and clean production zones comes before this step. However, the transfer from an unclean to a clean processing zone does not imply that the concentration of Listeria in the fish is zero. Carry-over of *L. monocytogenes* from the unclean zone cannot be excluded, as the fish skin may be contaminated. In addition, the fish flesh may be contaminated by *Listeria* from process surfaces, equipment, water, skin and, if not completely removed in previous steps, viscera. The fillets could have skin on or off, and be trimmed in various ways. The trimming can be done in the same factory as the slaughterhouse, but could also be done in a separate facility. Cross-contamination during filleting could therefore occur in more than one facility.

After processing, fish fillets can be super-chilled, stored on ice or chill-stored, or be further processed without storage. Fresh fish spoils rapidly, and even though facilities may have food and environmental sampling programmes, the option to wait for negative results for *L. monocytogenes* is often impractical.

Growth of *L. monocytogenes* introduced to the fish flesh in earlier steps can therefore already be expected before the processing to the final product starts. Survey studies of *L. monocytogenes* at the filleting stage indicate higher prevalence and concentrations after breaks in the production (e.g. after holidays) and in periods with warm weather (Skjerdal *et al.*, 2014). However, the concentrations tend to be low overall. In a study by Skjerdal *et al.* (2014) among 80 samples collected immediately after filleting, 29 were positive with detection level of 1 CFU/25 g, but none above 10 CFU/g. After a week of storage at 4 °C, 39 out of 80 samples contained more than 100 CFU/g.

Bacteria from food-equipment surface samples have been collected over years and sequenced. Strains with similar sequences are often found in many years, indicating persistent house strains in these types of facilities (Wagner *et al.*, 2022; Fagerlund *et al.*, 2021). There are indications that facilities that receive fish from many locations have a higher diversity of CC groups than those who only produce from one locality. Rørvik *et al.* (1997) reported that drains were well suited as indicators for the presence of *Listeria* spp. in a facility. Inspections conducted by the FDA revealed that facilities with numerous *L. monocytogenes* positive environmental samples had poor hygienic conditions (Cripe and Losikoff, 2021). However, companies receiving raw materials from many suppliers could also have many different strains in their facilities, even if their hygienic practices are good. Good hygienic practices will however reduce the probability of house strains.

Transportation to secondary processing

Eviscerated fish, with or without skin on, may be stored prior to secondary processing. Depending on the time-temperature conditions during storage and transport to secondary processing, contaminated bacteria can grow in the fish during this period. Several growth models and rates for *L. monocytogenes* on salmon have been published (Le Marc, 2001; Jia *et al.*, 2020). The secondary Food Spoilage & Safety Predictor model for *L. monocytogenes* can be applied to estimate growth in unprocessed fish fillets as well. The tool is developed for lightly preserved seafood, but according to the developers, it is well suited for raw, unprocessed fish given that the salt content, pH, additives, etc, can be set to the conditions in raw fish.

Module 2: Processing

Salting

Different salting technologies such as dry salting, injection of salt, and brining can be used in the production of RTE fish. Whichever the method, salt dissolves in water extracted from the salmon fillet and forms a concentrated salt solution in the liquid phase in the fish. For dry salting or brine salting, the gradient appears on the fillet surface. This gives a temporary concentration gradient between the salting medium itself and the muscle tissue. The absorbed salt then diffuses to the inner fish matrix. In the case of brine injection, salt uptake is faster, yet the inner part of the fillet may become contaminated with the bacteria via the injection needles. Considering the duration of this step and the high salt concentration on the surface where contamination occurs, this step is not favourable to the growth of *L. monocytogenes*, but the bacterium survives these salt concentrations (Lorentzen *et al.*, 2010a).

Smoking and maturation (relevant to smoked fish)

Smoking is an important component of fish preservation (Adeyeye, 2019), which can be applied before or after salting. Two different types of smoking can be considered: hot-smoking and cold-smoking (Lerfall and Hoel, 2021). Both smoking types can reduce the contamination of *L. monocytogenes*, although to different extents (Cripe and Losikoff, 2021). Reduction in *L. monocytogenes* due to cold-smoking has been observed by Eklund *et al.* (1995), Suñen *et al.* (2003), Neunlist *et al.* (2005) and Porsby *et al.* (2008). However, reduction means that a fraction survives during cold-smoking. The effect of hot-smoking will differ according to the temperature of the smoke. Hot-smoking is usually carried out with smoke above 60 °C (Cunha *et al.*, 2021). A model for inactivation in the

temperature range of 55–60 °C, developed for cod fish mince, prepared from fresh or rehydrated salt-cured cod is available (Lorentzen *et al.*, 2010b). As phenol does not stimulate survival, this model can be used to assess inactivation in cold-smoking and non-optimal hot smoking.

Maceration (relevant for gravad fish)

Gravad fish have similar process steps to smoked fish, but instead of smoking, salt is added to the fish (in similar concentrations as smoked products) and some sugar. This reduces the pH and stimulates the growth of lactic acid bacteria. This process is a short fermentation, lasting for a few days in chilled conditions. Growth of *L. monocytogenes* is expected to occur in gravad fish, since its final composition does not inhibit this pathogen.

Slicing

Slicing machines can be regarded as a source of *L. monocytogenes* contamination (Aarnisalo *et al.*, 2007). Earlier, Eklund *et al.* (1995) detected *L. monocytogenes* in product trimmings from slicers at a frequency of 48.5 percent (17/35) during production in five sampling visits to a fishery products processing plant. Nonetheless, immediately after cleaning, rinsing and sanitizing, no *L. monocytogenes* was detected on the slicing machines (0/6). Di Ciccio *et al.* (2012) repeatedly isolated *L. monocytogenes* serotypes 1/2a and 1/2b from slicer belts, distribution trays, slicing machines and slicing covers for three years in a smoked-salmon production facility. Out of 95 environmental samples tested, slicing machines (37 percent) and working tables (43 percent) had the highest frequencies of detection. In the United States of America, in a processing plant for catfish fillets, Chen *et al.* (2010) determined that in 15 percent (7/45) of the sampling times, skinning, slicing and blending equipment were contaminated with *L. monocytogenes*. In Ireland, Dass (2011) isolated Multiple-Locus Variable Number Tandem Repeat Analysis types c and b in the slicer and skinner machines over a one-year survey (2 positives out of 36).

Packaging

Vacuum-packaging and modified atmosphere packaging (MAP) are used to delay the proliferation of spoilage and pathogenic bacteria and to minimize oxidative reactions. However, *L. monocytogenes* can survive under such conditions (Roberts, 2020). If shelf-life is prolonged, as is the case of cold-smoked and gravad fish, even if coupled with refrigerated storage, reduced oxygen packaging may not guarantee complete inhibition of *L. monocytogenes* growth (Tocmo *et al.*, 2014).

Many authors have described cross-contamination during packaging, originating from surfaces that are in direct contact with the food being packaged. In cold-smoked

salmon plants, Autio *et al.* (1999), Vogel *et al.* (2001), Klaeboe *et al.* (2005), Nakamura *et al.* (2006), Thimothe *et al.* (2004) and Hu *et al.* (2006) recovered *L. monocytogenes* from direct food contact surfaces in the packaging equipment at the frequencies of 23.8 percent (20/84), 17.1 percent (140/818), 14.8 percent (23/155), 8.9 percent (9/101), 4.8 percent (6/125) and 1.4 percent (5/344), respectively. Nonetheless, cross-contamination during packaging will not be specifically modelled in the present proposal (see the following section "Cross-contamination during processing").

Alternative treatments

New technologies have been proposed as a post-packaging treatment for RTE fish, such as high-pressure processing (HPP) (Roobab *et al.*, 2022). For the present model, addition of lactic acid bacteria is considered as an adequate strategy to inhibit *L. monocytogenes*. It should be mentioned that not all lactic acid bacteria have the same inhibiting effect on *L. monocytogenes*. This should be taken into consideration in the model. A Jameson-effect growth model should be used to model the growth of *L. monocytogenes*, as affected by lactic acid bacteria. The effect of an alternative parameter on the level of *L. monocytogenes* can be modelled by a distribution of a reduction parameter.

Cross-contamination during processing

The incidence of specific *L. monocytogenes* strains that may persist in the different fish processing plants is widely documented in the scientific literature. Floors and drains are probably the sites most commonly contaminated by persistent strains, but *L. monocytogenes* can also be isolated from equipment such as gutting machines and slicing machines (Fagerlund *et al.*, 2022). Studies by Vogel et al (2001) suggest that end product contamination occurs mainly during processing, and probably not due to contamination from raw fish, although the authors did not exclude the possibility of raw fish being an important source of contamination. The same conclusion was reached by Nakamura *et al.* (2006), since they obtained the genetically related isolates of *L. monocytogenes* from the final products of smoked salmon, removed skin, swab samples from slicers and the floors of several rooms in the factory. However, *L. monocytogenes* was not isolated from the raw salmon. In Mędrala *et al.* (2003), contamination events mainly occurred in the brining and slicing steps. Persistence of specific strains was identified. An agent-based model for *Listeria* spp. cross-contamination in a smoked salmon facility revealed different contamination dynamics and risks among equipment surfaces in terms of the presence, level and persistence of *Listeria* spp. (Zoellner *et al.*, 2019). Although the available data clearly indicate that the persistence of *L. monocytogenes* in the food processing environment contributes to cross-contamination of finished products,

there is still no consensus on why "persistent strains persist" and no data on the transfer rate of these strains from environmental niches to products along the processing line.

Cross-contamination can occur at all steps from production/harvest to consumption. Different CCs predominate at sea, in processing environments and in different countries (Félix *et al.*, 2022). Hence, strains of CC-groups with different virulence potentials may be introduced at the different steps. Currently, there are not enough data to postulate the source of origin of *L. monocytogenes* contamination based on CC-groups, or full DNA sequences. However, such hypotheses are expected in the future. Furthermore, studies that indicate a link between phenotypic characteristics such as growth rate or disinfectant resistance and specific gene sequences can be expected. Inclusion of genetic information in the modules is therefore relevant.

Module 3: Cold chain

The models of Mejlholm and Dalgaard (2007a,b, 2009, 2013, 2015) and Mejlholm *et al.* (2010), can be used to estimate the growth of *L. monocytogenes* and the inhibitory effect of lactic acid bacteria throughout the distribution chain, taking into account the intrinsic factors (pH, aw, preservatives) and extrinsic factors (temperature and atmosphere in the package) of both the smoked fish and the gravad fish products.

4.4.3 Summary of assumption and data

Annex 6, Section 1 presents an extensive explanation of the data and the assumptions for building the model for *L. monocytogenes* in RTE smoked fish and gravad fish. Input variables, parameters, descriptions and equations are compiled in tables for each of the modules in Annex 6, Section 2.

5

Hazard characterization

5.1 INTRODUCTION

Dose-response (DR) modelling is undertaken to quantify the likelihood of infection and disease as a function of an ingested dose (Sanaa and Guillier, 2022). Widely-accepted DR models for toxic-infectious and invasive pathogens are based on two fundamental assumptions (Buchanan *et al.*, 2009; FAO/WHO, 2004b): the single-hit assumption and the independent action assumption (i.e. the probability that a given bacteria will cause an infection is not affected by dose, but the probability that infection will occur increases directly with the number of bacteria). Ingestion of *L. monocytogenes* cells, as for any pathogenic bacteria, does not always result in infection, illness or death. Various factors can act as barriers or increase the likelihood of infection or disease. In assessing the effects, several factors related to the pathogen, the human host and the environment (i.e. food matrix) should be considered (Sanaa and Guillier, 2022). This section aims to identify the factors of interest and the dose-response relations to be used in the quantitative risk assessment of listeriosis.

5.2 FACTORS INFLUENCING DOSE RESPONSE PARAMETERS

The DR relationship is not fully understood for human listeriosis. However, this relationship is determined by three main components, known as the triangle of infectious diseases (Buchanan *et al.*, 2000; Hoelzer *et al.*, 2013; McLauchlin *et al.*, 2004; FAO/WHO, 2004b):

- Food matrix, related to protection against physiological barriers, induction of stress response, and effects on transport through the gastrointestinal tract;
- Host, related to aspects such as susceptibility, immune status, medications, pregnancy, etc.; and
- Pathogen, including its survival properties, virulence, strain variability, and antibiotic resistance, among other things.

5.2.1. Food matrix

Foodborne listeriosis has been associated with the consumption of RTE foods such as milk products, fish products, processed meat and fruits and vegetables; and the risk is probably linked to their intrinsic characteristics that allow the growth of *L. monocytogenes*, and to their mode of consumption (Leclercq *et al.*, 2021). The food matrix may affect susceptibility to infection, including the pathogen's ability to survive the stomach's gastric acid action (McLauchlin *et al.*, 2004). Prior exposure of *L. monocytogenes* to moderately acidic conditions has been shown to increase the ability of *L. monocytogenes* to survive the conditions that they would be exposed to during their passage through the stomach and upper intestinal tract (Buchanan *et al.*, 2000). It has also been shown that foods that contain probiotics or/and prebiotics may reduce the expression of *L. monocytogenes*' virulence genes (Dong *et al.*, 2020; 2022; Wu *et al.*, 2022). Thus, an accurate assessment of DR relations may require consideration of the nature of the food matrix and its effect on the *L. monocytogenes* pathogenicity (Buchanan *et al.*, 2000). For instance, as stated in MRA38, although the food matrix could influence the expression of *L. monocytogenes* virulence genes, there are not enough data to include this as a variable in DR models.

5.2.2. Host

The response of human populations when exposed to *L. monocytogenes* is highly variable, according to the susceptibility of the considered populations (FAO/WHO, 2004a,b). In fact, human populations are highly diverse in relation to their response to infectious agents, as a consequence of their genetic background, general health, nutrition status, age, immune status, stress level, and prior exposure to the pathogen (Buchanan *et al.*, 2000). Some segments of the populations, groups with depressed immune systems, present higher risks; the very young (having immature responses) and the elderly (having reduced immune responses) are considered to be at increased risk of foodborne infectious agents (Buchanan *et al.*, 2000). Some more recent work on mechanistic dose models for *L. monocytogenes* have begun to quantify how differences in host gastrointestinal stress conditions, and other factors, lead to differences in *L. monocytogenes*

survival and therefore impact risks of illness at the same consumed dose (Rahman 2016; 2018). In addition to the extreme variability in the probability of infection among population subgroups with different predisposing factors, *L. monocytogenes* is characterized by a low probability of illness at low doses when averaging across the total population or broadly defined population subgroups (Pouillot *et al,* 2015a; FDA-FSIS, 2003).

The expert group expanded on the work on "Host Susceptibility" as described in MRA38 Section 4 (FAO/WHO, 2022). That work describes three basic groups of susceptible subpopulations:

1) Less susceptible subpopulations. These are "healthy individuals/adults", i.e. the general population, which include non-pregnant women, people younger than 65 years old, and those individuals not suffering from any condition leading to higher risk.

2) Susceptible subpopulations. These are populations with conditions that modestly increase risk for listeriosis. Previous work has typically defined these as including pregnant women, newborns (< 28 days old), and the elderly (≥ 65 years old).

3) Very susceptible subpopulations. These are populations with conditions that greatly increase the risk of listeriosis. Previous work has typically defined these as including people with weakened immune systems from conditions such as cancer treatment or organ transplant.

MRA38 (Section 4.2) (FAO/WHO, 2022) suggests developing updated DR models for susceptible sub-populations. Here, two specific approaches for expanded DR will be adopted.

The first approach will be to adopt the DR parameters that describe 11 sub-populations with a different intrinsic susceptibility to listeriosis, as given in MRA38 section 4.2.1 (Table 10). One interesting note from this table is that relative risk increases from about 5-fold for a person with heart disease to about 400-fold for a person with haematological cancer in comparison to the reference group (i.e. population < 65 years with no conditions). In addition, pregnancy has around a 100-fold risk increase, which would likely place this condition into the "very susceptible subpopulation" grouping (an increase in risk category from "susceptible subpopulation"), if one chose to disregard the relative risk from these 11 subpopulations in only three categories of susceptibility.

The second approach will be to adopt the DR parameters from the EFSA generic quantitative microbial risk assessment (QMRA) model (EFSA, 2018), which stratifies the population by age (typically in 10-year strata) and gender (male/female). This approach represents an additional option identified and prioritized by this expert group.

Table 5: Subpopulation descriptions and their respective relative risk values of invasive listeriosis in France taken from Pouillot *et al.* 2015

Subpopulation	**Description**	**Host susceptibility**[1]	**Relative Risk (95% CI)**[2]	**Estimates of r : mean**[3] ***Lognormal-Poisson DR Model***
Less than 65 years old, no known underlying condition, healthy adults	Population < 65 years with no conditions	LS	Reference group	7.90×10^{-12}
More than 65 years old, no known underlying condition	Population ≥ 65 years with no conditions	S	13.9 (8.6, 23.1)	1.49×10^{-10}
Pregnancy[4]	Total number of live births + fetal loss + abortions/population * 0.75	S	116 (71, 194.4)	2.01×10^{-9}
Cancer (haematological)	Leukaemia, Hodgkin's lymphoma, non-Hodgkin's lymphoma, multiple myeloma	VS	373.6 (217.3, 648.9)	9.60×10^{-9}
Cancer (non-haematological)	Breast, brain, ear, nose and throat, gastrointestinal, gynaecological, kidney, liver, lung, prostate cancers	VS	54.8 (34.2, 90.3)	7.76×10^{-10}
Diabetes	Type I, Type II	VS	7.6 (3.5, 15.6)	7.47×10^{-11}

Renal or liver failure	Dialysis: haemodialysis, peritoneal dialysis, liver disease: hepatitis A, B, C	VS	149.4 (82, 270.1)	2.79×10^{-9}
Heart disease	Self-reported heart disease	VS	5.4 (1.5, 14.4)	5.01×10^{-11}
HIV/AIDS	HIV or HIV/AIDS	VS	47.4 (10.5, 140.4)	6.50×10^{-10}
Inflammatory disease	Rheumatoid arthritis, Crohn's disease, colitis, ulcerative colitis, giant cell arteritis	VS	58.5 (25.2, 123.4)	8.43×10^{-10}
Solid organ transplant	Heart, intestinal, kidney, liver, lung, and pancreas transplant patients	VS	163.7 (26.3, 551.5)	3.14×10^{-9}

[1]Level of susceptibility of the subpopulations: LS: Less susceptible subpopulations; S: susceptible subpopulations; VS: Very susceptible subpopulations

[2]Estimated using a Poisson regression without adjustment. These 95% CIs should only be considered as indicative but suggest that all those groups have a significantly greater risk of listeriosis than the reference group

[3]Resulting statistics for r, the probability of illness following the ingestion of one cell of *L. monocytogenes* obtained from the Lognormal-Poisson dose-response model for invasive listeriosis following the ingestion of *L. monocytogenes* in different population subgroups. The distribution of r includes the individual within-group and the strain variability (Pouillot *et al.*, 2015a)

[4]Prevalence of pregnancy was determined using the Statistics Canada CANSIM database and a report from the Canadian Institute for Health Information. Estimates were determined by first summing live births, fetal loss and abortions for 2011. This value was then divided by the 2011 population. To account for the length of pregnancy, 9 months, the value was finally multiplied by 0.75. National, provincial, and territorial estimates were calculated. (For further detail, see Supplementary Material of Falk *et al*, available at: https://doi.org/10.1017/S0950268816000327)

The expert group chose to provide DR based on both underlying health conditions and basic demographic data, as it was suspected that different risk modellers would have access to different population data.

5.2.3. Pathogen

The expert group continue with the work on virulence differences between *L. monocytogenes* strains, as described in MRA38 Section 4 "Hazard Characterization". That work describes three basic groups of pathogen virulence:

1) Hypervirulent. Strains with increased virulence relative to most *L. monocytogenes* due to factors that are encoded in the genome. This group has previously been called "hypervirulent" or "highly virulent". The expert panel suggests one could the expert group also identify this group in plain language as "more virulent".
2) Virulent. Strains with typical variations in virulence relative to most *L. monocytogenes.* This group has been previously called "virulent".
3) Hypovirulent. Strains with reduced virulence relative to most *L. monocytogenes* due to factors that are encoded in the genome. This group has been called "hypovirulent" until now. The panel suggests one could also identify this group in plain language as "less virulent".

The classification of pathogenicity by CCs may be revised when additional data are obtained from further research, including in various regions. The dose-response relationship for *L. monocytogenes* in humans varies with genotypic lineage or subtype lineage. The lineage I isolates are more virulent than lineage II isolates, which has been supported by a risk assessment (Chen *et al.*, 2006) as well as by observations that lineage I isolates, on average, show higher measures of tissue culture pathogenicity as compared to lineage II isolates (Norton *et al.*, 2001).

5.3 MODELLING DOSE-RESPONSE RELATIONSHIPS

5.3.1 Classical modelling approaches

Several authors have focused their research on the development of DR models for listeriosis, either in an isolated perspective to compare the quality of fit of the various models, or integrated into more complex frameworks for risk assessment models. Most of the listeriosis DR relationships utilized were based in one of three mathematical functions: the exponential model (Buchanan *et al.*, 1997; Chen *et al.*, 2003; Haas *et al.*, 1999; Lindqvist and Westöö, 2000; Notermans *et al.*, 1998;

Pasonen *et al.*, 2019; Pouillot *et al.*, 2015a; Rahman *et al.*, 2018), the Weibull-gamma model (Farber *et al.*, 1996) and the Beta-Poisson model (Haas *et al.*, 1999).

5.3.2. New approaches to dose-response modelling

Towards mechanistic models

Rahman *et al.* (2016) presented a framework for the integration of host-pathogen interactions in the early stages of exposure, aiming to elucidate the DR paradigm of *L. monocytogenes* infection. These authors proposed to model the population dynamics of *L. monocytogenes* using the classical logistic model, coupled with two inhibitory factors: the carrying capacity and the immune pressure. The DR model parameters and the functional form were determined using data from animal feeding trials. The DR model proposed was merely illustrative. Rahman *et al.* (2018) then presented an extension of their model. This renewed framework was based on *in vitro* studies that simulate human physiology, modified mathematical forms and human listeriosis outbreak data. The model takes into account the gastrointestinal barriers for *L. monocytogenes* (gastric juice and low pH, commensal bacteria in the small intestine, and immune cells); thus, the mechanistic DR model considers the population dynamics of *L. monocytogenes* in the human gut pathway.

Advance to heterogeneity frameworks

Pouillot *et al.* (2015a) carried out a refinement of the exponential model to incorporate the population subgroups and strain virulence into the DR relationship. This framework can be seen as a mechanistic approach to the exponential dose-response model of listeriosis. The exponential model has the simplifying assumption of a constant probability of infection, following the ingestion of *L. monocytogenes* in a given population. Yet the framework proposed by Pouillot *et al.* (2015a) incorporates variability in strain virulence and host susceptibility into the DR relationship. The log-normal distribution was considered as a suitable distribution to represent the variability in host susceptibility and strain virulence in *r*. Thus, the log-normal distribution was used to separate the variability in *r* (probability of infection for a given individual following the ingestion of one cell of *L. monocytogenes*) into three sources (Pouillot *et al.*, 2015a):

(1) susceptibility across mutually exclusive population subgroups with a shared predisposing risk factor; (2) susceptibility across individuals within a given population subgroup; and (3) virulence among *L. monocytogene*s strains with different virulence determinants. The model is represented as follows:

$$P_{ill}(d) = \int_0^1 (1 - exp(-rd)) \sum_g \pi_g f(r;\theta_g) dr$$

where: d is the expected number of *L. monocytogenes* cells in one typical portion of an RTE food; $f(r;\theta_g)$ represents the remaining individual (within group) susceptibility variability and strain virulence variability in r; θ_g is characteristic of the subgroup g; $\pi_g, \sum_g \pi_g = 1$ is the proportional size of the population subgroup g within the total population. This DR relationship integrates, in addition to those factors accounted for by the subpopulation-specific dose-response model, the variability in mean susceptibility across population subgroups. Furthermore, r is defined as the product of two independent probabilities: P_i is the probability linked to events controlled by host factors (individuals); and P_S is the probability reflecting the bacterial factors that control virulence and pathogenicity.

$$r = P_i \times P_s$$

Log-normal distributions were then assumed for P_s and P_i, since their product is itself a log-normal random variable, and r is also log-normally distributed. For a given population subgroup and strain, r follows a log-normal distribution,

$$r \sim lognormal\left(\mu_i + \mu_s, \sqrt{\sigma_i^2 + \sigma_s^2}\right)$$

With these assumptions and data from Chen *et al.* (2003) and FDA-FSIS (2003), Pouillot *et al.* (2015a) obtained the parameters of the log-normal distributions for r by numerical integration for one healthy population group and for ten subgroups at increased risk. The advance of the lognormal-Poisson model devised by Pouillot *et al.* (2015a) resides in the explicitness of the variability in strain virulence and in susceptibility across population subgroups and, therefore, the model can accurately capture the risk of listeriosis in those population subgroups at highest risk. According to Pouillot *et al.* (2015a), the FAO/WHO model can be regarded as a marginal dose-response model for a population exposed to a cross-section of *L. monocytogenes* strains and, as such, the model averages across numerous individuals with different levels of susceptibility and multiple *L. monocytogenes* strains with varying levels of virulence. Pouillot *et al.* (2015a) went on to state that using the FAO/WHO model could be inappropriate for evaluating certain rare, but potentially highly relevant, events such as the ingestion of a highly virulent *L. monocytogenes* strain by a highly susceptible individual.

5.3.3 Adaptation of the Pouillot DR model to take virulence markers into account

As discussed in the WGS Section, classification of strains relying on CC/ST has proven to be a performance marker of the virulence power of *L. monocytogenes* strains. Fritsch *et al.* (2018) proposed an updated version of the Pouillot *et al.* (2015a) model. The model considers the variability of virulence levels among different *L.*

monocytogenes strains in mice, as related in studies by Pine *et al.* (1991), Pine *et al.* (1990), and Stelma *et al.* (1987). The median lethal doses (LD_{50}) for these 26 strains were obtained through intraperitoneal infection (FDA-FSIS, 2003) and were initially converted into *r* values. These *r* values represent the probability of a specific bacterial cell causing the adverse effect. The LD_{50} is inversely proportional to *r*, as indicated by Pouillot *et al.* (2015a). Consequently, $\log_{10}$ LD_{50} can be converted into $\log_{10}$ r with a scaling factor. Fritsch *et al.* (2018) clustered the 26 values observed in mice in three groups of virulence. This approach permitted three sets of values for $\log_{10}$ r to be defined instead of one. The $\log_{10}$(r) of the less than 65 without underlying conditions for the "more virulent", "virulent" and "less virulent" groups is characterized by normal distributions with the respective parameters *N*(-11.87, 0.52), *N*(-13.99, 0.63) and N(-16.70, 1.12). The relative risks proposed in Table 5 can be used to assess the DR for any other group of the population. Figure 5 allows both approaches to be compared. The range of values for the three distributions proposed by Fritsch *et al.* (2018) are globally equivalent to that proposed by Pouillot *et al.* (2015a).

Fritsch *et al.* (2018) associated these three subgroups, based on mice experiments, with three groups of ST/CCs by using the clinical frequency data of Maury *et al.* (2016). The clinical frequency considers both exposure and virulence. It was calculated by dividing the number of clinical isolates for a specific CC by the total number of clinical and food isolates belonging to that CC (Maury *et al.*, 2016). Recognizing that the frequency of clinical isolates relative to the total isolates served as a reliable indicator for hypervirulent clones such as CC1, CC2, CC4, and CC6, as well as hypovirulent CC9 and CC121, Fritsch *et al.* (2018) suggested extending this assumption to the other clones. Annex 7 provides the association between CC/ST and the three virulence groups. For CC/STs not present in that list, Fritsch *et al.* (2018) recommended classifying them in the "virulent" group.

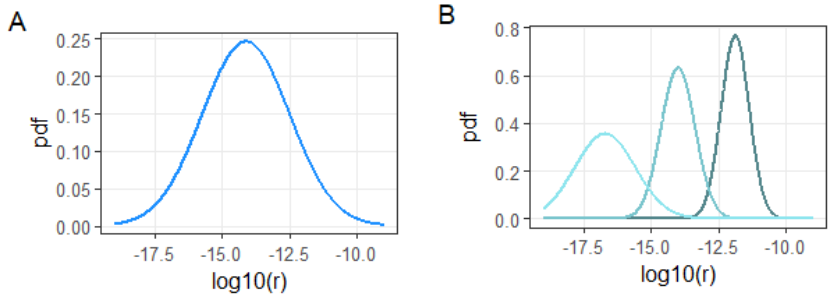

Figure 5: Variability of $\log_{10}$(r) for the less than 65 years of age without any known underlying condition according to Pouillot *et al.* (2015a) (A) and Fritsch *et al.* (2018) (B). In B, the three colours correspond to the three groups of virulence of *L. monocytogenes* strains.
Sources: See References section.

5.4 CONCLUSIONS - RECOMMENDATIONS

The fundamental element of hazard characterization is the DR relationship between the ingested dose and the manifestation and magnitude of the adverse health effect in exposed individuals. DR models that could take into consideration the pathogen, the host and the environment with adequate data, will contribute to modelling the biological mechanisms involved in listeriosis. The most frequent choice for dose-response functions to be integrated in listeriosis QRA models has been the exponential model.

Pouillot *et al.* (2015a) proposed a realistic approach for the *r* parameter of the exponential model, as it represents the variability in infectivity among different *L. monocytogenes* strains and host susceptibilities. Fritsch *et al.* (2018) proposed an adaptation of the parameter characterizing the strain variability based CC/ST marker. The expert group considered that the most appropriate DR approaches to implement in the QRA models proposed in the present work are the lognormal-Poisson model of Pouillot *et al.* (2015a) when no data are available for CC/ST, and the Fritsch *et al.* (2018) parameters (p_s) of the Pouillot *et al.* (2015a) model when CC/ST data are available.

As not all users of the QRA models will have data to fully describe host or pathogen differences, the implemented models should accommodate and be flexible to changing the population categories (i.e. by allowing the selection of some of them, e.g. > 65 years old and pregnant women). In the same way, the EFSA model should be made available to users in the situation where only age class data are available. The working group strongly recommends that work be done to verify that the conclusions on the classification of *Listeria* strains based on ST/CCs are valid in the different regions of the world.

6

Whole genome sequence

6.1 INTRODUCTION

Quantitative description of the behaviour of pathogens along the food chain, and the characterization of variability amongst pathogenic species are at the heart of the concerns of risk assessors in the area of microbiological food safety. Whole genome sequencing (WGS) is a molecular typing method that has developed rapidly in the last decade and offers great potential to support risk assessors in characterizing variability and in fine-tuning risk characterizations.

WGS is increasingly being used to genetically type pathogenic isolates from foods and patients, and is being used in surveillance and epidemiological investigations and root-cause analyses in some high-income countries. In addition, whole genome characterization of the strains has been shown to provide important information about pathogenic features, including virulence and antimicrobial resistance-associated genes. Therefore, WGS opens avenues for explaining the intraspecies variability of phenotypes when strong associations between genomic features and specific phenotypes are observed and validated. Quantitative microbiological risk assessment (QMRA) models generally describe the observed variability of a biological parameter (e.g. growth, resistance, virulence, prevalence) in a distribution at the species level. However, over-representation of specific subtypes of strains in specific food commodities, or correlation between subtypes and virulence are not considered, but they are relevant to the exposure assessment and hazard characterization steps of a QMRA, respectively. The expert group conducted a literature review to evaluate the benefits of WGS and explored which stages of a QMRA can be refined when WGS data on *L. monocytogenes* are implemented.

6.2 PREVALENCE

More evidence is becoming available that specific CCs of *L. monocytogenes* are over-represented in specific food commodities (Li *et al.*, 2018; Maury *et al.*, 2019; Painset *et al.*, 2019; Bechtel and Gibbons, 2021; Dufailu *et al.*, 2021; Barbuddhe *et al.*, 2022). This information is relevant for the exposure assessment step, where estimates and variability of prevalence are incorporated. The correlations between genotypes (i.e. CCs) and prevalence give support to replacing a global distribution of prevalence for *L. monocytogenes* as a whole by refined distributions per subgroups of CCs that are over-represented or under-represented in a specific commodity following the approach of Fritsch *et al.* (2018).

6.3 VIRULENCE

Genomic characterization of clinical isolates and food isolates has supported the grouping of *L. monocytogenes* into subgroups or classes that differ with respect to their virulence (Chen *et al.*, 2011). This information is relevant for the hazard characterization step of a QMRA, because differences in virulence classes may substantiate the use of different DR correlations for subgroups that differ in their virulence. The expert group concluded that there is sufficiently robust scientific information available to classify *L. monocytogenes* into different virulence classes by using information on CC or sequence type (ST). The expert group proposed taking up the three classes of virulence proposed in MRA38. The grouping of CC/STs into these three virulence classes is not fully conclusive (Wagner *et al.*, 2022), but may be taken as the first step towards differentiating CCs with respect to their virulence. The work of Fritsch *et al.* (2018) can be taken as an example for grouping CCs into three virulence classes, and thereby incorporating different *r* values for different virulence classes into the hazard characterization step. Recent Genome Wide Association Studies (GWAS) identified *de novo* markers of virulence (Njage *et al.*, 2019), but integration of this information to support the grouping of subtypes in virulence classes is not yet conclusive. Although the emergence of antibiotic resistance among *L. monocytogenes* strains requires attention (Barbuddhe *et al.*, 2022; Olaimat *et al.*, 2018), it is not yet considered as a factor worth taking into account for virulence, as most antibiotic therapies are successfully applied (Charlier *et al.*, 2017).

6.4 BEHAVIOUR IN THE FOOD CHAIN

New research is available that correlates genetic biomarkers to phenotypes relevant to the exposure assessment part of a QMRA (e.g. enhanced robustness, increased fitness, or persistence). More specifically, genetic associations have been reported for fitness in soil (Sévellec *et al.*, 2022), fitness at low temperatures (Fritsch *et al.*, 2019; Hingston *et al.*, 2017; Njage *et al.*, 2020), robustness towards acid and/or desiccation stress (Hingston *et al.*, 2017; Njage *et al.*, 2020), ability to form a biofilm and persist in environments (Lee *et al.*, 2019; Liu *et al.*, 2022) and tolerance to biocides (Palma *et al.*, 2022; Douarre *et al.*, 2022). However, validation of these potential markers is still needed to confirm their predictive potential in order to group strains with respect to differences in behaviour and genotype, and to integrate this information into the exposure assessment part of a QMRA.

6.5 RECOMMENDATIONS FOR THE IDENTIFICATION OF MARKERS IN RELATION TO EXPOSURE ASSESSMENT

Currently, the availability of sequenced genomes may be somewhat biased because sequenced isolates that have been isolated in some high-income countries by governmental agencies are over-represented in data bases. The biological material of the isolated strains is the basis for genotypic and phenotypic studies. While genotypic characterization (WGS) can ultimately be outsourced, resources to isolate pathogens should be present at the place of action and the biological material should become available for characterization. Phenotypic and genotypic characterization of strains requires considerable human and material resources. A broad collection of strains is needed to achieve unbiased genotype/phenotype correlation studies. The classification of virulence by CC/STs could be revised in due time when new scientific knowledge becomes available. Future revisions would also be useful in response to bacterial evolution, and the emergence of new subtypes.

The validation of genetic markers of relevant phenotypic traits is crucial for their use in risk assessment models (Guillier *et al.*, 2022; Lakicevic *et al.,* 2022), and markers are considered validated if robust statistical associations are proven using complementary experimental approaches. Experimental validation by reverse genetics of the markers associated with a phenotype of interest for microbial risk assessment is probably only feasible in some cases (Berendsen *et al.*, 2016), at least in the short term. It is likely that risk assessors will be satisfied with a statistical

validation (a p-value below the corrected significance threshold) of markers. Alternatively, validation of phenotype predictions using strains other than those used for marker identification also remains a realistic goal, even if it does not provide scientists with functional genomics justifications. Regarding exposure assessment models, a high number of sources of variability are usually modelled (levels, transfer rate, growth/survival rates, cardinal temperatures, etc.). Not all these sources have the same importance concerning the outcome of QMRA models. The implementation of biomarkers is thus specifically meaningful for the highest priority sources of microbiological variability (Fritsch, 2021), according to uncertainty or sensitivity analysis methods. Therefore, a QMRA performed for a species as a whole (*L. monocytogene*s) can point to the steps in the QMRA that have a major impact on the risk variability, and may provide justification for allocating resources for further elucidation of genetic markers for performance. In the short term, integration of information obtained from WGS will reduce sources of uncertainty in risk assessment. Strains' variability will be better grasped and modelled by identifying molecular markers of adaptation to environment/food stressors (in connection with predictive microbiology) or virulence markers (for the parameters of the DR relationship). In the longer term, it is conceivable that genomic markers could be used to establish management measures that are better adapted to the different potentials of subtypes. However, this implementation requires the development of validated microbiological methods for the identification of markers on isolated clones, and the standardization and validation of new methods is a long process.

7

Next steps

In conclusion, the expert group elaborated formal models for the risk assessment of *L. monocytogenes* for cantaloupe, lettuce, frozen vegetables and RTE seafood. As a next step, these models should be programmed, tested and reviewed. Based on a review of the published dose-response models for *L. monocytogenes*, the expert group proposed the use of existing models based on susceptible populations with underlying conditions that increase risk of listeriosis (or different risk of illness in different age-gender groups). The proposed dose-response model considers consumer susceptibility and virulence characterization based on the genomic data that are currently available. Furthermore, a sensitivity analysis should be performed to identify which model inputs and assumptions have the greatest impact on the model outputs (e.g. dose and risk for the different consumers).

As for dose-response, an updated set of parameters for each model that account for the three classes of virulence of *L. monocytogenes* should be put forward, incorporating current data on specific virulence profiles associated with sequence types (ST) and/or CCs. There is a need for additional data on *L. monocytogene*s in the food chain to better inform *L. monocytogenes* occurrence, virulence and dose response, so that a risk assessment for different ST/CCs of *L. monocytogenes* can be performed.

The review should verify that the models are flexible enough to run different scenarios and incorporate new data to account for national, regional and international contexts. Following the review, the models should be made publicly available. These novel models will advance the state of knowledge on *L. monocytogenes* risks by incorporating advances in next generation technologies and by going from primary production to consumption. Furthermore, these models could be parameterized to include a dose-response relationship accounting for train or host variability, vulnerable groups, and genomic data.

When the prevalence of vulnerable groups is not known, the model may provide an option for considering age-gender groups. To this end, it is desirable to provide a user-friendly interface to accommodate different model applications, with clear explanations provided to the users.

Once the models are tested, reviewed and verified, they could be used to run different scenarios assessing the effects of climate change, interventions along the full chain and sampling monitoring schemes. The model should help to provide specific recommendations highlighting certain epidemiological aspects such as the association between immunodeficiency diseases and the virulence of certain *L. monocytogenes* strains.

Regarding WGS, the global prevalence of *L. monocytogenes* in the exposure assessment part can be replaced by the specific prevalence for groups of ST/CCs when WGS data have demonstrated that these groups of ST/CCs are over-represented in a specific commodity. The three classes of virulence proposed in MRA38 should be used in the model. Scientific literature should be used to compile a list of ST/CCs belonging to different virulence groups. However, at this time, genetic biomarkers for enhanced robustness or increased fitness are not sufficiently conclusive for subgrouping *L. monocytogenes* with respect to differences in behaviour to be incorporated in the exposure assessment part. Additionally, the uncertainty between phenotypic and genotypic profiles of *L. monocytogenes* should be considered and carefully examined before being used in risk assessment.

8

Conclusion

Over the course of five days, the expert group reviewed the existing literature on risk assessment models, and discussed the model approach and exposure assessment, including hazard characterization and dose-response data and the most current knowledge on the genomic data for possible inclusion in the four risk assessment models encompassing: cantaloupe (whole cantaloupe and RTE diced cantaloupe), leafy greens (whole lettuce and RTE pre-cut lettuce), frozen vegetables (non-RTE blanched frozen vegetables), and RTE seafood (cold-/hot-smoked fish and gravad fish).

In light of the available data and current risk assessment approaches, the expert group discussed the stages from primary production to consumption to be represented in the four models, including approaches that accommodate the testing of scenarios, interventions and sampling schemes that could reduce the risk of listeriosis.

It was agreed that the full production chain, from primary production to consumption, be included in the risk assessment for cantaloupe, lettuce and RTE seafood, whereas for frozen vegetables the model would include processing to consumption stages. Using the Modular Process Risk Model framework and Monte Carlo simulation will allow for variability and uncertainty to be incorporated into the models, with "what-if" scenarios representing interventions to reduce the risks. Furthermore, it was concluded that the modules need to be structured in a flexible manner within each model, so that they can be shared among the four food commodities and for other similar commodities (e.g. fresh herbs, frozen beans, sashimi). Open-source software should be used to develop the models. When available, the input data for the risk assessment models should come from the scientific literature, previously published quantitative risk assessment models and industry sources. In cases where no data exist, it was

agreed that reasonable assumptions and expert knowledge elicitations be used.

Considering that surveillance and population data acquisition vary across different countries and geographical regions, it was agreed that the hazard characterization approach be adapted from existing models that consider variability in pathogen virulence and consumer susceptibility, and that this approach should be common to all food commodities. Two specific approaches for expanded dose-response (DR) model are proposed: 1) dose-response parameters that take into account different intrinsic susceptibilities to listeriosis (described in MRA38 section 4.2.1, Table 10); and 2) dose-response parameters from the EFSA QMRA model, which stratifies the population by age and gender.

A literature review of the current data on WGS related to *L. monocytogenes* was performed to assess whether enough information exists to inform the risk assessment models regarding strain variability in virulence and survival fitness. In lieu of current data, which suggest that some sequence types and CCs of *L. monocytogenes* are over-represented or under-represented in specific food commodities and that virulence differs among different CCs, the expert group concluded that this information should be included in the four risk assessment models. The three classes of virulence were proposed, in line with the grouping described in MRA38. While the expert committee agreed that the grouping of CC/STs into these three virulence classes is not fully conclusive, there is still value in using the existing data as a first step to differentiating CCs with respect to their virulence. It is recommended that the classification of virulence by CC/ST be revised in due course when new scientific knowledge becomes available.

While there is evidence that some genetic markers are associated with *L. monocytogenes* fitness in soil, low temperatures, acidic and dry environments, biofilm formation and tolerance of biocides, there is a lack of data on the validation of genetic markers of relevant phenotypic traits. At present, the genetic markers for increased fitness of *L. monocytogenes* in different environments are not sufficiently conclusive for subgrouping *L. monocytogenes* with respect to differences in behaviour to be incorporated into the exposure assessment within the model. These data would be useful in risk assessment models; however, this implementation requires the development of validated microbiological methods for the identification of markers within different ST/CCs, and the standardization and validation of new methods. It is recommended that the classification of *L. monocytogenes* based on genetic markers related to fitness be reviewed and revised as more conclusive data become available.

In conclusion, the formal models for the risk assessment of *L. monocytogenes* for cantaloupe, lettuce, frozen vegetables and RTE seafood developed by the expert

group should be programmed, tested and reviewed. The review should verify that the models are flexible enough to run different scenarios that assess interventions along the food chain, and the effects of climate change and changing farming and processing practices. The models should allow for flexibility to incorporate new data to account for national, regional and international contexts. In addition, the models should be conducive to providing specific recommendations for high-risk populations and should also account for changing conditions, such as the evolution of the pathogen and newly available genomic data on prevalence, strain fitness and virulence, changing consumer practices, and climate change.

9

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Annexes

Annex 1

Recommended revisions to the Annexes from the Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in foods (CAC/GL 61-2007)

Para.	CAC/GL 61-2007	JEMRA recommendations
Annex I: Recommendations for an environmental monitoring programme for *Listeria monocytogenes* in processing areas		
Title	Recommendations for an environmental monitoring programme for *Listeria monocytogenes* in processing areas	• To consider including primary production (handling, packing, with or without water) in the title. This could be done in a separate Annex or by modifying the existing Annex I to include a primary production consideration.
Introduction	"…foods that support *L. monocytogenes* growth and that are not given…"	• To consider the inclusion of other foods that do not support *L. monocytogenes* growth (e.g. frozen foods – frozen fruits/vegetables, ice cream). • To consider including information on the purpose of the environmental monitoring programme, as this will drive the sampling approach (i.e. verification of cleaning and sanitation, reaction to a positive sample, etc.).
a)	Type of product and process/operation	• To consider including the history of *L. monocytogenes* in raw materials in this section and including primary production. • To consider products, equipment (e.g. forklifts) and employee flow within the processing environment.
b)	Type of samples	• To consider highlighting the importance of non-food contact surfaces (NFCSs), and close-to-food contact surfaces (FCSs), in specific operations (i.e. frozen foods, condensation). • To consider adding examples of other NFCSs and FCSs (workers, shoes, gloves and cleaning equipment).
	Time of sampling	• To consider adding an additional bullet to capture the time of sampling during production or prior to the start of production (e.g. T = 0 and T = 3hr in production).

g)	Analytical methods	• To consider updating to include more relevant techniques (e.g. remove pulsed-field and ribotyping, replace with WGS and Multilocus Sequencing Typing).
i)	Actions in the event of positive results	• To consider including source tracing and isolate profiling to help understand the transient or persistent nature of contamination. • To consider the addition of a "Seek and destroy" approach. • To consider micro-climate (e.g. air movement and condensation). • To consider the addition of a recommendation for root cause analysis.
Annex II: Microbiological criteria for *Listeria monocytogenes* in ready-to-eat foods		
4.1 & 4.2	Table 1 & Table 2, Footnote c	• Delete the word "analytical standard deviation" and change to "standard deviation" and provide justification for the value used.
4.1 & 4.2	Table 1 (heading)	• To consider clarifying "m" by amending the text in the footnote to distinguish acceptable lots (≤m) from unacceptable lots (>m).
Annex III: Recommendations for the use of microbiological testing for environmental monitoring and process control verification by the competent authorities as a means of verifying the effectiveness of HACCP and prerequisite programmes for the control of *Listeria monocytogenes* in ready-to-eat foods		
Introduction		• To consider expanding the scope of the annex to include elaboration of the concepts from Section 3 of the main text of the Code, so as to capture primary production, given that some foods (e.g. cantaloupe, leafy greens) can go directly from farm to sale and on-farm activities (e.g. harvest, cutting, washing, packing) can be a source of contamination with *L. monocytogenes*.
a)	Environmental Monitoring	• To consider recommending storage of isolates for further characterization and sharing of data for risk assessment.
b)	Process Control Verification	• To consider recommending corrective actions for FBOs for when microbiological criteria are exceeded or for when *Listeria* spp. is detected (NFCS and FCS). *Listeria* spp. or other indicators are some of the options to include in the criteria for corrective action.

Table A 2.1: Features of quantitative risk assessment models of *L. monocytogenes* from consumption of produce by scope

Scope	Food	RTE	Cross-contamination: Stage	DR – End-point[1]	Type of DR model	DR Sub-populations	Strain variability	Temp profiles/ Lag time[2]	Country	Source
Production-to-consumption	Lettuce	No	Yes: Transport, market, restaurants and at home	Exp – I	FAO/WHO (2004b)	High-risk/ Low-risk	Strain diversity implicit in r	No/Yes	Republic of Korea	Ding *et al.* (2013)
Processing-to-consumption	RTE lettuce salad	Yes	No	WG – I	Farber *et al.* (1996)	High-risk/ Low-risk	-	No/No	Spain	Carrasco *et al.* (2010)
	Fresh-cut romaine lettuce	Yes	Yes: Processing – during packaging	WG – I	Farber *et al.* (1996)	High-risk/ Low-risk	-	No/Yes	United States	Guzel (2015)
	Fresh-cut cantaloupe	Yes	Yes: Processing – after cutting	WG – I	Farber *et al.* (1996)	High-risk/ Low-risk	-	No/Yes	United States	Guzel (2015)
	Fresh baby spinach	No	Yes: Processing – before packaging	Exp – I	Chen *et al.* (2006)	General	-	No/Yes	United States	Omac *et al.* (2017)

End process-to-consumption	Leafy green salads from salad bars	No	No	Exp – I	Chen *et al.* (2006)	General	-	Yes/No	Netherlands (Kingdom of the)	Franz *et al.* (2010)
	RTE leafy vegetables	Yes	No	Exp – I	Buchanan *et al.* (1997)	General	-	No/No	Brazil	Sant'Ana *et al.* (2014)
	Leafy green salads from salad bars	No	No	Exp – I Log – D	Chen *et al.* (2006), Williams *et al.* (2009)	General Perinatal	-	Yes/No	Netherlands (Kingdom of the)	Tromp *et al.* (2010)
Retail-to-consumption	Fruits and vegetables	Yes	No	Mouse Epi – I	FDA-FSIS (2003)	Multiple	Variability in the virulence of different strains represented in DR	No/No	United States	FDA-FSIS (2003)
	Lettuce	No	Yes: Handling at home	Exp – I	FAO/WHO (2004b)	High-risk	Strain diversity implicit in r	No/No	Spain	Domenech *et al.* (2014)
Consumption	Non-RTE frozen vegetables	No	No	Exp/WG – I	Buchanan *et al.* (1997), Bemrah *et al.* (1998)	High-risk/ Low-risk	-	No/Yes	United States	Zoellner *et al.* (2019)

[1]DR: dose-response; Exp: exponential; WG: Weibull-gamma; I: illness; D: death: Mouse-Epi: Mouse-Epidemiological
[2]Use of dynamic temperature profiles in the simulation model/Use of a predictive microbiology model for lag phase duration of *L. monocytogenes*

Sources: **Type of DR model** and **Source** columns cite the corresponding studies. See References section for further details.

Table A 2.2: Predictive microbiology models and main outcomes related to "what-if "scenarios and sensitivity analysis from quantitative risk assessment models of *L. monocytogenes* from consumption of produce

Scope	Food	Predictive microbiology models	"What-if" scenarios	Sensitivity	Source
Production-to-consumption	Lettuce	Growth (Gompertz model, polynomial model for lag phase, growth square root model)	-	-	Ding *et al.* (2013)
Processing-to-consumption	RTE lettuce salad	Growth (linear model, growth square root model)	(1) Use of MAP (5.5%, CO_2, 3% O_2; 92.5% N_2) as opposed to no packaging (baseline) reduces mean number of listeriosis cases by 95%; (2) Reducing the shelf-life from a maximum of 12 days to 4 days reduces number of cases by 84%; (3) Preventing high-risk consumers from consuming RTE salads reduces number of cases by 75%; (4) Applying microbiological criterion at primary production (n = 20; c = 0; absence in 25 g) reduces cases by 43%.	**Number of listeriosis cases**: Ranked in this order: serving size, storage temperature at home, storage time at home, LM concentration at consumption (nor provided)	Carrasco *et al.* (2010)
	Fresh-cut romaine lettuce	Growth (Baranyi model, growth square root model)	(1) LM counts at consumption are reduced by >99% after exposure to ionizing radiation (1 kGy at room temperature) and reduces log risk of illness in 1.66 log in the susceptible population; (2) Cold atmospheric plasma reduce LM population by 92% and log risk in 1.34 log; (3) Peroxyacetic acid reduces LM counts by 28%, and log risk in 0.35 log; (4) Cross-contamination during processing increases LM counts by 18% and log risk in 0.06 (because the transfer coefficient was very low at 0.002); (5) Home temperature abuse (20 °C x 24 h) increases mean LM counts by 56% and log risk in 1.1; (6) Consumption time up to a maximum of 14 days increases LM counts by 2100% and log risk in 2.6.	-	Guzel (2015)

	Fresh-cut cantaloupe	Growth (Baranyi model, growth square root model)	(1) Implementation of irradiation reduces LM at consumption by 99.9%, and mean cases of listeriosis by 99%; (2) cross-contamination during processing increases cases by 300%; (3) Home temperature abuse at home (20 °C x 24 h) increase LM at consumption by 300% and cases by 220%; (4) Extending consumption time up to a maximum of 10 days increases LM at consumption by 2300% and cases by 39000%.	-	Guzel (2015)
	Fresh baby spinach	Growth (Baranyi model, square root models for growth and lag phase, polynomial model for Ymax)	Baseline scenario represents neither interventions during processing nor cross-contamination. (1) Washing with water decreases mean cases of listeriosis by 7.5%; (2) Water with PAA or ClO_2 reduces mean risk of listeria cases by 22%; (3) Washing and cross-contamination still reduces mean cases by 12%; (4) Washing plus temperature abuse (at home, ambient temperature for 1.2 h) increases mean cases by 55%; (5) Washing plus irradiation reduce cases by 56%; (6) Washing plus irradiation plus MAP reduce cases by 65%; (7) Washing plus cross-contamination plus irradiation plus MAP plus temperature abuse reduce mean cases by 35%.	-	Omac *et al.* (2017)
End process-to-consumption	Leafy green salads from salad bars	Growth (Baranyi, growth square root model)	(1) A breakdown in the salad bar's cooling unit (temperature of 18 °C from the moment that the salad bar is filled) increases the mean number of cases by 23% (In the baseline scenario temperature of salad bar is assumed to fluctuate normally between 0–13 °C).	**Outcome - probability of infection from any serving:** Initial prevalence (r=0.75) and portion size (r= 0.62).	Franz *et al.* (2010)

RTE leafy vegetables	Growth (linear model, growth square root model)	(1) Reducing mean initial prevalence of LM from 1.7% to 0.17% decreases mean cases of listeriosis in 84%; (2) Reducing initial mean prevalence and keeping temperature strictly between 1–5 °C during processing and storage reduces cases by 85%; (3) Reducing maximum initial counts of LM from 2.74 to -1.04 log CFU/g reduces mean cases by 91%; (4) Reducing maximum initial counts and keeping temperature strictly between 1–5 °C reduce cases by 92%; (5) Reducing both prevalence and counts decreases mean cases by 98.7%.	-	Sant'Ana *et al.* (2014)
Leafy green salads from salad bars	Growth (linear model, growth square root model)	(1) The delivery frequency to a restaurant was increased from 2 days a week to 5 days a week. In this scenario, the catering outlet is allowed to order leafy green-based salad products every weekday. This scenario halved the mean number of cases.	**Outcome – the desired service level with regard to "out-of-stock"** (z parameter; the greater z is, the smaller the probability of being out of stock at the cold storage stage, but the higher the probability of having products of lower quality; z was set at 7, which results in hardly any out of stock situations and no products wasted): (1) The growth of LM is insensitive with a setting of z=6; (2) Increasing z to 8 will considerably increase the growth of LM; (3) The impact of increasing the delivery frequency to five times a week is rather insensitive to z.	Tromp *et al.* (2010)

Retail-to-consumption	Fruits and vegetables	Growth (linear model, square root model for EGR)	-	-	FDA-FSIS (2003)
	Lettuce	Growth (linear at 6 °C and 23 °C), Survival (empirical equation for water treatment)	-	**Outcome - LM counts at consumption**: Probability of washing (r=-0.46 - -0.43), surface contamination (r=0.23-0.29), time under running tap water (r=-0.09 – -0.14), board/knife transfer rate (r=0.07 - 0.13), contamination at retail (r=0.02 - 0.04)	Domenech *et al.* (2014)
Consumption	Non-RTE frozen vegetables	Growth (linear, EGR square root model, empirical model for lag phase)	The median log risk of listeriosis from consumption of frozen vegetables contaminated with LM is -12.7. (1) Within-package clustering parameter between 0.01 and 0.1 in the baseline - as opposed to 1 in the baseline - reduces median log risk to -15/-14.1; (2) Number of packages tested per lot of 20 or 10 - as opposed to 5 in the baseline - reduces median log risk to -14.4/-13.7; (3) Thawing at ambient temperature or in the fridge has a negligible effect on the risk; (4) Changing the number of servings per meal (s=0.5, 2) also resulted in no difference from the baseline (s=1) risk of listeriosis.	**Outcome - dose of LM consumed**: cooking the serving (r=-0.87), log reduction due to proper cooking (r=-0.48), LM counts in a serving from contaminated package (r=0.46), time stored at room temperature (r=0.02), time/temperature in the refrigerator (r=0.01)	Zoellner *et al.* (2019)

LPD: lag phase duration; RLT: relative lag time; EGR_x: exponential growth rate at x °C; RR: risk reduction; r: coefficient of correlation in sensitivity analysis

Sources: **Source** column cites the corresponding studies. See References section for further details.

Table A 2.3: Features of quantitative risk assessment models of *L. monocytogenes* from consumption of seafood products by scope

Scope	Food	RTE	Cross-contamination : Stage	DR – End-point[1]	Type of DR model	DR Sub-populations	Strain variability	Temp profiles/ Lag time[2]	Country	Source
End process-to-consumption	French cold smoked salmon	Yes	No	Exp – I	Pouillot *et al.* (2009)	Multiple	-	Yes/No	France	Pouillot *et al.* (2007) (2009)
Retail-to-consumption	Various: smoked seafood, raw seafood, preserved fish, cooked RTE crustaceans	Yes	No	Mouse Epi – I	FDA-FSIS (2003)	Multiple	Variability in the virulence of different strains represented in DR	No/No	United States	FDA-FSIS (2003)
	Packaged cold-/hot-smoked fish and gravad fish	Yes	No	Exp - I	Pouillot *et al.* (2015a)	Multiple	Challenge test data from a mixture of strains; h_0 distribution of variability in the physiological state of cells; variability in strain virulence and in susceptibility across population subgroups	Yes/Yes	Non-specific	Pérez-Rodríguez *et al.* (2017)

Consumption	Smoked/ gravad salmon/ rainbow trout	Yes	No	Exp – I	Buchanan *et al.* (1997)	General	All strains are virulent vs a proportion of virulent strains	No/No	Sweden	Lindqvist *et al.* (2000)
	Cold smoked fish	Yes	No	Exp – I	FAO/WHO (2004b)	High-risk/ Low-risk	Strain diversity implicit in r	No/Yes	Non-specific	FAO/WHO (2004b)
	Smoked fish and sliced cooked ham	Yes	No	Exp – I	FAO/WHO (2004b)	High-risk/ Low-risk	Strain diversity implicit in r	No/No	Spain	Garrido *et al.* (2010)
	Cold smoked salmon	Yes	No	BP – I	Haas *et al.* (1999)	General	-	No/Yes	Non-specific	Gospavic *et al.* (2010)
	VP cold smoked salmon	Yes	No	WG – I	Farber *et al.* (1996)	High-risk/ Low-risk	Challenge test data from a mixture of strains;	No/Yes	Ireland	Dass (2011)
	Traditional processed fish	No	No	WG – I	Farber *et al.* (1996)	High-risk/ Low-risk	-	No/No	Ghana	Bomfeh (2011)
	Cold smoked and salt-cured fishery products	Yes	No	Exp – I	Pasonen *et al.* (2019)	High-risk/ Low-risk	-	No/No	Finland	Pasonen *et al.* (2019)

[1]DR: dose-response; Exp: exponential; WG: Weibull-gamma; I: illness; D: death: Mouse-Epi: Mouse-Epidemiological; EGR_x: exponential growth rate at x °C
[2]Use of dynamic temperature profiles in the simulation model/Use of predictive microbiology model for lag phase duration of *L. monocytogenes*

Sources: **Type of DR model** and **Source** column cite the corresponding studies. See References section for further details.

Table A 2.4: Predictive microbiology models and main outcomes related to "What-if" scenarios and sensitivity analysis from quantitative risk assessment models of *L. monocytogenes* from consumption of seafood

Scope	Food	Predictive microbiology models	"What-if" scenarios	Sensitivity	Source
End processing-to-consumption	Cold smoked salmon	Growth (Jameson effect LM and background microflora, growth square root models for LM and background microflora)	**EXPOSURE ASSESSMENT:**(1) Reducing theoretical shelf-life from 28 to15 days reduced mean LM/g in contaminated servings by 10%; (2) The baseline scenario of 21.4% of shelf lives at home longer than 7 days was compared to a scenario of consumption within 7 days maximum; and reduces the mean LM/g by 10%; (3) Better refrigeration at retail from mean temperature 5.6 to 4 °C reduces the mean LM counts by 19%; (4) Better refrigeration at home from mean temperature 7 to 4 °C, reduces the mean LM counts by 36%; (5) Lower initial concentration from 0.46% of values above 1 CFU/g to a distribution truncated at 1 CFU/g, reduces the mean LM counts by 8%. **RISK ASSESSMENT: Output – Listeriosis cases compared to a base 100 for the baseline model:** (1) Shelf-life 15 days =23; (2) Prevalence of LM to a quarter=25; (3) Mean home refrigerator temperature 4 °C = 34; (4) Consumed 7 days after purchase = 37; (5) Prevalence of LM to a half = 50; (6) Mean retail temperature at 4 °C =67.	**EXPOSURE ASSESSMENT: Output – concentration of LM in contaminated servings:** (1) Total duration in the consumer phase ($p=10^{-30}$), (2) Mean temperature in the consumer phase ($p=10^{-20}$), (3) Initial LM counts ($p=10^{-20}$), (4) Mean temperature at retail phase ($p=10^{-14}$), (5) Total duration of the retail phase ($p=10^{-8}$), (6) Tmin for growth ($p=10^{-8}$), (7) Tmin microflora ($p=10^{-6}$), (8) Initial background flora counts (p=0.002), (9) Serving size (p=0.003), (10) MPD (p=0.008), (11) Ref GR at 25 °C (p=0.015), (12) Ref GR of flora at 25 °C (p=0.025). **RISK ASSESSMENT: Output - listeriosis cases in the reference population:** (1) r value of DR model ($p=10^{-300}$), (2) SD(MPD) ($p=10^{-137}$), (3) Ref of GR 25 °C for LM ($p=10^{-101}$), (4) MPD of LM ($p=10^{-76}$), (5) Tmin of LM ($p=10^{-12}$), (6) GR of flora 25 °C ($p=10^{-8}$), (7) Prevalence of LM ($p=10^{-6}$), (8) Servings/year ($p=10^{-2}$).	Pouillot *et al.* (2007), (2009)

Retail-to-consumption	Various: smoked seafood, raw seafood, preserved fish, cooked RTE crustaceans	Growth (linear model, EGR5 square root models)	For cold smoked salmon, reducing the max home storage time from 45 to 30 days, reduces the mean cases by 38% in the elderly population.	-	FDA-FSIS (2003)
	Packaged cold-/hot-smoked fish and gravad fish	Growth (Baranyi model with Jameson effect LM and LAB, EGR5 square root model and effect of lactate)	(1) Decreasing the maximum initial LM counts by 2 log decreases listeriosis cases per million servings by >99%; (2) Decreasing time to consumption in 25% decreases listeriosis by 80%; (3) Decreasing 1–2 °C in the dynamic temperature profiles reduces cases by 75%; (4) Including lag time in the model has no effect on listeriosis cases.	-	Pérez-Rodrí-guez *et al*. (2017)
Consumption	Smoked/ gravad salmon/ rainbow trout	-	(1) The minimum level of LM resulting in a risk of illness greater than 10^{-7} or 10^{-8} was 25 or 2 CFU/g; (2) If the assumption that all strains are virulent was reduced to 1-10%, the annual listeriosis cases is reduced by 84% in the both high-risk and the low-risk populations.	**Output – annual risk of illness**: ranked as initial LM counts, prevalence, serving size, proportion of virulent strains	Lindq-vist *et al.* (2000)
	Cold smoked fish	Growth (LM growth model affected by LAB growth, Square root model for GR as a function of temperature, pH, aw, undissociated lactic acid)	(1) If LM growth rate inhibition due to LAB growth is between 80-100% , the increase of listeriosis per 100 000 people is 684-fold in the overall population, in comparison to the baseline scenario of no-growth of LM between purchase and consumption; (2) If LM growth rate inhibition due to LAB growth is 95%, the increase of listeriosis per 100 000 people is 67-fold in the overall population in comparison to the no-growth of LM baseline scenario; (3) Reducing the mean shelf-life of smoked fish from 14 to 7 days, results in an 80% reduction in listeriosis.	-	FAO/ WHO (2004b)

Consumption	Smoked fish (salmon and trout)	Growth (Logistic model without delay, growth cardinal model)	(1) Reducing home storage time from a max of 30 to 7 days, reduces the annual cases by 15% for salmon and by 45% for trout; (2) If all domestic temperatures have a mean temperature of 4.5 °C, the mean number of annual cases is reduced by 65% for salmon and by 70% for trout; (3) Combining the two measures above reduces the mean annual cases by 75% for salmon and by 87% for trout; (4) If at purchase, LM counts did not exceed 100 CFU/g (truncating the baseline N~(1.01, 0.71) for smoked salmon and N~(1.35,1.40) for smoked trout, the mean annual cases would decrease by 22% for salmon and by 99% for trout.	-	Garrido *et al*. (2010)
	Cold smoked salmon	Growth (Baranyi model with Jameson effect LM and background microflora, extended GR models for LM and LAB as a function of temperature, pH, aw, un-dissociated lactic acid, undissociated diacetate, phenols, dissolved CO_2 and nitrite)	(1) At a mean initial LM count in smoked fish of 4 CFU/g, reducing the time of consumption from 28 to 14 days reduces the risk of illness by 64%; (2) If mean time of consumption is 14 days, reducing the mean initial counts from 25 CFU/g to 4 CFU/g reduces the risk of illness by 67%.	-	Gospavic *et al*. (2010)

Consumption	Vacuum-packed cold smoked salmon	Growth (Baranyi model, growth square root model)	(1) If initial LM counts at retail (1-1000) were truncated at >100 CFU/g, the risk of illness would reduce by 0.3/0.9 log (high-risk and low-risk populations); (2) Reducing the maximum consumer shopping time from 3 hours to 30 min reduces risk of illness by 0.8/0.8 log; (3) Reducing consumer storage days from 21-30 to 7-15 days reduces risk of illness by 0.5/0.6 log; (4) Fixing storage temperature from 3–10 °C to 4 °C reduces risk of illness by 1.0/1.1 log; (5) If LM counts were not higher than 2 log CFU/g and the maximum shopping time were reduced to 30 min, reducing consumer storage to 7-15 days and temperature to 4 °C, would reduce the risk of illness by 1.32/1.39 log.	**Output – annual risk of illness in the high-risk population**: (1) LM counts at retail (r=0.97); (2) Temperature in consumer fridge (r=0.13); (3) Time in consumer fridge (r=0.06).	Dass (2011)
	Cold smoked and salt-cured fishery products	Growth (Logistic growth model, growth cardinal parameter model as a function of temperature, salt content, pH and phenolic compounds)	(1) If home storage temperature decreased from 7 °C to 3 °C, the median cases of listeriosis per 100 000 of the elderly population would decrease by 70%; (2) If home storage temperature decreased from 7 °C to 3 °C, the median cases of listeriosis per 100 000 of the working-age population would decrease by 40%.	-	Pasonen *et al.* (2019)

LPD: lag phase duration; RLT: relative lag time; EGR_x: exponential growth rate at x °C; RR: risk reduction; r: coefficient of correlation in sensitivity analysis

Sources: **Source** column cites the corresponding studies. See References section for further details.

Annex 3

Technical description of the exposure assessment model for *L. monocytogenes* in whole cantaloupe and RTE diced cantaloupe

SECTION 1: ASSUMPTIONS AND DATA

Module 1: Pre-harvest

Data relevant to this module that have been retrieved from the literature are those on: prevalence and concentration of *L. monocytogenes* in irrigation water; prevalence, concentration and survival of *L. monocytogenes* in soil; likelihood of *L. monocytogenes* being detected in soils of produce fields; and survival of *L. monocytogenes* on cantaloupe rind. Table A 3.1 lists some sources and data regarding the occurrence of *L. monocytogenes* in irrigation water used for crop production.

Table A 3.1: Data on *L. monocytogenes* in water environments ordered by prevalence

Country	Type of water	Positive/Total (% Prevalence)	Source
Austria	River and pond	0/68 (0.0)	Linke *et al.* (2014)
United States	Engineered water	0/28 (0.0)	Strawn *et al.* (2013a)
United States	Engineered water	0/14 (0.0)	Strawn *et al.* (2013b)
Malaysia	Irrigation water for veg farms	0/15 (0.0)	Jeyaletchumi *et al.* (2011)
India	River water	8/100 (8.0)	Soni *et al.* (2013)
Switzerland	River, stream, inland canal	25/191 (13.1)	Raschle *et al.* (2021)
Canada	Rural and urban watersheds	56/329 (17.0)	Stea *et al.* (2015)
United States	Pond, river used for irrigation	2/9 (22.2)	Strawn *et al.* (2013b)
South Africa	Roof-harvested rainwater	72/297 (22.0)	Jongman and Korsten (2016)
Canada	Surface (river)	32/134 (23.9)	Lyautey *et al.* (2007)
United States	Surface water	48/146 (33.0)	Strawn *et al.* (2013a)
United States	Lake, stream, river, pond	605/1405 (43.1)	Cooley *et al.* (2014)
South Africa	Irrigation canal and river	19/36 (52.8)	Ijabadeniyi *et al.* (2011)
United States	Surface water for irrigation	33/52 (63.5)	Weller *et al.* (2015)

Sources: **Source** column cites the corresponding studies. See References section for further details.

Three studies have quantified the concentrations of *L. monocytogenes* in the collected water samples positive for the pathogen. Sharma *et al.* (2020) and Acheamfour *et al.* (2021) reported very low levels of *L. monocytogenes* in surface water creeks, ponds, tidal brackish river and non-tidal fresh rivers, with MPN values ranging from <0.03 to 11 MPN/L. In Iwu *et al.* (2022), the mean concentration of *L. monocytogenes* in irrigation water samples from 19 agricultural sites in South Africa was 1196 CFU/100 mL, ranging from 0 - 5667 CFU/100 mL.

Table A 3.2 compiles available data on the prevalence of *L. monocytogenes* in agriculture soils. Fields that contain animal manure are more likely to be contaminated with *L. monocytogenes*, as shown by Szymczak *et al.* (2014) (Table A 3.2), because of their ability to survive in soils for months. Quantification of *L. monocytogenes* populations in soil samples was found in Dowe *et al.* (1997) and Iwu *et al.* (2022). The former reported a mean of 4 MPN/g (95% CI: <1.0 – 28 MPN/g) in the only *L. monocytogenes* positive cultivated field, whereas the latter reported the mean concentration of this pathogen in soil samples from 19 agricultural sites in South Africa to be higher, at 1964 CFU/g, ranging from 133 - 6233 CFU/g.

Table A 3.2: Data on *L. monocytogenes* prevalence in soil

Country	Characteristics	Positive/Total (%Prevalence)	Source
Canada	Cultivated fields, 7 fields fertilized with animal manure in addition to inorganic fertilizer	1/13 (7.7)	Dowe *et al.* (1997)
Malaysia	Vegetables fields in traditional farming	4/21 (19.0)	Jeyaletchumi *et al.* (2011)
United States	Organic/Irrigate/Manure/Compost Farm 1: no/no/yes/yes Farm 2: yes/yes/yes/yes Farm 3: no/yes/yes/no Farm 4: no/yes/no/no Farm 5: no/no/no/no (data broken down by farm not available)	16/178 (8.9)	Strawn *et al.* (2013a)
France	Cultivated soils from Burgundy	9/53 (17.0)	Locatelli *et al.* (2013a)

Poland	Lands fertilized with manure	2/173 (1.2)	Szymczak *et al.* (2014)
	Lands fertilized with artificial fertilizers	0/173 (0.0)	
	Garden plots intensively fertilized with manure	5/47 (10.6)	
	Wastelands	0/120 (0.0)	
Austria	Soil types (humus, sand and clay)	28/467 (6.0)	Linke *et al.* (2014)
United States	Soil samples from spinach fields		Weller *et al.* (2015)
	Low-risk fields	24/546 (4.4)	
	High-risk fields	62/546 (11.4)	

Sources: **Source** column cites the corresponding studies. See References section for further details.

Specific farm management practices may impact the risk of pathogen contamination in the pre-harvest environment. Three published studies, which conducted longitudinal or cross-sectional studies, assessed some management practices that can be associated with the presence of *L. monocytogenes* in soil (Strawn *et al.*, 2013b; Weller *et al.*, 2015, 2016). The effect of risk factors related to farm management, as odds ratio (OR) outcomes, are compiled in Table A 3.3 and can be useful as factors for the correction of prevalence of *L. monocytogenes* in soil.

Table A 3.3: Data on likelihood of *L. monocytogenes* being detected in soils in produce fields

Source	Risk factor	Description	OR	95% CI	p-value
Strawn *et al.* (2013b)	Manure	Last time manure was applied			
		Within 365 days	7.0	[3.1 – 15.4]	<0.001
		Over 365 days	0.6	[0.2 – 1.7]	0.381
		Not applied	1.0		
	Irrigation	Last time field was irrigated			
		Within 3 days	6.0	[2.0 – 18.1]	0.010
		4-7 days	1.2	[0.3 – 4.5]	0.793
		8-14 days	0.4	[0.1 – 2.0]	0.288
		Over 14 days/not irrigated	1.0		
	Soil cultivation	Last time soil was cultivated			

Strawn *et al.* (2013b)		Within 7 days	2.9	[1.1 – 8.6]	0.050
		8-14 days	1.4	[0.4 – 5.1]	0.660
		15-30 days	0.4	[0.1 – 1.7]	0.224
		Over 30 days	1.0		
Weller *et al.* (2015)	Irrigation/ rain	Time since irrigation/rain occurred			
		24 hours	25	[5.7 – 99]	0.010
		48 hours	2.5	[0.49 – 12]	0.27
		72 hours	3.4	[0.74 – 15]	0.11
		144-192 hours	1.0		
	Amount of irrigation water (mm) applied to field 2 days before sample collection[1]		1.2	[1.1 – 1.3]	0.010
Weller *et al.* (2016)	Areas within 37.5 m of surface water		3.0	[2.0 – 4.6]	<0.001
	Areas within 62.5 m of pasture		2.9	[1.4 – 6.0]	0.005

[1]Change in the odds of isolating *L. monocytogenes* associated with a 1-mm increase in the amount of irrigation water applied

Sources: **Source** column cites the corresponding studies. See References section for further details.

Module 2: Harvest

After harvesting, *L. monocytogenes* may slowly decline on the cantaloupe rind when the surface is intact. Even after washing, remaining cells may still survive, and as shown by Ukuku and Fett (2002) and Nyarko *et al.* (2016a), the extent of their survival will be affected by storage temperature. *L. monocytogenes* populations are greater on melons stored at 25 °C compared to those at 4 °C. Both articles have demonstrated that *L. monocytogenes* dies off on the surface of cantaloupe rind. Table A 3.4 compiles the data digitized from the two articles. On the contrary, *L. monocytogenes* has been shown to grow when inoculated in stem scars, according to an experiment carried out by Nyarko *et al.* (2016a) at three storage temperatures. Digitized data from this source are compiled in Table A 3.5.

Table A 3.4: Data on the survival of *L. monocytogenes* on intact cantaloupe rind

Source	Inoculation	Cultivar	Temp. (°C)	Time (day)	Counts
					log_{10} CFU per melon
Nyarko *et al.* (2016a)	Spot inoculated (6 log) on drawn circles (11 cm^2) of cantaloupe. Then allowed to dry for 1 h.	Athena / Rocky Ford	4	0	5.77 / 5.77
				1	4.96 / 5.57
				3	3.65 / 4.35
				5	2.78 / 3.48
				7	2.87 / 3.39
				9	0.78 / 2.00
				15	1.75 / 2.96
		Athena / Rocky Ford	10	0	5.80 / 5.80
				1	5.59 / 5.52
				3	4.83 / 4.35
				5	4.14 / 4.62
				7	3.73 / 4.28
				9	2.76 / 4.00
		Athena / Rocky Ford	25	0	5.85 / 5.85
				1	5.10 / 5.10
				3	5.10 / 2.03
				5	3.30 / 2.10
				7	3.23 / 3.23
					log_{10} CFU/cm^2
Ukuku and Fett (2002)	Inoculated by immersion in 3 L suspension of 8 log CFU/ml. Then allowed to dry for 1 h.		4	0	3.47
				1	3.47
				3	3.08
				6	2.93
				9	2.77
				15	2.46
			20	0	3.47
				1	3.47
				3	3.08
				6	2.70
				9	2.31
				15	1.50

Sources: **Source** column cites the corresponding studies. See References section for further details.

Table A 3.5: Data on the growth of *L. monocytogenes* on stem scars of cantaloupe

Source	Inoculation	Cultivar	Temp. (°C)	Time (day)	Counts
					log_{10} CFU per melon
Nyarko *et al.* (2016a)	Spot inoculated (6 log) on drawn circles (11 cm^2) of cantaloupe. Then allowed to dry for 1 h.	Athena / Rocky Ford	4	0	5.77 / 5.77
				1	5.80 / 6.15
				3	6.00 / 6.05
				5	6.05 / 5.72
				7	6.22 / 6.22
				9	6.82 / 6.60
				15	6.10 / 6.71
		Athena / Rocky Ford	10	0	5.80 / 5.80
				1	6.37 / 6.03
				3	6.64 / 6.47
				5	6.12 / 6.47
				7	5.82 / 6.55
				9	6.38 / 6.38
Nyarko *et al.* (2016a)	Spot inoculated (6 log) on drawn circles (11 cm^2) of cantaloupe. Then allowed to dry for 1 h.	Athena / Rocky Ford	25	0	5.85 / 5.85
				1	7.13 / 7.55
				3	6.85 / 7.78
				5	6.71 / 7.68
				7	6.75 / 8.33

Sources: **Source** column cites the corresponding studies. See References section for further details.

Module 3a, b: Cleaning, washing and sanitizing (relevant for RTE diced cantaloupe and whole cantaloupe)

The reduction of *L. monocytogenes* on the cantaloupe rind due to washing with water and sanitizers can be modelled using the data compiled in Table A 3.6. This table gathers literature data on the reduction effect of washing from inoculation experiments, along with determinant features of the washing treatment, such as exposure time, temperature, and sanitizer concentration.

Table A 3.6: Reduction of *L. monocytogenes* (and standard error) on cantaloupe surface rind after treatment with sanitizers (water, chlorine dioxide gas [ClO_2], hydrogen peroxide [H_2O_2], sodium hypochlorite [SH] and a sanitizer mixture) at a given sanitizer concentration, exposure time and temperature

Sanitizer	Study	Concentration (%)	Exposure time (min)	Temperature (°C)	Reduction (log CFU/cm^2)	St. Error (Reduction)	n[1]
Tap	Guzel *et al.* (2017)	-	3	25	0.18	0.053	8
water	Ukuku *et al.* (2012)		5	25	0.20	-	3
	Ukuku *et al.* (2005)[2]						
	Stored at 5 °C x 0 day		5	20	0.20	-	3
	Stored at 5 °C x 7 days		5	20	0.15		3
	Ukuku and Fett (2002)[2]						
	Stored at 4 °C x 1 day	-	2	25	0.30	-	3
	Stored at 4 °C x 5 days	-	2	25	0.22	-	3
	Stored at 4 °C x15 days	-	2	25	0.22	-	3
ClO_2 gas	Mahmoud *et al.* (2008)	0.00005	2	25	1.2	-	6
		0.0001	2	25	1.8	-	6
		0.00015	2	25	2.1	-	6
		0.0003	2	25	2.1	-	6
		0.0005	2	25	2.2	-	6
		0.00005	10	25	3.3	-	6
		0.0001	10	25	3.2	-	6
		0.00015	10	25	3.7		6
		0.0003	10	25	3.8	-	6
		0.0005	10	25	4.3	-	6

SH	Guzel *et al.* (2017)	0.020	5	25	0.57	0.250	8
	Ukuku and Fett (2002)[2]						
	Stored at 4 °C x 1 day	0.100	2	25	>3.0	-	3
	Stored at 4 °C x 5 days	0.100	2	25	>3.0	-	3
	Stored at 4 °C x15 days	0.100	2	25	>3.0	-	3
H_2O_2	Ukuku *et al.* (2012)	2.5	5	25	2.8		3
	Ukuku *et al.* (2005)[2]						
	Stored at 5 °C x 0 day	2.5	5	20	2.3	-	3
	Stored at 5 °C x 7 days	2.5	5	20	2.8		3
	Ukuku and Fett (2002)[2]						
	Stored at 4 °C x 1 day	5.0	2	25	>3.0	-	3
	Stored at 4 °C x 5 days	5.0	2	25	>3.0	-	3
	Stored at 4 °C x15 days	5.0	2	25	>3.0	-	3
HPLNC[3]	Ukuku *et al.* (2005)[2]						
	Stored at 5 °C x 0 day	-	5	20	>4.0	-	3
	Stored at 5 °C x 7 days	-	5	20	>4.0	-	3

Sources: **Study** column cites the corresponding studies. See References section for further details.

[1]Number of samples in inoculation experiments

[2]Studies where inoculated cantaloupes were stored at different temperatures and times before the washing treatment

[3]Solution of 1% H_2O_2, 25 µg/ml nisin, 1% sodium lactate and 0.5% citric acid

Module 4a: Processing of RTE diced cantaloupe

Transfer of *L. monocytogenes* from cantaloupe rind to flesh

Patil (2017) devised an experiment whereby *L. monocytogenes*-contaminated cantaloupe rinds (5 log CFU/cm^2), after 24h storage at 4 and 30 °C, were cut with an 18-mm diameter cork-borer to obtain 25-mm long cylinders, and *L. monocytogenes* counts were determined at different depths: 0-5 mm, 6-10 mm, 11-15 mm, 16-20 mm and 21-25 mm. The level of transfer from rind surface to the 5-mm depth pieces varied from 2.8 to 3.6 log CFU/cm^2 in cantaloupe stored at 4 °C and from 1.9 to 4.0 log CFU/cm^2 in cantaloupe stored at 30 °C. There was no statistical difference in the transferred populations between storage temperature conditions and, furthermore, there was no statistical difference in the amount of cells transferred at the different depths studied, since *L. monocytogenes* was detected at depths from 5 mm to 25 mm from 1.9 to 2.8 log CFU/cm^2 at 4 °C (from an initial inoculum on cantaloupe rind of 5.9 log CFU/cm^2), and from 0.9 to 2.0 log CFU/cm^2 at 30 °C (from an initial inoculum on cantaloupe rind of 5.2 log CFU/cm^2). This suggests that all edible flesh surfaces at any depth of cut are equally susceptible to contamination from a contaminated rind surface. Summary results from Patil's experiment are shown in Table A 3.7 along with rind-to-flesh surface transfer rates calculated thereof.

Table A 3.7: Available data to build a transfer equation for *L. monocytogenes* load from cantaloupe rind to flesh (dices in g or surface slices in cm^2)

Source[1]	*L. monocytogenes* on the rind (log CFU/cm^2)	*L. monocytogenes* in cantaloupe flesh	Transfer rate (%) ($10^{flesh}/10^{rind}$)×100
To fresh cut (dices)		(log CFU/g)	
Ukuku and Fett (2002)	2.16	0.23	1.175
	3.26	0.154	0.191
	3.98	1.31	0.214
	4.52	1.46	0.087
Ukuku *et al.* (2012)	4.60	2.60	1.000
	4.40	2.20	0.631

To slices (flesh surface)			(log CFU/cm^2)	
Patil (2017) (melons stored at 4 or 30 °C before slicing)	4 °C	5.94	2.45	0.032
		5.44	1.42	0.010
	30 °C	5.22	1.64	0.026
		5.44	1.17	0.005

Sources: **Source** column cites the corresponding studies. See References section for further details.

[1]All values digitized from charts

Other studies aimed to quantify the transfer of *L. monocytogenes* from cantaloupe rind to fresh-cut pieces (Ukuku and Fett, 2002; Ukuku *et al.*, 2012). Inoculated whole cantaloupes, which were stored at 4 °C, were cut into four sections, and subsequently each section was further cut into 3-cm cubes, and the rind was removed. Table A 3.7 compiles the results of the populations of *L. monocytogenes* on the inoculated cantaloupe rind and those of the fresh-cut pieces that were extracted from the two studies. Afterwards, a transfer rate was calculated as %CFU to be ~0.08-1.18 percent. When the results are plotted together (Figure A 3.1), however, it is evident that the amount of contamination transferred to the fresh cuts depends on the initial load on the surface. Hence, using a relationship between these two variables would be more accurate than using a transfer rate. As can also be observed in Figure A 3.1, Ukuku *et al.* (2012) stated that the transfer of the pathogen can occur on a fairly consistent basis if the number of *L. monocytogenes* on the rind is above 2 log CFU/cm^2.

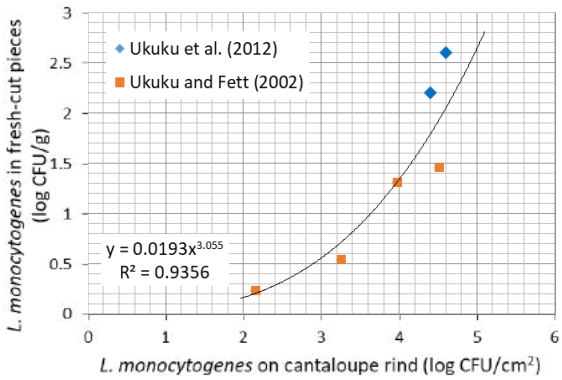

Figure A 3.1: Effect of the *L. monocytogenes* contamination on the cantaloupe rind on the extent of transfer to the fresh-cut pieces

Sources: See References section.

Cross-contamination from equipment

Patil (2017) assessed the transfer of *L. monocytogenes* from contaminated equipment (slicing and dicing machines) to non-inoculated cantaloupe halves in consecutive slicing and dicing events. Whole cantaloupes were dip-inoculated for the slicing experiment to simulate surface contamination from soil or irrigation water, whereas in the dicing experiment, rind-free cantaloupe halves were dip-inoculated to simulate contamination during rind removal. Slicing two cavity-cleaned inoculated cantaloupe halves, from cantaloupes inoculated at 5.2 log CFU/cm^2 and stored 1 day at 4 °C, resulted in 2.2 log CFU/g in the edible flesh after the first slicing event. The 8 non-inoculated halves that were sliced afterwards using the same contaminated slicing equipment presented levels of *L. monocytogenes* that were lower every time (1.5-0.1 log CFU/g) but not significantly different. Dicing of the two cavity-cleaned inoculated cantaloupe halves, from rind-free cantaloupes inoculated at 5.5 log CFU/cm^2 and stored 1 day at 4 °C, led to *L. monocytogenes* populations of 4.7 log CFU/g in the fresh-cut pieces after the first dicing event. A significantly lower *L. monocytogenes* transfer from the contaminated dicing equipment to non-inoculated cantaloupe halves was found in the fresh-cut pieces in the consecutive dicing events, diminishing from 3.7 log CFU/g at the first dicing event of non-inoculated cantaloupe to 0.7 log CFU/g at the fourth dicing event.

In this study, *L. monocytogenes* growth was also assessed in the diced cantaloupe halves produced at the sequential dicing events during subsequent storage at 4, 7 and 10 °C. After 3 days of storage at 7 °C, the levels of *L. monocytogenes* in these dices were not significantly different between sequential slicing events, having reached levels of 1.4-2.8 log CFU/g. The same was observed in the dices: after 3 days of storage at 10 °C they had reached contamination levels between 3.6 and 6.0 log CFU/g not significantly affected by the order of the sequential dicing event. Data digitized from Patil (2017) (Table A 3.8) can be re-used to model the equipment-mediated transfer of *L. monocytogenes* during consecutive slicing and dicing, as performed in Qi *et al.* (2020) and Zilelidou *et al.* (2015).

Table A 3.8: Data on equipment-mediated transfer of *L. monocytogenes* from one contaminated cantaloupe half in sequential slicing or dicing of non-inoculated cantaloupe halves

Process	Characteristics of the experiment	Inoculation outer surface (log CFU/cm^2)	Slicing/ Dicing event	Counts (log CFU/g)
Slicing	Whole cantaloupe rinds inoculated, air-dried for 1 h and stored at 4 °C for 24 h. For event 1: **Inoculated** cantaloupes were halved and seeds removed For events 2-9: **Non-inoculated** cavity-clean cantaloupe halves	5.2	1	2.20
			2	1.50
			3	0.46
			4	0.37
			5	0.33
			6	0.19
			7	0.56
			8	0.37
			9	0.10
Dicing	Off-rind, cantaloupes inoculated, air-dried for 1 h and stored at 4 °C for 24 h. For event 1: **Inoculated** cantaloupes were halved and seeds removed For events 2-9: **Non-inoculated** cavity clean cantaloupe halves	5.5	1	4.70
			2	3.45
			3	2.34
			4	1.50
			5	0.61
			6	0.79
			7	0.84
			8	1.31
			9	1.08

Source:
Patil, R.D. 2017. Transfer of *Listeria monocytogenes* during cutting, slicing, dicing, and subsequent storage of cantaloupe and honeydew melons. Michigan State University. Master's Thesis.

Modules 5a and 5b: Cold chain of RTE diced cantaloupe and temperature profile of whole cantaloupe

The effect of temperature on *L. monocytogenes* growth in cantaloupe in Modules 5a and 5b will be determined by predictive microbiology models. Secondary models will be built for lag phase duration and growth rate using published data.

Lag phase duration

Scolforo *et al.* (2017) determined the lag phase duration of *L. monocytogenes* serovar 1/2b (UFV_2) inoculated into the pulp of Canary melons stored at different temperatures. Data are compiled in Table A 3.9.

Table A 3.9: Lag phase duration data of *L. monocytogenes* in Canary melon

Temperature (°C)	Lag phase duration (Standard error) [h]	Specific growth rate (Standard error) [h^{-1}]
5.0	53.6 (5.8)	0.025 (0.001)
15.0	4.2 (0.9)	0.114 (0.021)
20.0	6.1 (0.6)	0.183 (0.008)
30.0	1.5 (0.3)	0.373 (0.065)
35.0	0.56 (0.2)	0.671 (0.086)

Source: **Scolforo, C.Z., Bairros, J.V., Rezende, A C.B., Silva, B S., Alves, R.B.T., Costa, D.S., Andrade, N.J., et al.** 2017. Modeling the fate of *Listeria monocytogenes* and *Salmonella enterica* in the pulp and on the outer rind of Canary melons (*Cucumis melo* (Indorus Group)). *LWT – Food Science and Technology,* 77: 290–297. https://doi.org/10.1016/j.lwt.2016.11.059

In the Baranyi model, Q_0 defines the initial concentration of a hypothetical substrate needed for starting microbial growth, as a function of specific growth rate (μ_{max}) and lag phase duration (λ).

$$Q_0 = \frac{1}{\exp(\mu_{max}\lambda)-1} \tag{3.1}$$

The parameter Q_0 of *L. monocytogenes* in cantaloupe flesh can be approximated by feeding the data from Table A 3.9 in Equation (3.1).

Growth rate

The most comprehensive challenge study on *L. monocytogenes* 4b, 1/2a and 1/2b was carried out by Fang *et al.* (2013), who assessed the effect of serotypes on growth rates across all temperature conditions. They did not find any serotype effect or interaction with temperature, and they subsequently determined the cardinal parameters for the temperature of *L. monocytogenes* in fresh-cut cantaloupe (T_{min}=1.90 C, T_{opt}=38.3 C, T_{max}=45.7 C, μ_{opt}=0.975 h^{-1}). Nevertheless, other challenge studies have been undertaken (Hong *et al.*, 2014; Ukuku and Fett, 2002, Danyluk *et al.*, 2014; Guzel *et al.*, 2017; Patil, 2017; Moreira, 2019). Table A 3.10 compiles these results plus those of Farber *et al.* (1998) carried out in squash (a fruit also from the *Cucurbitaceae* family), since their estimates compare very well with those of cantaloupe. Figure A 3.2 plots the specific growth rate estimates against storage temperature. Through meta-analysis, overall T_{min}, T_{opt}, T_{max} and μ_{opt} can be estimated by fitting the cardinal parameter model for temperature.

The differential form of the Baranyi and Roberts model will be employed to predict the growth of *L. monocytogenes* in cantaloupe, providing a value for q_0 (Equation 3.1) to assume the presence of a lag phase.

$$\frac{dN}{dt} = \mu_{max}\frac{Q}{1+Q}\left(1 - \frac{N}{10^{MPD}}\right)N$$

$$\frac{dQ}{dt} = \mu_{max}Q \tag{3.2}$$

Table A 3.10: Growth kinetics data on *L. monocytogenes* in cantaloupe flesh or similar from published articles

Study	Medium	Strain	Stressed	Temperature (°C)	Specific GR (h^{-1})
Fang *et al.* (2013)	Cantaloupe	F2365, H7858, ATCC19115	Stressed (rifampicin-resistant and cold resistant)	4.0	0.0120
				8.0	0.0470
				12	0.1260
				16	0.1860
				20	0.2930
				25	0.5250
				30	0.7300
				33	0.8150
				40	0.9160
				37	0.8600
				43	0.6920
		F4260	Stressed (rifampicin-resistant and cold resistant)	4.0	0.0110
				8.0	0.0580
				12	0.1230
				16	0.1940
				20	0.3210
				25	0.5300
				30	0.7470
				33	0.9000
				37	0.9900
				40	0.9730
				43	0.7590
				38	0.9750
		V7	Stressed (rifampicin-resistant and cold resistant)	43	0.7540
				40	0.9980
				33	0.9010
				37	0.9640
				30	0.7860
				25	0.5430
				20	0.3220
				16	0.1970

Study	Medium	Strain	Stressed	Temperature (°C)	Specific GR (h^{-1})
				12	0.1350
				8.0	0.0640
Hong *et al.* (2014)	Cantaloupe	ATCC BAA839, ATCC BAA839, ATCC 19111, ATCC 13932	Stressed (rifampicin-resistant and cold resistant)	10	0.0852
				15	0.1983
				20	0.4030
				25	0.5803
				10	0.0852
				15	0.2118
				20	0.4030
				25	0.5112
Ukuku and Fett (2002)	Cantaloupe dices	Scott A, H7778, ATCC-15313, CCR1LG	Not stressed	20	0.3720
Danyluk *et al.* (2014)	Fresh-cut cantaloupe	LCDC 81-861, Scott A, 101M, V7	Not stressed	5.0	0.0850
				10	0.2000
				15	0.2300
				20	0.3900
				25	0.4745
Guzel *et al.* (2017)	Fresh-cut cantaloupe	NRCC B33076	Not stressed	5.0	0.0368
				10	0.0898
				25	0.6240
				30	0.7161
				36	0.9233
Patil (2017)	Cantaloupe dices	J22F, J29H, M3	Not stressed	4.0	0.0520
				7.0	0.0670
				10	0.1840
Moreira (2019)	Fresh-cut watermelon	LCDC 81-861, V7, 101M, Scott A	Not stressed	4.0	0.0318
				13	0.1213
				13	0.1438
Farber *et al.* (1998)	Squash	Not stated	Not stressed	4.0	0.0370
				10	0.0910

Sources: **Study** column cites the corresponding studies. See References section for further details.

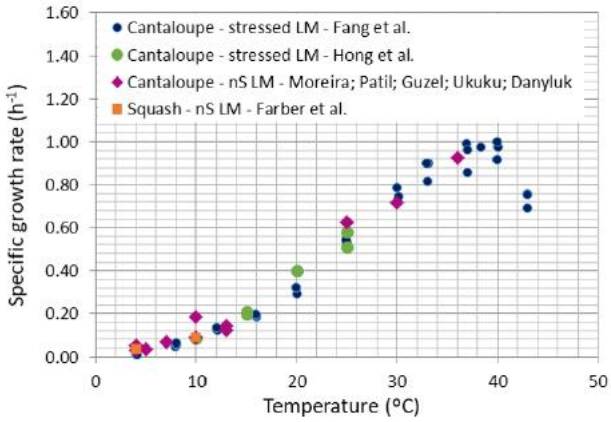

Figure A 3.2: Specific growth rate estimates of *L. monocytogenes* (LM) in cantaloupe flesh against temperature, extracted from the literature. nS: non-stressed.

Sources: See References section.

Transportation from end processing to retail

Fresh-cut cantaloupe is transported in refrigerated trucks, whose temperature should be lower than 7 °C (FSAI, 2022). The temperature can be assumed to be between 3 °C and 10.3 °C with a mean of 5 °C (Ding *et al.*, 2013; Sant'Ana *et al.*, 2014).

Modules 6a and 6b: Retail of RTE diced cantaloupe and whole cantaloupe

RTE diced cantaloupe is a product that is kept under refrigeration at retail. Time–temperature measurements indicate that cold storage in display cabinets at retail is generally not the most efficient step in the cold chain, and that temperatures frequently rise above the recommended limit (Mercier *et al.*, 2017). Derens-Bertheau (2015), measuring the temperature along the cold chain for yogurt and meat products in France, found that ~31% of the measurements were above 6 °C. They also reported temperatures during display at retail from 1.4 °C to 9.8 °C, with an average of 5.6 °C. Other studies (McKellar *et al.*, 2012; 2014) have reported average display temperatures of 4.1 °C and 3.5 °C for fresh-cut lettuce during winter and summer, respectively.

Whole cantaloupes are commonly displayed at ambient temperature. Reasonable assumptions should be made for modelling the transfer of *L. monocytogenes* from other produce and from food contact surfaces onto surface spots of the cantaloupe as well as other assumptions to represent common practices of merchandizing whole and sliced cantaloupe in informal markets.

Modules 7a and 7b: Consumer handling of RTE diced cantaloupe and whole cantaloupe

Nauta *et al.* (2003) indicated that the mean temperature of European domestic refrigerators is 6.64 °C. In Brazil, Silva *et al.* (2008) determined a mean temperature of 6 °C, oscillating between 3.1 °C and 10.8 °C, whereas in a Korean QRA risk assessment model for lettuce, mean refrigerator temperatures at home were assumed to be between 4 °C and 8.3 °C (Ding *et al.*, 2013). Carrasco *et al.* (2010) assumed a maximum temperature of home refrigerators of 11.3 °C.

The consumer can purchase whole cantaloupes or RTE diced cantaloupes. If they purchase fresh-cut cantaloupes, it can be assumed that it is more likely to be consumed promptly, and the product is less likely to be stored in the refrigerator until consumption before the end of its shelf-life. *L. monocytogenes* growth in cantaloupe flesh can be modelled using data from Table A 3.9. If consumers purchase whole cantaloupe, they may or may not wash the cantaloupe before slicing. Reduction factors from Table A 3.6 can be used for washing with tap water. If cantaloupe rind is contaminated with *L. monocytogenes*, the transfer rates used in Table A 3.7 can be used to model the transfer from rind to sliced surface, or from rind to dices, accordingly.

SECTION 2: THE EXPOSURE ASSESSMENT MODEL

The pre-harvest module considers that *L. monocytogenes* can end up on cantaloupe rind via two routes: contaminated water from the last irrigation event directly in contact with the cantaloupe rind, and contamination from soil reaching the cantaloupe rind indirectly through soil splash from the last irrigation (Table A 3.11). If the irrigation water is contaminated with *L. monocytogenes* at a probability P_{irrig}, the concentration of *L. monocytogenes* on the rind right after irrigation (C_{rind_irrig}) is determined by knowing the concentration of *L. monocytogenes* in the irrigation water (C_{irrig}) and the volume of water remaining on the cantaloupe right after irrigation ($W_{surface}$). The soil route starts just before irrigation and considers that the irrigation water, if it contains *L. monocytogenes*, can contaminate the soil, or increase its level of contamination, if previously contaminated. The prevalence of *L. monocytogenes* in soil can increase if manure was applied within one year of cultivation (P_{soil_manure}), and if irrigation is carried out one or two days before harvesting ($P_{irrig24}$, $P_{irrig48}$). An *L. monocytogenes* transfer rate ($TR_{soil\text{-}rind}$) from soil to cantaloupe rind due to soil splash following irrigation is then considered, although the extent of transfer can be reduced by R_{foil}% if cantaloupe is grown on protective foil. From soil splash, the concentration of LM on cantaloupe is C_{rind_soil}. Therefore,

considering these two routes of contamination, the total concentration of LM on cantaloupe C_{rind_tot} is calculated, under the combination of probabilities that irrigation water was contaminated or not, and soil pre-irrigation was contaminated or not. In the lapse of time between the last irrigation and harvesting (t_{irrig}), *L. monocytogenes* cells on cantaloupe rind are assumed to decline until reaching a concentration of C_{rind_harv} at the time of harvesting. A cantaloupe rind is negative for *L. monocytogenes* if both the soil and the irrigation water are free of *L. monocytogenes*. Therefore, the prevalence of cantaloupes with *L. monocytogenes* on the rind at harvest is calculated (P_{rind_harv}).

During harvesting (Table A 3.12), conveyors, crates or plastic surfaces, if contaminated with *L. monocytogenes* at a probability P_{cch}, can transfer cells (N_{trans}) to the rind of cantaloupes, regardless of their being previously contaminated or not. If cross-contamination occurs, the prevalence of cantaloupe with *L. monocytogenes* on the rind post-harvest increases to P_{rind_post}. At this point, it is assumed that a fraction of cantaloupes (P_{bruise}) can be bruised due to harvesting or transport. Cantaloupes can then be directed to informal markets, can be washed at the packinghouse to be sold at formal retail, or can be further processed to be sold as RTE diced cantaloupe.

At the packinghouse or processing plant (Table A 3.13), there could be a short storage time ($t_{storage}$). During this holding time, the *L. monocytogenes* population on cantaloupe rind continues to decline until reaching a pre-wash concentration on rind of $C_{on\_pre\text{-}wash}$. Cleaning with brushes and subsequent washing with water reduces *L. monocytogenes* in R_{clean} and R_{ww}, respectively. In some cases, this treatment can completely eliminate *L. monocytogenes*, thereby decreasing the prevalence on the rind to P_{whole}, and when contaminated, the concentration of *L. monocytogenes* on cantaloupe rind is C_{whole}. Subsequently, cantaloupes are transported to retail. Cantaloupes that are to be used to produce RTE diced cantaloupe are subjected to sanitizing, where the *L. monocytogenes* concentration on the rind can be further reduced (C_{on_sanit}). During dicing, two sources of contamination are modelled in an additive way: (1) the transfer from contaminated cantaloupe rind to dices, by using a transfer rate $TR_{rind\text{-}dices}$ as a function of the *L. monocytogenes* concentration on rind; and (2) the cross-contamination from the dicing machine to dices ($TR_{machines\text{-}dices}$), which can be contaminated at a probability P_{ccd}. To model the partitioning during packaging, the numbers of *L. monocytogenes* transferred to all dices (N_{total}) from dicing one cantaloupe are assumed to be randomly distributed to packs in numbers (N_{pack}) that follow a beta-binomial distribution with a clustering parameter b. Due to randomness, some of the RTE diced cantaloupe packs will be free of *L. monocytogenes* (Pi_{pack}). At the end of processing, RTE diced cantaloupe packs have a prevalence and a

concentration of *L. monocytogenes* of P_{RTE} and C_{RTE}, respectively.

For the contaminated fraction (Table A 3.14), the growth of *L. monocytogenes* in RTE diced cantaloupe is modelled using a cardinal model for temperature (secondary model) and a Baranyi-Roberts model (primary model), considering that the lag phase starts at the end of processing. The cold chain encompasses transportation to retail, display at retail, transportation to home and home storage. No cross-contamination events are modelled to represent home preparation, and the concentration of *L. monocytogenes* in RTE diced cantaloupe is higher at C_{RTE_home}.

Informal markets are assumed to sell whole cantaloupes and slices (Table A 3.15). There is a probability that cross-contamination can occur (P_{ccm}) from surfaces such as boxes or floors when contaminated with *L. monocytogenes* in numbers N_{floor}. When in contact with such contaminated surfaces, a fraction of cells ($N_{f\text{-}c}$) will be transferred onto the cantaloupe rind, regardless of whether they were *L. monocytogenes* free or previously contaminated. During display, *L. monocytogenes* on rind can grow if the surface is injured or can decline if it is intact. When slicing, *L. monocytogenes* cells on rind can be transferred ($TR_{rind\text{-}slice}$) to the slice. From the informal markets module, the concentration of *L. monocytogenes* on the rind of a whole cantaloupe just purchased is C_{whole_market}, whereas the concentration on a slice (when sliced in the market) is $C_{slice\text{-}market}$.

As assumed for whole cantaloupes sold in informal markets, for whole cantaloupes intended to be commercialized at formal retail, *L. monocytogenes* cells on rind can grow and/or decline conditional on the existence of bruises on the rind or not (Table A 3.16). Such bacterial kinetics are solved for the contaminated fraction of whole cantaloupes during transport to retail, display at formal retail and transport to home. No lag phase for the growth model is assumed, and no further cross-contamination events are assumed along such distribution chains. Consumer handling considers three possible scenarios: (1) that the consumer washes the cantaloupe and then stores it until consumption; (2) that the consumer stores the cantaloupe, and washes the rind before consumption; and (3) that the consumer does not wash the cantaloupe at all. Taking into account a reduction in *L. monocytogenes* (R_{ww}) due to washing, the concentration of *L. monocytogenes* on cantaloupe rind just before slicing ($C_{on\_slicing}$) is estimated. At this point, it is considered that slices of cantaloupe can be contaminated with *L. monocytogenes* due to transfer from the rind ($TR_{rind\text{-}slice}$) and/or due to cross-contamination from the knife ($TR_{knife\text{-}slice}$). The prevalence ($P_{slice\text{-}home}$) and concentration ($C_{slice\text{-}home}$) of the pathogen in cantaloupe slices to be consumed are calculated.

Table A 3.11: Variables related to the pre-harvest module for cantaloupe melons

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Route of LM: Irrigation water on rind	P_{irrig}	Probability that water from last irrigation before harvest is contaminated with LM	-	-	Table A 3.1
	C_{irrig}	Concentration of LM in irrigation water	CFU/ml	-	<0.03 to 11 MPN/L (Sharma *et al.*, 2020; Acheamfour *et al.*, 2021) 1196 CFU/100 mL ranging from 0 - 5667 CFU/100 mL (Iwu *et al.*, 2022).
	t_{irrig}	Time between last irrigation and harvest	day	-	EKE
	$W_{surface}$	Volume of water remaining on cantaloupe rind right after irrigation	ml/cm^2	-	Assumption to be made
	B_{irrig+}	Status of last irrigation water contaminated with LM	-	Bernoulli (P_{irrig})	-
	C_{rind_irrig}	Concentration of LM on rind after direct contact with irrigation water	CFU/cm^2	$W_{surface} \times C_{irrig}$	-

Route of LM: soil to rind	P_{foil}	Probability that cantaloupe is grown using protective foil	-	-	Assumption to be made
	P_{soil}	Prevalence of LM in soil	-	-	Table A 3.2
	C_{soil}	Concentration of LM in soil just before last irrigation of cantaloupe field	CFU/g	-	4 MPN/g (95% CI: <1.0 – 28 MPN/g) (Dowe *et al*. 1997) 1964 CFU/g (range: 133 - 6233 CFU/g (Iwu *et al.*, 2022)
	W_{soil}	Volume of water remaining in soil after last irrigation	ml/g soil	-	Assumption to be made
	$F_{irrig24}$	Impact of last irrigation occurring 24 h before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	$F_{irrig48}$	Impact of last irrigation occurring 48 h before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	F_{manure}	Impact of manure applied within a year before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	$TR_{soil-rind}$	Transfer rate from soil to rind due to soil splash after last irrigation	(CFU/ cm^2 rind / CFU/g q te soil)	-	Assumption to be made
	$B_{irrig24+}$	Field irrigated 24 h before harvest	-	Bernoulli ($P_{irrig24}$)	Assumption of frequency to be made
	$B_{irrig48+}$	Field irrigated between 24 and 48 h before harvest	-	Bernoulli ($P_{irrig48}$)	Assumption of frequency to be made

$B_{manure+}$	Field where manure was applied within one year of the cultivation of leafy greens	-	Bernoulli (P_{manure})	Assumption of frequency to be made

P_{soil_manure}	Prevalence of LM in soil after correction for application of manure	-	IF ($B_{manure+}$=1)
			(F_{manure} x P_{soil})/
			(1-P_{soil}+(F_{manure} x P_{soil})
			IF ($B_{manure+}$=0)
			P_{soil}
				-

P_{soil_irrig}	Prevalence of LM in soil after correction for irrigation close to harvest	-	IF ($B_{irrig24+=}$1)
			($F_{irrig24}$ x P_{soil_manure})/(1-P_{soil_manure}+($F_{irrig24}$ x P_{soil_manure})
			IF ($B_{irrig48+=}$1)
			($F_{irrig48}$ x P_{soil_manure})/(1-P_{soil_manure}+($F_{irrig48}$ x P_{soil_manure})
			ELSE
			P_{soil_manure}
				-

B_{soil+}	Status of soil contaminated with LM at irrigation	-	Bernoulli (P_{soil_irrig})	-
$Delta_{soil}$	Increase in LM in soil due to contaminated water from last irrigation	CFU/g	C_{irrig} x W_{soil}	-

C_{soil_irrig}	Concentration of LM in soil after last irrigation (contaminated soil only)	CFU/g	IF (B_{irrig+}=1) & (B_{soil+}=1)
			$Delta_{soil}$ + C_{soil}
			IF (B_{irrig+}=1) & (B_{soil+}=0)
			$Delta_{soil}$ + 0
			IF (B_{irrig+}=0) & (B_{soil+}=1)
			0 + C_{soil}
			ELSE
			Remove
				-

	B_{foil+}	Status of cantaloupe grown on protective foil	-	Bernoulli (P_{foil})	-
	R_{foil}	Percentage of reduction of LM numbers when protective foil is used	%	-	EKE

	$TR_{soil\text{-}rind\text{-}c}$	Corrected transfer rate from soil to rind due to soil splash after last irrigation	(CFU/ cm^2 rind / CFU/soil)	IF (B_{foil+}=1)
				$TR_{soil\text{-}rind}$ x (100-R_{foil})/100
				ELSE
				$TR_{soil\text{-}rind}$
					EKE

	C_{rind_soil}	Concentration of LM on rind after irrigation following soil water splash	CFU/cm^2	C_{soil_irrig} x $TR_{soil\text{-}rind\text{-}c}$	-

Total LM concentration on rind	C_{rind_tot}	Total concentration of LM on rind	CFU/cm^2	IF (B_{irrig+}=1) & (B_{soil+}=1)
				C_{rind_irrig} + C_{rind_soil}
				IF (B_{irrig+}=1) & (B_{soil+}=0)
				C_{rind_irrig} + 0
				IF (B_{irrig+}=0) & (B_{soil+}=1)
				0 + C_{rind_soil}
				ELSE
				Remove
					-

	C_{rind_harv}	Concentration of LM on rind at the moment of harvest	Log CFU/cm^2	f_{rind1}(log $C_{rind\text{-}tot}$, t_{irrig})	f_{rind1}(C_0, t): Decay function of LM on the rind as a function of time t. Fitted from Ukuku and Fett (2002) data (Table A 3.4)
Prevalence	P_{rind_irrig}	Prevalence of cantaloupes contaminated on the rind at irrigation	-	1 – (1 – P_{irrig}) x (1 - P_{soil_irrig})	-
	P_{rind_harv}	Prevalence of cantaloupes with LM on rind at the moment of harvest	-	P_{rind_irrig}	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 3.12: Variables related to the harvest module for cantaloupe melons

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Cross-contamination	P_{cch}	Probability that conveyors / crates, plastic surfaces are contaminated with LM	-	0.08; 0.17	*Listeria* spp. on plastic surfaces in contact with cantaloupe (Strawn and Danyluk, 2017)
	TR_{plas_canta}	Transfer coefficient from food-contact surface to cantaloupe	Log (CFU/ cm^2 cantaloupe/ CFU/ cm^2 surface)	Normal (-1.42, 0.52)	Hoelzer *et al.* (2012a)
	N_{plas}	Numbers of CFUs on food-contact surface (conveyor or belt) touching the cantaloupe	CFU	-	Assumption to be made
	B_{cant+}	Status of a cantaloupe being contaminated with LM	-	Bernoulli ($P_{rind\text{-}harv}$)	
	B_{cch+}	Status of cross-contamination taking place after harvest	-	Bernoulli (P_{cch})	-
	N_{trans}	Numbers of CFUs transferred to cantaloupe rind	CFU/cm^2	$N_{plas} \times 10^{TRplas\_canta}$	-
	C_{rind_post}	Concentration of LM on cantaloupe on the rind post-harvest	Log CFU/cm^2	IF (B_{cant+}=0) & (B_{cch+}=1) Log (0 + N_{trans}) IF (B_{cant+}=1) & (B_{cch+}=1) Log (10^{Crind_harv} + N_{trans}) IF (B_{cant+}=1) & (B_{cch+}=0) Log (10^{Crind_harv} + 0) ELSE Remove	
	P_{rind_post}	Prevalence of cantaloupes with LM on the rind post-harvest	-	1 – (1 – $P_{rind\text{-}harv}$) x (1 – P_{cch})	-
Bruising	P_{bruise}	Probability that a cantaloupe gets bruised during harvest and transport	-	-	EKE
	$B_{bruise+}$	Status of a cantaloupe suffering bruising due to handling after harvest	-	Bernoulli (P_{bruise})	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 3.13: Variables and functions related to the packinghouse/processing module for RTE and whole cantaloupe

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Predictive microbiology models	$f_{rind\text{-}survival}(C_0, t)$	Survival of LM on intact cantaloupe rind as a function of initial concentration and time, at ambient temperature	log CFU/cm^2	-	To be fitted to data from Table A 3.4
	$f_{rind\text{-}growth}(C_0, t)$	Growth of LM on bruised cantaloupe rind as a function of initial concentration and time, at ambient temperature	log CFU/cm^2	-	To be fitted to data from Table A 3.5
Holding time	$t_{storage}$	Time of storage of cantaloupes from entering packinghouse until start of processing	h	-	To be assumed
	$C_{on\_pre\text{-}wash}$	Concentration of LM on cantaloupe rind pre-wash	log CFU/cm^2	$f_{rind\text{-}survival}(C_{rind\_post}, t_{storage})$	-
Cleaning	R_{clean}	Reduction in LM due to brushing	log CFU/cm^2		To be assumed
	$C_{on\_cleaned}$	Concentration of LM on cantaloupe rind after cleaning	log CFU/cm^2	$C_{on\_pre\text{-}wash} - R_{clean}$	-
Washing	R_{ww}	Reduction in LM due to washing with water	log CFU/cm^2	-	Table A 3.6
	C_{on_washed}	Concentration of LM on cantaloupe rind after washing	log CFU/cm^2	$C_{on\_cleaned} - R_{ww}$	-
Whole cantaloupe					
	A_{cant}	Surface area of a cantaloupe	cm^2	-	To be determined
	N_{on_washed}	Number of CFUs of LM on cantaloupe rind after washing	CFU	$A_{cant} \times 10^{C\,on\_washed}$	-
	$N_{on\_washed\_c}$	Number of CFUs of LM on cantaloupe rind after washing	CFU	IF ROUND(N_{on_washed})>0 N_{on_washed} ELSE 0	-

	Pi_{on_washed}	Proportion of cantaloupes free of LM on the rind	-	$P(N_{on\_washed\_c}=0)$	-
	P_{whole}	Prevalence that rind of whole cantaloupe after washing is contaminated with LM	-	P_{rind_post} x (1 – Pi_{on_washed})	-
	C_{whole}	Concentration of LM on contaminated cantaloupe rinds after washing	Log CFU/ cm^2	IF ROUND(N_{on_washed})>0 C_{on_washed} ELSE Remove	-
RTE diced cantaloupe					
Sanitizing	Sanit	Probability that the sampled batch sanitizes whole cantaloupes with ClO_2, H_2O_2, SH or does not sanitize at all	-		EKE
	R_{ClO2}	Reduction of LM due to sanitizing with ClO_2	log CFU/cm^2	-	Table A 3.6
	R_{H2O2}	Reduction of LM due to sanitizing with H_2O_2	log CFU/cm^2	-	Table A 3.6
	R_{SH}	Reduction of LM due to sanitizing with SH	log CFU/cm^2	-	Table A 3.6
	$Sanit_{type}$	Type of sanitizer used to sanitize cantaloupe rind	-	Discrete (Sanitizer)	-

	C_{on_sanit}	Concentration of LM on cantaloupe rind after sanitization	log CFU/cm^2	IF ($Sanit_{type}$ = ClO2)
				C_{whole} – R_{ClO2}
				IF ($Sanit_{type}$ = H2O2)
				C_{whole} – R_{H2O2}
				IF ($Sanit_{type}$ = SH)
				C_{whole} – R_{SH}
				IF ($Sanit_{type}$ = ”0”)
				C_{whole}
					-

Dicing, cross-contamination from dicing equipment and partitioning	$TR_{rind-dices}$	Transfer rate of contaminated cantaloupe rind to cantaloupe dices	%	$f_{transfer}$ (C_{on_sanit})	Function modelled using data from Table A 3.7
	W_{cant}	Weight of a rind-free cantaloupe	g	-	To be determined
	W_{dices}	Weight of the diced cantaloupe RTE product	g	-	Assumption to be made
	b	Dispersion factor related to the heterogeneous distribution of LM in diced cantaloupe	-	-	Assumption to be made
	P_{ccd}	Probability that dicing machine is contaminated	-	-	To be determined from literature
	$TR_{machine-dices}$	Cross-contamination transfer coefficient from dicing machine to dices	%	-	To be determined using data from Table A 3.8 (Dicing only)
	N_{dicer}	Numbers of LM cells on dicing machine ready to be transferred	CFU		Assumption to be made
	B_{cant+}	Status of a cantaloupe rind being contaminated with LM	-	Bernoulli (P_{whole})	-
	B_{ccd+}	Status of dicing machine being contaminated	-	Bernoulli (P_{cch})	-
	Packs	Number of cantaloupe packs that can be produced from one cantaloupe	-	ROUND (W_{cant}/W_{dices})	-

N_{dices}	Numbers of LM cells in the mass of dices originating from a cantaloupe contaminated on the rind	CFU	IF (B_{cant+}=1) & (B_{ccd+}=1) $N_{dices} = 10^{C\ on\_sanit} \times TR_{rind\text{-}dices} \times W_{cant}$ N_{from_dicer} = Binomial (N_{dicer}, $TR_{machine\text{-}dices}$)
N_{from_dicer}	Numbers of LM cells transferred to all dices of one cantaloupe from a contaminated dicing machine	CFU	$N_{total} = N_{dices} + N_{from\_dicer}$ N_{pack} = Binomial (N_{total}, Beta (b, b x Packs – 1)) $Pi_{pack} = (Packs - 1)/(N_{total} + Packs - 1)$
N_{total}	Total numbers of LM cells in the mass of dices originating from a cantaloupe	CFU	IF (B_{cant+}=1) & (B_{ccd+}=0) $N_{dices} = 10^{C\ on\_sanit} \times TR_{rind\text{-}dices} \times W_{cant}$
N_{pack}	Total number of LM cells distributed in a pack of diced cantaloupe	CFU	N_{pack} = Binomial (N_{dices}, Beta (b, b x Packs – 1)) $Pi_{pack} = (Packs - 1)/(N_{dices} + Packs - 1)$

Pi_{pack}	Probability that a pack of diced cantaloupe is LM-free		IF (B_{cant+}=0) & (B_{ccd+}=1)
			N_{from_dicer} = Binomial (N_{dicer}, $TR_{machine\text{-}dices}$)
			
			N_{pack} = Binomial (N_{from_dicer}, Beta (b, b x Packs – 1))
			
			$Pi_{pack} = (Packs - 1)/(N_{from\_dicer} + Packs - 1)$
			
			ELSE
			Remove

P_{RTE}	Prevalence of RTE diced cantaloupe packs contaminated with LM	-	$[1\text{-}Pi_{pack}] \times [1 - (1 - P_{whole})x(1 - P_{ccd})]$
C_{RTE}	Concentration of LM in RTE diced cantaloupe packs	Log CFU/g	Log (N_{pack} /W_{dices})

Table A 3.14: Variables and functions related to the cold chain of RTE diced product from end processing to consumption

Domain	Variable name	Description	Unit	Equations	Data/ Assumptions
Growth parameters	CM(T)	Secondary model: Cardinal parameter model to estimate maximum growth rate (μ) of LM in cantaloupe flesh as a function of temperature	h^{-1}		Model fitted to data from Table A 3.10 to determine T_{min}, T_{opt}, T_{max}, μ_{opt}
	$f_{Baranyi}(t; q_0, C_0, \mu, MPD)$	Primary model: Baranyi model to predict concentration of LM in cantaloupe flesh in time t, using parameters μ, MPD, q_0	log CFU/g	Equation (3.2)	
	q_0	Initial concentration of a hypothetical substrate	-	Equation (3.1)	Distribution modelled using data from Table A 3.9
	MPD	Maximum population density of LM in cantaloupe	CFU/g	-	Assumption to be made
Refrigerated transport	$Temp_{RTE\_trans}$	Temperature of RTE diced cantaloupe during transport	°C	-	
	t_{RTE_trans}	Transportation time of RTE diced cantaloupe from end processing to retail	h	-	
	C_{RTE_trans}	Concentration of LM in RTE diced cantaloupe at the end of transport	Log CFU/g	$\mu = \mathbf{CM}(Temp_{RTE\_trans})$ $C_{RTE\_trans} = \mathbf{f_{Baranyi}}(C_{RTE}, t_{RTE\_trans}; q_0, \mu, MPD)$	-

Retail	$Temp_{RTE\_retail}$	Temperature of RTE diced cantaloupe at retail	°C	-	
	t_{RTE_retail}	Time of RTE diced cantaloupe on display during retail	h	-	
	C_{RTE_retail}	Concentration of LM in RTE diced cantaloupe at the end of retail	Log CFU/g	$\mu = \mathbf{CM}(Temp_{RTE\_retail})$ $C_{RTE\_retail} = \mathbf{f_{Baranyi}}(C_{RTE\_trans}, t_{RTE\_retail}; q_1, \mu, MPD)$	-
Transport to home	$Temp_{RTE\_rh}$	Temperature of RTE diced cantaloupe in the transportation from retail to home	°C	-	
	t_{RTE_rh}	Transportation time of RTE diced cantaloupe from retail to home	h	-	
	C_{RTE_rh}	Concentration of LM in RTE diced cantaloupe at the end of transport to home	Log CFU/g	$\mu = \mathbf{CM}(Temp_{RTE\_rh})$ $C_{RTE\_rh} = \mathbf{f_{Baranyi}}(C_{RTE\_retail}, t_{RTE\_rh}; q_2, \mu, MPD)$	-
Home	$Temp_{RTE\_home}$	Temperature of RTE diced cantaloupe in the home	°C	-	
	t_{RTE_home}	Time to consumption of RTE diced cantaloupe	h	-	
	C_{RTE_home}	Concentration of LM in RTE diced cantaloupe at the point of consumption	Log CFU/g	$\mu = \mathbf{CM}(Temp_{RTE\_home})$ $C_{RTE\_home} = \mathbf{f_{Baranyi}}(C_{RTE\_rh}, t_{RTE\_home}; q_3, \mu, MPD)$	-
	P_{RTE_home}	Prevalence of RTE diced cantaloupe contaminated with LM at the point of consumption	-	P_{RTE}	

Table A 3.15: Variables and functions related to the merchandizing of whole and sliced cantaloupe in informal markets

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Predictive microbiology models for rind	$f_{rind\text{-}survival}(C_0, t)$	Survival of LM on intact cantaloupe rind as a function of initial concentration and time, at ambient temperature	log CFU/cm^2		To be fitted to data from Table A 3.4
	$f_{rind\text{-}growth}(C_0, t)$	Growth of LM on bruised cantaloupe rind as a function of initial concentration and time, at ambient temperature	log CFU/cm^2		To be fitted to data from Table A 3.5
Cross-contamination	P_{ccm}	Probability that cross-contamination with LM occurs due to poor handling in informal markets	-		EKE
	TR_{floor_canta}	Transfer coefficient from any surface (floor, boxes) to cantaloupe rind in informal markets	%		EKE
	N_{floor}	Numbers of LM on any surface (floor, boxes) ready to be transferred to cantaloupe rind in informal markets	CFU		EKE
	B_{cant+}	Status of a cantaloupe being contaminated with LM	-	Bernoulli ($P_{rind\text{-}post}$)	-

	B_{ccm+}	Status of cross-contamination taking place in informal markets	-	Bernoulli (P_{ccm})	-
	$N_{f\text{-}c}$	Numbers of LM transferred to cantaloupe rind	CFU	ROUND(N_{floor} x TR_{floor_canta})	-

	C_{rind_market}	Concentration of LM on cantaloupe rind in informal markets	Log CFU/ cm^2	IF (B_{cant+}=0) & (B_{ccm+}=1)
				Log [(0 + $N_{f\text{-}c}$)/A_{cant}]
				IF (B_{cant+}=1) & (B_{ccm+}=1)
				Log [(10^{Crind_post} + $N_{f\text{-}c}$)/A_{cant}]
				IF (B_{cant+}=1) & (B_{ccm+}=0)
				Log [(10^{Crind_post} + 0)/A_{cant}]
				ELSE
				Remove
					-

	P_{rind_market}	Prevalence of cantaloupes with LM on the rind in informal markets	-	1 – (1 – $P_{rind\text{-}harv}$) x (1 – P_{cch})	-
Whole cantaloupe	t_{market}	Time that whole cantaloupes are displayed until purchase	h	-	Assumption to be made
	B_{cant+}	Status of a cantaloupe being contaminated with LM	-	Bernoulli (P_{rind_market})	-
	$B_{bruise+}$	Status of a cantaloupe having suffered bruising due to handling after harvest	-	Bernoulli (P_{bruise})	-

	C_{whole_market}	Concentration of LM on the rind of cantaloupe purchased in informal markets (contaminated fraction)	Log CFU/ cm^2	IF (B_{cant+}=1) & ($B_{bruise+}$=1)
				Log [10^$f_{rind\text{-}survival}$ (C_{rind_post}, t_{market}) + 10^$f_{rind\text{-}growth}$ (C_{rind_post}, t_{market})]
				IF (B_{cant+}=1) & ($B_{bruise+}$=0)
				$f_{rind\text{-}survival}$ (C_{rind_post}, t_{market})
				ELSE
				Remove
					-

	P_{whole_market}	Prevalence of cantaloupe contaminated with LM on the rind, purchased in informal markets	-	$P_{rind\text{-}market}$	-
Slices of cantaloupe	$TR_{rind\text{-}slice}$	Transfer rate of LM from cantaloupe rind to slice, at ambient temperature	CFU/cm^2 slice / CFU/ cm^2 rind		Data from Patil (2017), from Table A 3.7
	W_{slice}	Weight of a slice of cantaloupe without rind	g		To be determined
	A_{slice}	Surface area of a cantaloupe slice (edible)	cm^2		To be determined
	N_{slice}	Number of LM cells on a cantaloupe slice	CFU	ROUND (C_{rind_market} x $TR_{rind\text{-}slice}$ x A_{slice})	-
	$C_{slice\text{-}market}$	Concentration of LM cells on a slice from a contaminated cantaloupe	Log CFU/g	Log (N_{slice}/W_{slice})	-
	$P_{slice\text{-}market}$	Prevalence of cantaloupe slices contaminated with LM	-	P_{whole_market}	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 3.16: Variables and functions related to retail, transport and home preparation of whole cantaloupe

Domain	Variable name	Description	Unit	Equations	Data/ Assumptions
Transport to retail	$Temp_{whole\_trans}$	Temperature of whole cantaloupe during transport	°C	-	
	t_{whole_trans}	Transportation time for whole cantaloupe from end processing to retail	h	-	

	C_{whole_trans}	Concentration of LM on the rind of cantaloupe at the end of transport (contaminated fraction)	Log CFU/cm^2	IF (B_{cant+}=1) & ($B_{bruise+}$=1)
				Log [10^$f_{rind\text{-}survival}$ (C_{whole}, t_{whole_trans}) + 10^$f_{rind\text{-}growth}$ (C_{whole}, t_{whole_trans})]
				IF (B_{cant+}=1) & ($B_{bruise+}$=0)
				$f_{rind\text{-}survival}$ (C_{whole}, t_{whole_trans})
				ELSE
				Remove	

	P_{whole_trans}	Prevalence of cantaloupe with LM on the rind after transport		P_{whole}	
Retail	$Temp_{whole\_retail}$	Temperature of whole cantaloupe at retail	°C	-	
	t_{whole_retail}	Time of whole cantaloupe on display during retail	h	-	

	C_{whole_retail}	Concentration of LM on the rind of cantaloupe at the end of retail (contaminated fraction)	Log CFU/cm^2	IF (B_{cant+}=1) & ($B_{bruise+}$=1)
				Log [10^$f_{rind\text{-}survival}$ (C_{whole_trans}, t_{whole_retail}) + 10^$f_{rind\text{-}growth}$ (C_{whole_trans}, t_{whole_retail})]
				IF (B_{cant+}=1) & ($B_{bruise+}$=0)
				$f_{rind\text{-}survival}$ (C_{whole_trans}, t_{whole_retail})
				Remove	

	P_{whole_retail}	Prevalence of cantaloupe with LM on the rind at the end of retail		P_{whole_trans}	

Transport to home	$Temp_{whole\_rh}$	Temperature of whole cantaloupe during transport to home	°C	-	
	t_{whole_rh}	Transportation time of whole cantaloupe from retail to home	h	-	
	C_{whole_rh}	Concentration of LM on the rind of cantaloupe at the end of transport to home (contaminated fraction)	Log CFU/cm^2	IF (B_{cant+}=1) & ($B_{bruise+}$=1) Log [10^$f_{rind\text{-}survival}$(C_{whole_retail}, t_{whole_rh}) + 10^$f_{rind\text{-}growth}$(C_{whole_retail}, t_{whole_rh})] IF (B_{cant+}=1) & ($B_{bruise+}$=0) $f_{rind\text{-}survival}$(C_{whole_retail}, t_{whole_rh}) Remove	
	P_{whole_rh}	Prevalence of cantaloupe with LM on the rind at the end of transport to home		P_{whole_retail}	
Home	SL_{cant}	Maximum lifetime of a cantaloupe under optimum storage conditions	h		To be determined
	$Temp_{home}$	Storage temperature at home	°C		Assumption to be made
	t_{home}	Storage time before slicing	h		Assumption to be made
	R	Negative correlation between $Temp_{home}$ and t_{home}	-	0.25	FDA-FSIS (2003)
Handling type	Handling	Probability that the consumer: washes first then stores (Wash-st) stores first then washes (St-wash) does not wash rind at all (No-wash)	-	Discrete ()	Assumption to be made
	R_{ww}	Reduction in LM due to washing with water	log CFU/cm^2	-	Table A 3.6
	St-wash(+)	Status of the consumer storing the whole cantaloupe and washing rind just before consumption	-	P(Handling=St-wash)	-

Wash-st(+)	Status of the consumer washing rind as soon as cantaloupe arrives home, and then storing for later consumption	-	P(Handling=Wash-st)
No-wash(+)	Status of the consumer not washing cantaloupe at all	-	P(Handling=No-wash)

C_{on_stored}	Concentration of LM on cantaloupe rind until first slicing	log CFU/cm^2	IF ($B_{bruise+}$=0)
			IF (St-wash(+)=1 OR No-wash(+)=1)
			$f_{survival}(t_{home}, C_{whole\_rh})$
			IF ($B_{bruise+}$=1)
			IF (St-wash(+)=1 OR No-wash(+)=1)
			$f_{survival}(t_{home}, C_{whole\_rh})$ + $f_{growth}(t_{home}, C_{whole\_rh})$

C_{on_washed}	Concentration of LM on cantaloupe after surface washing	log CFU/cm^2	IF ($B_{bruise+}$=1) OR ($B_{bruise+}$=0)
			IF (Wash-st(+)=1)
			$C_{whole\_rh} - R_{ww}$
			IF (St-wash(+)=1)
			$C_{on\_stored} - R_{ww}$
			IF (No-wash(+)=1)
			C_{on_stored}

$C_{on\_slicing}$	Concentration of LM on cantaloupe rind just before the slicing (opening)	log CFU/cm^2	IF (Wash-st(+)=1)
			IF ($B_{bruise+}$=1)
			$f_{survival}(t_{home}, C_{on\_washed})$ + $f_{growth}(t_{home}, C_{on\_washed})$
			IF ($B_{bruise+}$=0)
			$f_{survival}(t_{home}, C_{on\_washed})$
			IF (St-wash(+)=1)
			C_{on_washed}
			IF (No-wash(+)=1)
			C_{on_stored}

Slicing (transfer from rind to slice and potential cross-contamination)	$TR_{rind-slice}$	Transfer rate of LM from contaminated cantaloupe rind to slice, at ambient temperature	CFU/cm^2 slice / CFU/cm^2 rind	-	Data from Patil (2017), from Table A 3.7
	P_{cck}	Probability of cross-contamination event at home (from contaminated knife)	-		To be determined
	$TR_{knife-slice}$	Cross-contamination transfer coefficient from knife to cantaloupe flesh during slicing	-		To be determined using data from Table A 3.8 (Slicing only)
	N_{knife}	Numbers of LM on knife ready to be transferred upon contact with cantaloupe flesh	CFU		Assumption to be made
	B_{cck+}	Status of knife being contaminated with LM	-	Bernoulli (P_{cch})	-
	N_{slices}	Total numbers of LM transferred to cantaloupe slice (cantaloupe and/or knife)	CFU	IF (B_{cant+}=1) & (B_{cck+}=1) $N_{dices} = ROUND (10^{Con\_slicing} \times TR_{rind-slice} \times A_{slice} + TR_{knife-slice} \times N_{knife})$ IF (B_{cant+}=1) & (B_{cck+}=0) $N_{dices} = ROUND (10^{Con\_slicing} \times TR_{rind-slice} \times A_{slice})$ IF (B_{cant+}=0) & (B_{cck+}=1) $N_{dices} = ROUND (TR_{knife-slice} \times N_{knife})$ IF (B_{cant+}=0) & (B_{cck+}=0) Remove	
	$C_{slice-home}$	Concentration of LM cells on a slice from a contaminated cantaloupe	Log CFU/g	$Log(N_{slices}/W_{slice})$	-
	$P_{slice-home}$	Prevalence of cantaloupe slices contaminated with LM	-	$1 - (1 - P_{whole\_rh}) \times (1 - P_{cck})$	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Annex 4

Technical description of the exposure assessment model for *L. monocytogenes* in whole lettuce and RTE pre-cut lettuce

SECTION 1: ASSUMPTIONS AND DATA

Module 1: Primary production

Irrigation water, fertilized soil and farm management practices as risk factors for *L. monocytogenes*

The occurrence of *L. monocytogenes* in lettuce at the pre-harvest stage is considered to depend on the type of cultivation. Sources of contamination in conventional and protected cultivation are irrigation water and fertilized/amended soil, contaminated equipment, workers, and wildlife intrusion. Occurrence data for this pathogen in water and soil are compiled in Tables A 3.1 and A 3.2 from Annex 3, respectively. Data on management practices that can be associated with an increased probability of occurrence of *L. monocytogenes* in soil are compiled in Table A 3.3 (Annex 3), and will be used in the harvest module to adjust the prevalence in soil for recent application of manure, recent last irrigation, and vicinity of surface water or pasture.

Survival of *L. monocytogenes* in soil

The survival of *L. monocytogenes* in soil is another phenomenon that needs to be incorporated into the model. Various studies have investigated the factors impacting *L. monocytogenes* survival in soil (Locatelli *et al.*, 2013b; Oliveira *et al.*, 2011; McLaughlin *et al.*, 2011; Girardin *et al.*, 2005; Nicholson *et al.*, 2005), and their experiments involved direct inoculation of *L. monocytogenes* or *L. innocua* in soil, by adding previously spiked fertilizer in soil, or by irrigating the soil with spiked water. Table A 4.1 compiles data on the survival of *L. monocytogenes* or *L. innocua* in soil. These studies revealed that the survival of *L. monocytogenes* in soil is affected by soil pH, ambient temperature, season, the irrigation method that disseminates the contaminated water and the soil texture. Nonetheless, *L. monocytogenes* survival in soil does not seem to be affected by the type of manure added to the soil (Nicholson *et al.*, 2005). They found out that, regardless of the type of manure spread on soil, *L. monocytogenes* persisted over 16 days in a clay loam grassland soil, while survival was lower in a sandy arable soil. Furthermore, Oliveira *et al.* (2011) proved that there was no difference in *L. monocytogenes*

survival in soil between contaminating the soil through spiked compost and contaminating the soil through irrigation water with the surface method.

Girardin *et al.* (2005) investigated the behaviour of *L. innocua* in parsley grown in fields fertilized with contaminated amendments. Before being applied to the soil, animal manure or municipal waste sewage underwent a composting process, and irrigation was carried out through sprinkling using groundwater. After 90 days, the population of *L. innocua* had diminished in >7 log from the inoculation level. The data extracted from the aforementioned studies allow a model to be built of the survival of *L. monocytogenes* or its surrogate in soil, as affected by season.

Table A 4.1: Data on survival of *L. innocua* or *L. monocytogenes* in soil taken from soil inoculation or irrigation studies

Source	Bacterium	Characteristics of the study	Time	Mean concentration (log CFU/g dw soil)
Locatelli *et al.* (2013b)	*L. monocytogenes*	Soil microcosms directly spiked with the pathogen (non-sterile soil)		
		pH < 5.5	0 day	6.00
			7	1.40
			14	0.60
			84	0.19
		5.5 < pH < 7.0	0 day	6.00
			7	3.40
			14	2.40
			84	1.00
		pH > 7.0	0 day	6.00
			7	5.00
			14	4.30
			84	2.80
Oliveira et. al. (2011)	*L. innocua* (experiments with lettuce)	Soil with spiked compost		
		Autumn	0 wk	7.00
			4	6.13
			6	5.60
			8	5.43
			9	5.25
		Spring	0 wk	6.00
			3	5.81
			5	5.47
			7	4.62
			9	3.76

		Soil irrigated with surface irrigation (data for one irrigation event)		
			0 wk	6.13
		Autumn	2	5.95
			4	5.43
			0 wk	6.16
		Spring	2	5.13
			4	4.10
		Soil irrigated with sprinkle irrigation (one irrigation event)		
			0 wk	4.20
		Autumn	2	1.00
			4	0.88
			0 wk	5.30
		Spring	2	2.74
			4	1.37
McLaughlin *etal.* (2011)	*L. monocytogenes E*GDe + CD83+CD1038	Soil directly spiked with the pathogen (stored at 25 °C)	0 day	6.13
			1	5.77
			2	5.16
			4	3.87
			6	1.70
Girardin *et al.* (2005)	*L. innocua* (experiments with parsley)	Soil with organic fertilizers (composted sewage sludge and composted bovine manure)	0 day	5.60
			7	5.40
			30	1.00
			55	-0.75
			75	-0.55
			90	-1.50
Nicholson *et al.* (2005)	*L. monocytogenes*	Soil after manure application		
			0 day	2.50
			1	2.50
		Applied on surface of sandy soil	2	1.37
			4	1.56
			8	0.00
			16	0.00
			0 day	2.50
			1	2.00
		Applied on surface of clay loam grassland soil	2	1.20
			4	1.04
			8	0.00
			16	0.28

Sources: **Source** column cites the corresponding studies. See References section for further details.

Transfer of *L. monocytogenes* to leafy greens by soil splash

Listeria monocytogenes may be transferred from the soil to fresh produce through soil splash from irrigation (Smith *et al.*,2018). In an experiment designed to assess the survival and transfer of *L. innocua*, Girardin *et al.* (2005) demonstrated that bacterial transfer to parsley leaves occurred mainly through soil splash from rain and irrigation after the bacterium was inoculated into the soil, instead of through internalization. *Listeria innocua* was detected on parsley leaves once there is 1 CFU/g in the soil. Oliveira *et al.*'s (2011) study also sought to explore the transfer of *L. innocua* from soil to lettuce. To achieve this, the authors either applied artificially contaminated compost or irrigated with contaminated water. Although both studies (Girardin *et al.*, 2005; Oliveira *et al.*, 2011) quantified the fate of *L. innocua* on the edible parts of the crop, as a consequence of contamination from soil, neither of them quantified or modelled a transfer rate per se. Table A 4.2 presents transfer rates calculated after extraction of data from both studies. They were calculated as the microbial density that ended up on leaves after irrigation, divided by the microbial counts in soil at the moment of irrigation. Defined as such, the leaf-soil transfer rates are punctual and do not represent the subsequent decay of *L. innocua* deposited on the leaves. On reassessing the data of Oliveira *et al.* (2011), it can be presumed that, regardless of the manner in which the soil was contaminated with *L. innocua*, the transfer rates are higher in autumn than in spring.

Table A 4.2: Data on transfer of *L. innocua* from soil to edible parts of fresh produce

Source	Characteristics sampling time	Soil	Log CFU/g in soil at irrigation	Log CFU/g leaves after irrigation	Transfer rate: log ($10^{leaves}/10^{soil}$)
Girardin *et al.* (2005)	Crop: parsley Irrigation: sprinkler Sampling: 25 days after planting	Soil spiked with compost	1.80	-1.50	-3.30
Oliveira *et al.* (2011)	Crop: lettuce Sampling: 3-4 weeks after transplanting	Soil spiked with compost	Autumn 6.13 Spring 5.81	2.25 1.40	-3.88 -4.41
		Soil spiked with surface irrigation	Autumn 6.13 Spring 6.16	1.4 1.2	-4.73 -4.96

Sources: **Source** column cites the corresponding studies. See References section for further details.

Survival of *L. monocytogenes* on leafy greens

Once *Listeria* cells have been deposited on the edible part of the crop, they are seen to decline. Table A 4.3 compiles the results of two experiments conducted by Girardin *et al.* (2005) and Oliveira *et al.* (2011) on the fate of *L. innocua* once inoculated on produce leaves, either through splashing of contaminated soil during irrigation or through sprinkle irrigation with contaminated water. It can be noted that, although the outcomes from the two experiments point towards a decline in the pathogen's persistence on leaves, the times for reaching log reductions are variable between experiments. Parsley leaves contaminated through splashing of contaminated soil during irrigation exhibited a reduction of 0.5-1.5 logs in the population of *L. innocua* in 5 days, whereas lettuce leaves contaminated through sprinkle irrigation with contaminated water achieved reductions of 3.3-4.7 logs in 4 weeks. Models of the fate of *L. innocua* as a surrogate for *L. monocytogenes* will be obtained from those results. The first data set will be used to model the fate of *L. monocytogenes* on leaves contaminated by soil splashing due to irrigation, and the second data set will be used to model the decay of the pathogen on leaves following irrigation with contaminated water.

Nutrient solution in hydroponics as source of *L. monocytogenes*

Koseki *et al.* (2011) and Standing *et al.* (2013) demonstrated that *L. monocytogenes* can internalize in hydroponic cultivation through roots in contact with a contaminated nutrient solution. As test samples, Koseki *et al.* (2011) used spinach that was hydroponically grown for 10 weeks. Plants were then watered once with the nutrient solution spiked with 3 or 6 log CFU per ml of *Salmonella* spp., *L. monocytogenes* or *E. coli* O157:H7, and were held at 23° C and 50% RH. After 48 hours, spinach leaves were analysed for detection and enumeration of the pathogen. Applying a logistic regression, Koseki *et al.* (2011) modelled the probability of internalization (P_{int}) through spinach roots after 48 hours of exposure to a contaminated nutrient solution, as a function of the pathogen and inoculum load in the solution ($LM_{solution}$). The following simplified equation can be used to estimate the probability of internalization of *L. monocytogenes* in spinach leaves:

$$\text{logit}(P_{int}) = -4.793 + 0.759\ LM_{solution} \qquad (4.1)$$

Table A 4.3: Data on the survival of *L. innocua* on produce leaves during cultivation

Source	Characteristics		Time	Log CFU/ g leaves
Girardin *et al.* (2005)	Crop: Parsley Experiment: **Splashing from soil** with inoculated compost (10^7 CFU *L. innocua*/g soil) on aerial surfaces; Irrigation: from a central point at 1.8 m height N=220 plants; Temp:14–30 °C	Distance from water impact point (cm)		
		35	1 day	4.4
			2	4.0
			4	3.5
			5	2.9
		40	1 day	2.2
			2	1.7
			4	1.7
			5	1.3
		45	1 day	2.0
			2	1.6
			4	1.6
			5	1.5
Oliveira *et al.* (2011)	Crop: Lettuce Experiment: Contaminated (10^7 CFU *L. innocua*/ml) **water sprinkled on leaves**, mimicking irrigation Data from one irrigation event carried out on week 3 (spring) or 4 (autumn) N=20 pots	Season		
		Autumn	0 wk	4.59
			2	1.69
			4	1.35
		Spring		
			0 wk	4.73
			2	0.47
			4	0.00

Sources: **Source** column cites the corresponding studies. See References section for further details.

It should be noted that the doses in the studies were for experimental purposes and we need to see how we can apply them to the real situation; the degree of internalization therefore depends on the concentration of the pathogen in the nutrient solution. At a concentration of 3 log CFU/ml in a nutrient solution, the positive spinach leaves presented concentrations of *L. monocytogenes* <1.7 log CFU/leaf, whereas roots exposed to a solution with a concentration of 6 log CFU/ml lead to concentrations in spinach leaves ranging between <1.7 – 4 log CFU/leaf.

Module 2: Harvest

In both Modules 2 and 3, cross-contamination from multiple surfaces in contact with lettuce during harvesting is considered. Cross-contamination transfer coefficients and probabilities will be assumed from other foodstuffs undergoing similar stages or from expert opinions. The extent of removal of *L. monocytogenes* cells due to tearing lettuce leaves with attached soil will be assumed as being minimal.

Module 3a,b: Chilling

Koseki and Isobe (2005) determined that the necessary cooling time for iceberg lettuce under moderate conditions was 3 hours. Cooling systems in leafy greens

production include forced air, hydrocooling and vacuum cooling systems. Hydrocooling might entail a risk for contamination with pathogens (Gil *et al.*, 2015). It will be assumed that lettuces will be cooled down promptly (maximum 180 minutes) with chilling temperatures of between 4–7 °C (Terry *et al.*, 2011).

Module 3a,b is applicable to whole lettuce to be sold in formal retail and to lettuce to be sent to a processing facility. For modelling *L. monocytogenes* growth in/on lettuce, the lag phase duration (λ [h]) will be initialized at this point of the chain using any of the two following equations developed for non-RTE leafy greens (Koseki and Isobe, 2005; Omac *et al.* 2017, respectively),

$$\ln\lambda = -0.155\ (T-31.91) \qquad (4.2)$$

$$\lambda = [0.0099(T+4.26)]^{-2} \qquad (4.3)$$

Alternatively, the value of the parameter (α_0) of the physiological initial state of the cell of 0.072 for *L. monocytogenes* in lettuce can be used (Koseki and Isobe, 2005). The model should endeavour to represent real temperature fluctuations during transportation as in Zeng *et al.* (2014) if data become available.

Module 4a,b,c: Transportation

It is presumed that the transportation conditions are dependent on the lettuce pathway. Whole lettuce to be sold in formal retail and lettuce to be sent to a processing facility will be transported under refrigeration temperatures. In the case of whole lettuce sold in informal retail, the transportation assumes the distribution from farm to informal markets at room temperature. Predictive microbiology models from Module 3a,b will be used in Module 4a,b,c. Table A 4.4 should be considered with caution as no washes or post-processing chemical and physical treatments are thought to deliver significant reductions reliably (FAO/WHO, 2023b).

Module 5a: Processing: cutting, washing and sanitizing, packaging and cooling (relevant for RTE pre-cut lettuce)

Washing and sanitizing

The reduction in *L. monocytogenes* concentration in lettuce can be approached from the effectiveness of washing with water and with tested sanitizers (chlorine dioxide gas [ClO_2], slightly acidified electrolysed water [SAEW] and sodium hypochlorite/chlorinated water [SH]) compiled in Table A 4.4 from published inoculation experiments. The log reductions after washing with water or

treatment with CD, SAEW and SH can be meta-analysed to obtain an estimate by sanitizer.

Table A 4.4: Reduction of *L. monocytogenes* concentration (and standard error) in leafy greens after treatment with water and sanitizers (chlorine dioxide gas [ClO2], slightly acidified electrolysed water [SAEW] and sodium hypochlorite [SH]) at a given sanitizer concentration, exposure time and temperature

Sanitizer	Study	Produce	Concentration (%)	Exposure time (min)	Temperature (°C)	Reduction (log CFU/g)	St. Error (Reduction)
Water	Guzel *et al.* (2017)	Romaine lettuce	-	10	25	0.63	0.170
			-	10	25	0.55	0.160
			-	10	25	0.53	0.050
	Rahman *et al.* (2010)	Spinach	-	3	23	1.00	0.212
	Sagong *et al.* (2011)	Lettuce	-	5	20	0.23	0.069
	Xu *et al.* (2007)	Lettuce	-	4	22	0.24	0.130
				4	22	0.25	0.104
	Bari *et al.* (2005)	Cabbage	-	1	4	0.95	0.074
			-	3	23	0.35	0.160
		Lettuce	-	3	23	0.40	0.070
		Spinach	-	3	23	0.20	0.070
ClO_2 gas	Lee *et al.* (2004)	Lettuce	0.00043	30	22	5.00	-
			0.00067	60	22	5.20	-
			0.00087	180	22	5.40	-
	Sy *et al.* (2005)	Cabbage	0.00014	10	22	1.76	-
			0.00027	20	22	3.31	-
			0.00041	30	22	3.60	-
		Lettuce	0.00014	10	22	0.81	-
			0.00027	20	22	1.23	-
			0.00041	30	22	1.53	-
SAEW	Forghani and Oh (2013)	Cabbage	0.00215	3	23	1.19	0.032
		Lettuce	0.00215	3	23	1.20	0.098
		Spinach	0.00215	3	23	1.48	0.135
	Rahman *et al.* (2010)	Spinach	0.0005	3	23	2.80	0.250
			0.005	3	23	2.70	0.335

SH	Rahman *et al.* (2010)	Spinach	0.01	3	23	2.10	0.250
	Su and D'Souza (2012)	Lettuce	0.02	0.5	25	0.78	0.200
			0.02	1	25	0.94	0.065
	Guzel *et al.* (2017)	Romaine lettuce	0.02	10	25	0.89	0.040
			0.02	10	25	1.05	0.110
			0.02	10	25	1.01	0.120

Sources: **Study** column cites the corresponding studies. See References section for further details.

Cross-contamination

There are not many contamination transfer coefficients for *L. monocytogenes* applicable to leafy greens in the literature; those estimated by Hoelzer *et al.* (2012a) have been commonly used in QRA models. Table A 4.5 compiles transfer coefficients for different donors and recipients relative to *L. monocytogenes* as estimated by Hoelzer *at al.* (2012a), Zilelidou *et al.* (2015) and Kuan *et al.* (2017b).

Cross-contamination during shredding can be approximated using the knife-to-lettuce transfer rate, and cross-contamination from equipment and sorting tables will be approached using the stainless steel-to-vegetables transfer rate (Table A 4.5). As in Module 4b, pre-cut lettuce bags can be assumed to be cooled down promptly (maximum 180 minutes) with chilling temperatures between 4–7 °C (Terry *et al.*, 2011).

Table A 4.5: Transfer rate (TR) estimates for representing cross-contamination in vegetables found in the literature

Donor	Recipient	Unit	Mean	Standard deviation	Source
Lettuce	Knife	Log TR	-1.587	0.390	Zilelidou *et al.* (2015)
Knife	Lettuce	Log TR	-0.801	0.536	
Stainless steel	Vegetables	Log TR	-0.440	0.400	Hoelzer *et al.* (2012a)
Hand	Vegetables	Log TR	-1.720	1.070	
Chopping board	Vegetables	Log TR	-1.420	0.520	
Lettuce	Chopping board	%TR	4.70	1.30	Kuan *et al.* (2017b)
Chopping board	Cabbage	%TR	31.7	6.90	

Sources: **Source** column cites the corresponding studies. See References section for further details.

Modules 6a, b: Temperature chain for RTE pre-cut lettuce and whole lettuce sold at formal retail

The evolution of *L. monocytogenes* in RTE pre-cut lettuce and in/on whole lettuce will be modelled using microbial kinetic data. Lag phase duration will be considered from the point when pre-cut lettuce packs are chill-stored at the processing plant, using the model developed by Sant'Ana *et al.* (2012) for MAP leafy greens:

$$\ln\lambda = -0.145\ (T - 35.3) \qquad (4.4)$$

In the case of whole lettuce to be sold at formal retail, the lag phase duration will be initialized at this point using either of the two available models (Equation 4.2 or 4.3).

Kinetic data and growth models for *L. monocytogenes*

Some experiments to characterize the growth kinetics of *L. monocytogenes* in leafy greens have been carried out. Microbial growth rate estimates taken from the literature are shown graphically in Figure A 4.1. Table A 4.6 tabulates the growth rate estimates at different temperatures and conditions. One of the studies (Li *et al.*, 2002) employed an experimental setting, whereby lettuce samples were inoculated with the pathogen before or after treatment in a water bath at 20 °C or 50 °C with or without chlorine 20 mg/L. The results of this study are highlighted in Figure A 4.1 to show that its growth rate estimates lie below the others. Figure A 4.1 also highlights data from Carrasco *et al.* (2008) and Sant'Ana *et al.* (2012), since they both inoculated in shredded lettuce in MAP conditions (CO_2:5%; O_2:5%). These data points lie reasonably close to the tendency shown by the other experiments, where non-bagged (non-MAP) leafy greens were inoculated. These data – except from the previously mentioned data from Li *et al.* (2002) – could be integrated into a meta-analytical regression extracting the cardinal temperature parameters for lettuce and MAP lettuce.

In order to limit the exponential growth of *L. monocytogenes* at a maximum population density (MPD), the following equation from Koseki and Isobe (2005) can be used:

$$MPD = 0.037\ T + 12.434 \qquad (4.5)$$

where MPD is given in ln CFU/g and T is temperature in °C.

Table A 4.6: Growth rate (GR) estimates of *L. monocytogenes* in leafy greens or similar media from published studies

Study	Medium	Strain	Stressed	Temperature (°C)	Specific GR (h^{-1})	pH	Specific conditions
Conner *et al.* (1986)	Clarified cabbage juice	LCDC81-861	No	30	0.4560	6.2	
		Scott A	No	30	0.4279	6.2	NaCl(%):0.5
Carlin *et al.* (1995)	Fresh endive	Scott A	No	3	0.0170		
		Scott A	No	6	0.0290		
		Scott A	No	3	0.0160		
		Scott A	No	6	0.0230		
Carlin and Nguyen-The (1994)	Butterhead lettuce	Scott A	No	10	0.0550		
	Curly endive	Scott A	No	10	0.0230		
Carlin *et al.* (1996a)	Fresh endive	Scott A	No	10	0.0210		
Carlin *et al.* (1996b)	Fresh endive	Scott A	No	3	0.0048	6	
		Scott A	No	3	0.0089	6	
		Scott A	No	10	0.0333	6	
Francis *et al.* (2001)	Lettuce extract medium	ATCC-19114	No	8	0.0403	7	
		ATCC-19114	No	8	0.0361	7	
		ATCC-19114	No	8	0.0329	7	CO_2(%):25
		ATCC-19114	No	8	0.0363	7	CO_2(%):25

Li *et al.* (2002)	Lettuce	F8027/H022/F8255/ G1091/F8369	Yes	5	0.0320	Inoculation before treatment in water bath at 50 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	5	0.0030	Inoculation before treatment in water bath at 20 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	5	0.0170	Inoculation before treatment in water bath at 50 °C without chlorine
		F8027/H022/F8255/ G1091/F8369	Yes	15	0.1920	Inoculation before treatment in water bath at 50 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	15	0.0840	Inoculation before treatment in water bath at 20 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	15	0.1910	Inoculation before treatment in water bath at 50 °C without chlorine
		F8027/H022/F8255/ G1091/F8369	Yes	5	0.0190	Inoculation after treatment in water bath at 50 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	5	0.0080	Inoculation after treatment in water bath at 20 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	5	0.0210	Inoculation after treatment in water bath at 50 °C without chlorine
		F8027/H022/F8255/ G1091/F8369	Yes	5	0.0070	Inoculation after treatment in water bath at 20 °C without chlorine
		F8027/H022/F8255/ G1091/F8369	Yes	15	0.0910	Inoculation after treatment in water bath at 20 °C with chlorine 20 mg/L
		F8027/H022/F8255/ G1091/F8369	Yes	15	0.1080	Inoculation after before treatment in water bath at 50 °C without chlorine

Koseki and Isobe (2005)	Lettuce	ATCC-13932 ATCC-15313 ATCC-19111 ATCC-19117 ATCC-19118 ATCC-35152	No	10	0.1050	6.4	raw, cut
		ATCC-13932 ATCC-15313 ATCC-19111 ATCC-19117 ATCC-19118 ATCC-35152	No	15	0.2020	6.4	raw, cut
		ATCC-13932 ATCC-15313 ATCC-19111 ATCC-19117 ATCC-19118 ATCC-35152	No	20	0.3500	6.4	raw, cut
		ATCC-13932 ATCC-15313 ATCC-19111 ATCC-19117 ATCC-19118 ATCC-35152	No	25	0.4460	6.4	raw, cut
		ATCC-13932 ATCC-15313 ATCC-19111 ATCC-19117 ATCC-19118 ATCC-35152	No	5	0.0196	6.4	raw, cut
Carrasco *et al.* (2008)	MAP shredded lettuce	ATCC 13932	No	5	0.1428		CO_2(%):5; O_2(%):5
				13	0.0230		CO_2(%):5; O_2(%):5
Sant'Ana *et al.* (2012)	MAP shredded lettuce	413, 494, 581	No	7	0.0379		CO_2(%):15; O_2(%):5
			No	10	0.0560		CO_2(%):15; O_2(%):5
			No	15	0.1347		CO_2(%):15; O_2(%):5
			No	20	0.2418		CO_2(%):15; O_2(%):5
			No	25	0.3385		CO_2(%):15; O_2(%):5
			No	30	0.4835		CO_2(%):15; O_2(%):5
Guzel *et al.* (2017)	Fresh-cut romaine lettuce	*L. innocua* NRCC B33076	No	5	0.0253		raw, cut
			No	10	0.0460		raw, cut

Sources: **Study** column cites the corresponding studies. See References section for further details.

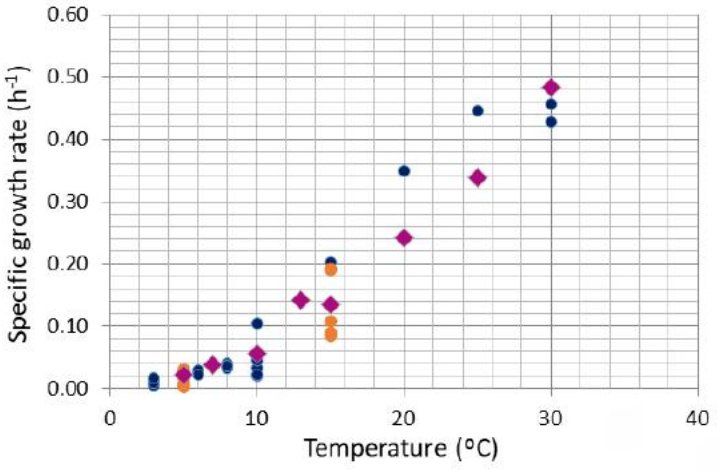

Figure A 4.1: Specific growth rate estimates of *L. monocytogenes* in leafy greens against temperature, extracted from the literature. Purple diamond markers represent data from MAP shredded lettuce, blue markers represent combined data from lettuce, fresh endive, lettuce extract medium and cabbage juice, and orange circles represent data on lettuce from Li *et al.* (2002). *Source:* See References section.

Time and temperature from processing to retail

Transportation from processing facilities to retail can be assumed to be short in distance (Ding *et al.*, 2013; Sant'Ana *et al.*, 2014). Minimally-processed produce is generally stored in refrigerators (4–6 °C) until it is transferred to trucks, which should maintain the same temperature, and should be lower than 7 °C (FSAI, 2022). The temperature during transportation can be assumed to be between 3 °C and 10.3 °C with a mean of 5 °C. The time for transportation to retail depends on geographical distances, but by bringing together the assumptions of Ding *et al.* (2013) and Sant'Ana *et al.* (2014), a short time of between 2 to 10 hours with a mean of 3 hours can be assumed.

Time and temperature during retail

Derens-Bertheau (2015), measuring the temperature along the cold chain for yogurt and meat products in France, found that ~31 percent of the measurements were above 6 °C. They also reported temperatures during display at retail from 1.4 °C to 9.8 °C, with an average of 5.6 °C. Other studies (McKellar *et al.*, 2012; 2014) have reported average display temperatures of 4.1 °C and 3.5 °C for fresh-cut lettuce during winter and summer, respectively. Nonetheless, the model should consider that whole lettuce can be displayed at ambient temperature.

Time and temperature from retail to home

Time of transportation from retail to home ($t_{r\text{-}h}$ [h]) can be modelled as in Sant'Ana *et al.* (2014) and Nauta *et al.* (2003) (mean: 43 mins., SD: 19 mins.). The mean change in temperature ($\Delta T_{r\text{-}h}$ C) during transportation from retail to home can be modelled as a function of the transportation time using the Audit International (2000) equation, previously used by Carrasco *et al.* (2010) for MAP leafy greens, and by Omac *et al.* (2017) for fresh spinach:

$$\Delta T_{r\text{-}h} = -1.318\ t_{r\text{-}h}^2 + 5.8701\ t_{r\text{-}h} \qquad (4.6)$$

Module 7c: Informal retail of whole lettuce

Listeria monocytogenes growth kinetics on whole lettuce will be assessed with the same data, equations and assumptions as Module 6b. Probability of cross-contamination, cross-contamination transfer coefficients and pathogen numbers on surfaces such as reusable plastic boxes/crates will be taken from similar QRA models.

Module 8a: Consumer handling (relevant for RTE pre-cut lettuce)

Growth during home cold storage

Nauta *et al.* (2003) indicated that the mean temperature of European domestic refrigerators is 6.64 °C. In Brazil, Silva *et al.* (2008) determined a mean temperature of 6 °C, oscillating between 3.1 °C to 10.8 °C, whereas in a Korean QRA risk assessment model for lettuce, mean fridge temperatures at home were assumed to be between 4 °C and 8.3 °C (Ding *et al.*, 2013). Carrasco *et al.* (2010) assumed a maximum temperature of home fridges of 11.3 °C.

Ding *et al.* (2013) assumed that Koreans would store lettuce in their home fridges for 12 h, 48 h and 168 h (7 days) as minimum, mean and maximum, respectively. Sant'Ana *et al.* (2014) assumed home storage time for RTE leafy greens produced in Brazil from 0 to 192 h (8 days). It is important, as in FDA-FSIS (2003), to establish a negative correlation between home temperature storage and time storage, since the stored lettuce will deteriorate faster at higher temperatures and this in turn will prompt consumers to consume them sooner. A negative correlation value of -0.25 can be employed (FDA-FSIS, 2003; Carrasco *et al.*, 2010).

In every iteration to sample home storage time, it should be checked that the sum of home storage time, time spent at retail and time from retail to home does not exceed the maximum shelf-life of RTE or non-RTE leafy greens, since these are

highly perishable products. According to Tsironi *et al.* (2017), pre-packed leafy greens purchased in retail establishments have a shelf-life of 8-10 days under refrigeration temperatures lower than 5 °C. It can be assumed that the consumer will not consume a product that shows evident signs of deterioration. The growth of *L. monocytogenes* during home cold storage is to be modelled using the same data, equations and assumptions as Module 6b.

Washing of RTE pre-cut lettuce

At the time of consumption, leafy greens may or may not be washed. Although RTE pre-cut lettuce is intended to be consumed without washing, there is a probability that the consumer opts for washing. If that is the case, the consumer's practice of washing under running tap water may produce a reduction (R_{wash}[log CFU/g]) in the pathogen's population depending on the washing time (t_{wash} [min]) (Domenech *et al.*, 2013). These authors proposed the following equation to estimate the log reduction in *L. monocytogenes* achieved by washing with tap water:

$$R_{wash} = \log_{10}\left(\frac{N(t)}{N_0}\right) = -0.28 \ln(t_{wash}) - 0.0103 \quad (4.7)$$

Yet as there is a limited effect, reduction during washing was not considered.

Module 8b,c: Consumer handling (relevant for whole lettuce)

Growth during home cold storage and washing

L. monocytogenes is assumed to grow in/on whole lettuce during home storage. It will be modelled using the same data, equations and assumptions as Module 6a. To be prepared, whole lettuce will first be washed with tap water. Equation (4.7) of Module 8a will be used to estimate *L. monocytogenes* concentration in lettuce leaves after washing.

Cross-contamination during handling

Poor hygiene practices at home, such as lack of cleaning of kitchen equipment/utensils or using the same chopping board to slice raw meat and raw vegetables, may lead to cross-contamination. The transfer rate estimates from chopping board-to-vegetables and knife-to-lettuce presented in Table A 4.5 can be applied at a given probability of poor hygiene handling by the consumer. The contamination levels of chopping boards and knives should be taken from published articles.

SECTION 2: THE EXPOSURE ASSESSMENT MODEL

The primary production of lettuce considers three types of cultivation: conventional or open field cultivation (Table A 4.7), protected cultivation (Table A 4.8) and hydroponic cultivation (Table A 4.9). Conventional and protected cultivation share a very similar model, except for the input variables of prevalence of *L. monocytogenes* in soil at sowing (P_{soil}) and concentration of *L. monocytgenes* in soil at sowing (C_{soil}), which can be different between the two types of cultivation, in addition to the possibility of proximity to pasture or surface water affecting open field cultivation and not protected cultivation.

In conventional and protected cultivation, the simulation unit is a small parcel of lettuce. In both production systems, the model considers that *L. monocytogenes* can only end up on lettuce by two routes: contaminated water from the last irrigation directly falling on leaves; and contamination from the soil reaching the leaves indirectly through soil splash from the last irrigation. Such routes are regulated by the following factors, which are known to affect the prevalence or concentration of *L. monocytogenes* on lettuce:

1) **Season** (autumn versus spring), which affects both the survival of *L. monocytogenes* population on leaves from the last irrigation, and the survival of *L. monocytogenes* in soil from the moment of sowing. The probability that the sampled parcel of lettuce is grown in spring or autumn is defined as P_{season}. Furthermore, season affects the transfer rate of LM from soil to the edible part of fresh produce due to soil splash at the last irrigation.

2) **Soil**, used in farming practices that are known to increase the prevalence of *L. monocytogenes* in soil, namely: proximity to pasture area or surface water (only for open field cultivation), application of manure within a year before harvest, and last irrigation occurring 24 or 48 hours before harvesting. The odds ratios for the above practices ($F_{pasture}$, F_{manure}, $F_{irrig24}$ and $F_{irrig48}$, respectively) are applied in the model to increase the prevalence of *L. monocytogenes* in soil, if any of the practices are carried out (OR estimate in Table A 3.3). In addition, the decay of *L. monocytogenes* in soil from the moment of sowing until the last irrigation (where soil splash can occur) is quantified.

3) **Irrigation**, through the contamination of both soil and leaves, if the irrigation water from the last irrigation event is contaminated with *L. monocytogenes* (P_{irrig}). Total contamination in lettuce just after the last irrigation is the sum of the number of *L. monocytogenes* on the leaves through direct contamination of irrigation water (zero if irrigation water is not contaminated) and the number transferred to lettuce from soil from the last irrigation (zero if soil is not contaminated).

Route of direct contamination of LM by irrigation water

For the route of direct contamination of *L. monocytogenes* by irrigation water falling onto the leaves at the moment of irrigation (t=0), the concentration of *L. monocytogenes* on leaves right after irrigation ($N_{leaves\_irrig\_0}$) is calculated from the concentration of *L. monocytogenes* in irrigation water (C_{irrig}) and the volume of water remaining on lettuce right after irrigation (W_{leaves}).

Route of indirect contamination of LM by contaminated soil

For the soil route – assuming soil is contaminated – the concentration of *L. monocytogenes* in soil just before irrigation is calculated using a decay function of *L. monocytogenes* in soil, evaluated at the initial concentration of *L. monocytogenes* in soil at the moment of sowing in the field (C_{soil}) and the time elapsed between sowing and the last irrigation (t_{cult}-t_{irrig}). Since this function is different for the two seasons, the outputs (numbers of LM in soil just before irrigation) can be computed for autumn ($N_{soil\_bef\_irrig\_f}$) and spring ($N_{soil\_bef\_irrig\_s}$).

Since irrigation water can be contaminated with *L. monocytogenes*, at a probability of P_{irrig}, the irrigation water can contaminate or increase the contamination in soil. Such an increase in contamination ($Delta_{soil}$) is calculated from the load of *L. monocytogenes* in the irrigation water (C_{irrig}) and the amount of irrigation water absorbed per gram of soil (W_{soil}). If the irrigation water is not contaminated with *L. monocytogenes*, $Delta_{soil}$ is zero. If contaminated, $Delta_{soil}$ is added to the previously computed *L. monocytogenes* load in soil just before irrigation, keeping the distinctions by season. Thus, the numbers of *L. monocytogenes* in soil just after irrigation with contaminated water will be $N_{soil\_aft\_irrig\_f}$ for the autumn season and $N_{soil\_aft\_irrig\_s}$ for the spring season.

At the moment of irrigation, there is some transfer of *L. monocytogenes* from soil to the edible parts of the lettuce, which differ between seasons (TR_f, TR_s). Applying these transfer rates, the contamination on lettuce from soil splash can also be computed for any season on a conventional farm ($N_{leaves\_splash\_f}$ and $N_{leaves\_splash\_s}$). At this point (i.e. just after last irrigation), the total concentration of *L. monocytogenes* via soil and/or via irrigation water on lettuce leaves are computed, separately for autumn (C_{leaves_f}) and spring (C_{leaves_s}). Cells deposited on lettuce die off in time. By evaluating the decay function of *L. monocytogenes* on lettuce leaves at time t_{irrig} (time between last irrigation and harvest), the concentrations of *L. monocytogenes* on lettuce leaves at harvest in autumn ($C_{leaves\_irrig\_f}$) and in spring ($C_{leaves\_irrig\_s}$) are determined.

The prevalence of *L. monocytogenes* in soil is amenable to being increased by the odds ratios $F_{pasture}$, F_{manure}, $F_{irrig24}$ and/or $F_{irrig48}$. For a small parcel of lettuce to be

negative for *L. monocytogenes*, both soil and irrigation water have to be negative for *L. monocytogenes*, although allowances must be made for those parcels which, despite soil or irrigation water being contaminated with *L. monocytogenes*, will end up producing lettuce without *L. monocytogenes* due to the microbial decay processes. In conventional or open field production, the prevalence and concentration of *L. monocytogenes* on lettuce leaves at harvest are P_{harv_conv} and C_{leaves_conv} (Table A 4.7), whereas based on a similar model, the prevalence and concentration of *L. monocytogenes* on harvested lettuce grown in protected cultivation are P_{harv_prot} and C_{leaves_prot} (Table A 4.8).

Hydroponic cultivation (Table A 4.9) may produce *L. monocytogenes*-positive lettuce if the nutrient solution contains *L. monocytogenes* (at the probability $P_{nutrient}$), and if internalization occurs later ($P_{internalize}$). The probability of internalization, $P_{internalize}$, is a logistic function of the concentration of *L. monocytogenes* in the nutrient solution ($C_{nutrient}$). The prevalence of *L. monocytogenes* in a small parcel of hydroponic lettuce (P_{harv_hp}) is therefore the multiplication of the probabilities $P_{nutrient}$ and $P_{internalize}$. If positive for *L. monocytogenes*, the hydroponic lettuce will have a concentration of the pathogen of C_{leaves_hp}.

Lettuces originating from any of the production systems are set to have an overall prevalence and concentration of *L. monocytoges* at harvest of P_{harv} and C_{harv}, respectively, taking into account the proportion of lettuces produced in conventional, protected and hydroponic cultivation (P_{prod}). In the harvest module (Table A 4.10), the outer leaves of lettuces that are dirty or damaged are removed, which causes a fraction of *L. monocytogenes* population on lettuce to be eliminated (R_{outer}), thereby producing lettuces with a lower concentration of the pathogen (C_{clean}). However, during the handling of lettuces post-harvest and their placing in boxes or crates, *L. monocytogenes* can enter through hands/gloves or surfaces of boxes/crates at a probability P_{cch}. The number of cells transferred to a lettuce leaf (N_{trans}) will increase both the concentration of *L. monocytogenes* on lettuce post-harvest to C_{post_harv} and the prevalence to P_{post_harv}.

At the packinghouse, lettuces that are destined for formal retail as whole lettuces and for further processing may be chilled (at temperature $Temp_{chill}$) and briefly stored (during t_{chill} hours) before being dispatched (Table A 4.11). The physiological state of *L. monocytogenes* in lettuce is initialized during chilling, and the module only considers the growth of this pathogen in lettuce during storage, and not the survival. It is worth mentioning that the growth parameters applied in this module pertain to lettuce (and not to pre-cut or shredded lettuce). The value of Q at time t_{chill} is retained for the sampled lettuce in order to give continuity to the growth kinetics of *L. monocytogenes* in the next module. The same approach is followed in the module of transportation of whole lettuce to formal retail,

informal retail and processors (Table A 4.12), whereby no cross-contamination is assumed, but only growth of *L. monocytogenes* in the contaminated units. In this transportation module, the main difference between products is that the transportation temperature for whole lettuce to formal retail may be chilled or ambient, for further processing is chilled, and for whole lettuce to informal markets is ambient. In the contaminated fraction of lettuces, the concentrations of *L. monocytogenes* at the end of transport to formal retail is C_{for}, at the end of transport to the processor is C_{proc}, and at the end of transport to informal markets is C_{inf}. Prevalences are not affected.

The module of processing of RTE pre-cut lettuce is presented in Table A 4.13. Lettuces undergo washing with water and could be further sanitized with chemical substances, which cause a reduction in *L. monocytogenes* until a certain concentration level of the pathogen in lettuce (C_{sanit}) is reached. *L. monocytogenes* reduction factors are available for water (R_{ww}), chlorine dioxide (R_{ClO2}), slightly acidified electrolysed water (R_{SAEW}), hydrogen peroxide (R_{H2O2}) and sodium hypochlorite (R_{SH}). During processing, it is presumed that there is a probability P_{cc} that dryers or conveyors in contact with lettuce are contaminated with *L. monocytogenes*, and that a fraction of such contamination can be transferred to lettuce ($TR_{fac\text{-}lettuce}$). A dispersion factor (b) related to the heterogeneous distribution of *L. monocytogenes* in bulk shredded lettuce is assumed in order to randomly determine the number of cells in a portion of pre-cut lettuce for the partitioning process that is carried out during packaging. The numbers of *L. monocytogenes* in the bulk (batch) of pre-cut lettuce originate from the fraction of contaminated lettuces (P_{post_harv}) having a concentration C_{sanit}. If a cross-contamination event occurs, the cells transferred from dryers or conveyors (N_{from_fac}) increase the numbers in the bulk to N_{total}. Through a beta-binomial distribution, the concentration of *L. monocytogenes* in a pack of pre-cut lettuce is calculated (C_{RTE}). The calculated proportion of packs without *L. monocytogenes* is then used to compute the prevalence of RTE pre-cut lettuce packs contaminated with the pathogen (P_{RTE}).

The cold chain module only pertains to the distribution of RTE pre-cut lettuce, comprising storage at the end of processing, transportation to retail, display to retail and transportation to home (Table A 4.14). The only process modelled in the cold chain module is growth, but using *L. monocytogenes* kinetic parameters specifically for pre-cut or shredded lettuce. The Baranyi-Roberts primary model is employed, and the physiological state of cells in pre-cut lettuce is initialized

at the end of processing. The temperature chain module (Table A 4.15), which only applies to whole lettuce sold at formal retail, includes display at retail and transportation from retail to home. Growth is modelled using *L. monocytogenes* kinetic parameters specifically for lettuce, and the value of Q(t) is carried on from the previous stage of transportation to formal retail ($Q(t_{trans\text{-}for})$).

In informal markets (Table A 4.16), when placing lettuces on the stalls, cross-contamination to lettuce can occur from crates, boxes, tables or hands; at probability P_{ccm}., *L. monocytogenes* cells ($N_{s\text{-}l}$) can be transferred to lettuces, regardless of whether they were *L. monocytogenes* free or already contaminated, in which case the contamination will increase. While lettuces are on display and exposed to ambient temperature, *L. monocytogenes* will grow in the contaminated fraction of lettuces. This growth will continue in the stage of transportation to home.

Consumer handling of lettuce is modelled in separate modules for whole lettuce (Table A 4.17) and RTE pre-cut lettuce (Table A 4.18). Whole lettuces purchased in formal retail or informal markets present an overall prevalence P_{whole} and overall concentration C_{whole} of *L. monocytogenes* when entering the home (Table A 4.17). During home storage, the growth of *L. monocytogenes* in lettuce is modelled until a concentration of C_{prep} is reached at the start of preparation. Washing the leaves with tap water reduces the concentration of *L. monocytogenes* by a factor of R_{wash}, which depends on the time of washing (t_{wash}). When cutting the lettuce, there is a probability that a chopping board (P_{chopp}) is contaminated with *L. monocytogenes*, and that a transfer of a fraction of it ($TR_{chopp\text{-}lettuce}$) can therefore occur to the serving being prepared (SS_{whole}). If cross-contamination occurs while preparing, and lettuce leaves after washing are still contaminated with *L. monocytogenes*, the serving size will receive contamination from both sources. At the point of consumption, the prevalence and concentration in servings of lettuce (from whole lettuce) are estimated at $P_{whole\text{-}serv}$ and $C_{whole\text{-}serv}$, respectively.

Consumer handling of RTE pre-cut lettuce also involves home cold storage before consumption, where the growth of *L. monocytogenes* in contaminated packs is computed (Table A 4.18). There is a probability $P_{RTE\text{-}wash}$ that the consumer washes the pre-cut lettuce before consumption. If that is the case, the extent of reduction in the pathogen ($R_{RTE\text{-}wash}$) depends on the time of washing with tap water ($t_{RTE\text{-}wash}$). At the point of consumption, the prevalence and concentration in servings of RTE pre-cut lettuce are estimated at $P_{RTE\text{-}serv}$ and $C_{RTE\text{-}serv}$, respectively.

Table A 4.7: Variables related to the primary production module of lettuce in conventional cultivation

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Cultivation	P_{season}	Probability that lettuce is grown in spring or winter	-		Assumption to be made
	P_{soil}	Probability that soil at sowing is contaminated with LM	-		Table A 4.2
	t_{cult}	Cultivation period of lettuce	Week	-	Available data
Route of LM: Irrigation water directly on leaves	P_{irrig}	Probability that water from last irrigation before harvest is contaminated with LM	-	-	Table A 4.1
	C_{irrig}	Concentration of LM in irrigation water (last irrigation)	CFU/ml	-	<0.03 to 11 MPN/L (Sharma *et al.*, 2020; Acheamfour *et al.*, 2021) 1196 CFU/100 mL ranging from 0 - 5667 CFU/100 mL (Iwu *et al.*, 2022).
	t_{irrig}	Time between last irrigation and harvest	day	-	EKE
	W_{leaves}	Volume of water remaining on lettuce leaves right after irrigation	ml/g leaves	-	Allende *et al.* (2017)
	B_{irrig+}	Status of last irrigation water contaminated with LM	-	Bernoulli (P_{irrig})	-
	B_{soil+}	Status of soil contaminated with LM		Bernoulli (P_{soil})	-
	B_{fall}	Cultivation in autumn		Bernoulli (P_{season})	-
	B_{spring}	Cultivation in spring		1-Bernoulli (P_{season})	
	$N_{leaves\_irrig\_0}$	Numbers of LM on lettuce leaves after direct contact with irrigation water	CFU/g leaves	IF (B_{irrig+}=1) W_{leaves} x C_{irrig}	-

Route of LM: From soil to leaves (1) in soil until irrigation	$f_{soil}(t, C_0, S)$	Decay function: LM concentration in soil as a function of time t and season S	Log CFU/g soil	-	Data from Oliveira *et al.* (2011) in Table A 4.1.
	C_{soil}	Concentration of LM in fertilized soil at sowing	Log CFU/g soil		Pre-calculation can be done from LM in manure. Alternatively: 4 MPN/g (95% CI: <1.0 – 28 MPN/g) (Dowe *et al.* 1997) 1964 CFU/g (range: 133 - 6233 CFU/g (Iwu *et al.*, 2022)
	$N_{soil\_bef\_irrig\_f}$	Numbers of LM in soil in autumn just before irrigation	CFU/g soil	IF (B_{fall}=1) IF (B_{soil+}=1) $10^{fsoil\ (tcult\text{-}tirrig,\ Csoil,\ autumn)}$	-
	$N_{soil\_bef\_irrig\_s}$	Numbers of LM in soil in spring just before irrigation	CFU/g soil	IF (B_{spring}=1) IF (B_{soil+}=1) $10^{fsoil\ (tcult\text{-}tirrig,\ Csoil,\ spring)}$	-
(2) Irrigation water to soil	W_{soil}	Volume of water remaining in soil after last irrigation	ml/g soil	-	EKE
	$Delta_{soil}$	Increase in LM in soil after last irrigation event	CFU	IF (B_{irrig+}=1) C_{soil} x W_{soil} ELSE 0	-
	$N_{soil\_aft\_irrig\_f}$	Numbers of LM in soil in autumn just after irrigation	CFU/g	IF (B_{fall}=1) IF (B_{soil+}=1) $N_{soil\_bef\_irrig\_f}$ + $Delta_{soil}$	-
	$N_{soil\_aft\_irrig\_s}$	Numbers of LM in soil in spring just after irrigation	CFU/g	IF (B_{spring}=1 IF (B_{soil+}=1) $N_{soil\_bef\_irrig\_s}$ + $Delta_{soil}$	-

(3) Soil splash to leaves	TR_f	Transfer rate from soil to leaves due to soil splash after last irrigation in autumn	Log (CFU on leaves/CFU in soil)	-	Table A 4.2
	TR_s	Transfer rate from soil to leaves due to soil splash after last irrigation in spring	Log (CFU on leaves/CFU in soil)	-	Table A 4.2

	$N_{leaves\_splash\_f}$	Numbers of LM on lettuce leaves after irrigation following splash in autumn	CFU/g leaves	IF (B_{fall}=1)
				IF (B_{soil+}=1)
				10^{TRf} x $N_{soil\_aft\_irrig\_f}$
					-

	$N_{leaves\_splash\_s}$	Numbers of LM on lettuce leaves after irrigation following splash in spring	CFU/g leaves	IF (B_{spring}=1)
				IF (B_{soil+}=1)
				10^{TRs} x $N_{soil\_aft\_irrig\_s}$
					-

Total LM on leaves	C_{leaves_f}	Total concentration on leaves (from irrigation and soil) after last irrigation in autumn	Log CFU/g leaves	IF (B_{fall}=1)
				IF (B_{soil+}=1) & (B_{irrig+}=1)
				Log($N_{leaves\_splash\_f}$ + $N_{leaves\_irrig\_0}$)
				IF (B_{soil+}=1) & (B_{irrig+}=0)
				Log($N_{leaves\_splash\_f}$ + 0)
				IF (B_{soil+}=0) & (B_{irrig+}=1)
				Log(0 + $N_{leaves\_irrig\_0}$)
					-

	C_{leaves_s}	Total concentration on leaves (from irrigation and soil) after last irrigation in spring	Log CFU/g leaves	IF (B_{spring}=1)
				IF (B_{soil+}=1) & (B_{irrig+}=1)
				Log($N_{leaves\_splash\_s}$ + $N_{leaves\_irrig\_0}$)
				IF (B_{soil+}=1) & (B_{irrig+}=0)
				Log($N_{leaves\_splash\_s}$ + 0)
				IF (B_{soil+}=0) & (B_{irrig+}=1)
				Log(0 + $N_{leaves\_irrig\_0}$)
					-

Decline in LM from irrigation to harvest	$f_{leaves}(t, C_0, S)$	Decay function: LM concentration on leaves as a function of time t and season S	Log CFU/g leaves	-	Data from Oliveira *et al.* (2011) in Table A 4.3. Combine results
	$C_{leaves\_irrig\_f}$	Concentration of LM on lettuce leaves at harvesting in autumn	Log CFU/g leaves	IF (B_{fall}=1) $f_{leaves}(t_{irrig}, C_{leaves\_f}$, autumn)	-
	$C_{leaves\_irrig\_s}$	Concentration of LM on lettuce leaves at harvesting in spring	Log CFU/g leaves	IF (B_{spring}=1) $f_{leaves}(t_{irrig}, C_{leaves\_s}$, spring)	-
	C_{leaves_conv}	Concentration of LM on lettuce leaves at harvesting in any season in conventional cultivation	Log CFU/g leaves	IF (B_{fall}=1) $C_{leaves\_irrig\_f}$ IF (B_{spring}=1) $C_{leaves\_irrig\_s}$	-
Prevalences	$F_{irrig24}$	Impact of last irrigation occurring 24 h before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	$F_{irrig48}$	Impact of last irrigation occurring 48 h before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	F_{manure}	Impact of manure applied within a year before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	$F_{pasture}$	Impact of proximity to pasture area or surface water	-	-	OR estimate (Table A 3.3)
	$B_{irrig24+}$	Field irrigated 24 h before harvest	-	Bernoulli ($P_{irrig24}$)	Assumption of frequency to be made
	$B_{irrig48+}$	Field irrigated between 24 and 48 h before harvest	-	Bernoulli ($P_{irrig48}$)	Assumption of frequency to be made

Prevalences	$B_{manure+}$	Field where manure was applied within one year of the cultivation of leafy greens	-	Bernoulli (P_{manure})	Assumption of frequency to be made
	$B_{pasture+}$	Field close to pasture area or surface water	-	Bernoulli ($P_{pasture}$)	Assumption of frequency to be made
	$P_{pasture}$	Prevalence of LM in soil after correction for proximity to pasture of surface water	-	IF ($B_{pasture+}$=1) ($F_{pasture}$ x P_{soil})/(1-P_{soil}+($F_{pasture}$ x P_{soil})) IF ($B_{pasture+}$=0) P_{soil}	-
	P_{soil_manure}	Prevalence of LM in soil after correction for application of manure	-	IF ($B_{manure+}$=1) (F_{manure} x $P_{pasture}$)/(1-P_{soil}+(F_{manure} x $P_{pasture}$) IF ($B_{manure+}$=0) $P_{pasture}$	-
	P_{soil_irrig}	Prevalence of LM in soil after correction for irrigation close to harvest	-	IF ($B_{irrig24+=}$1) ($F_{irrig24}$ x P_{soil_manure})/(1-P_{soil_manure}+($F_{irrig24}$ x P_{soil_manure}) IF ($B_{irrig48+=}$1) ($F_{irrig48}$ x P_{soil_manure})/(1-P_{soil_manure}+($F_{irrig48}$ x P_{soil_manure}) ELSE P_{soil_manure}	-
	P_{harv_conv}	Prevalence of lettuce with LM on leaves at the moment of harvest in conventional production	-	1 – (1 – P_{irrig}) x (1 - P_{soil_irrig})	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.8: Variables related to the primary production module for lettuce in protected cultivation

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Cultivation	P_{season}	Probability that lettuce is grown in spring or winter	-		Assumption to be made
	P_{soil}	Probability that soil at sowing is contaminated with LM	-		Table A 3.2
	t_{cult}	Cultivation period of lettuce	Week	-	Available data
Route of LM: Irrigation water direct on leaves	P_{irrig}	Probability that water from last irrigation before harvest is contaminated with LM	-	-	Consider lower prevalence than in conventional agriculture Table A 3.1
	C_{irrig}	Concentration of LM in irrigation water (last irrigation)	CFU/ml	-	Consider lower counts than in conventional agriculture <0.03 to 11 MPN/L (Sharma *et al.*, 2020; Acheamfour *et al.*, 2021) 1196 CFU/100 mL ranging from 0 - 5667 CFU/100 mL (Iwu *et al.*, 2022).
	t_{irrig}	Time between last irrigation and harvest	day	-	EKE
	W_{leaves}	Volume of water remaining on lettuce leaves right after irrigation	ml/g leaves	-	Consider lower volume of water than in conventional production. Allende *et al.* (2017)

Route of LM: Irrigation water directly on leaves	B_{irrig+}	Status of last irrigation water contaminated with LM	-	Bernoulli (P_{irrig})	-
	B_{soil+}	Status of soil contaminated with LM		Bernoulli (P_{soil})	-
	B_{fall}	Cultivation in autumn		Bernoulli (P_{season})	-
	B_{spring}	Cultivation in spring		1-Bernoulli (P_{season})	
	$N_{leaves\_irrig\_0}$	Numbers of LM on lettuce leaves after direct contact with irrigation water	CFU/g leaves	IF (B_{irrig+}=1) W_{leaves} x C_{irrig}	-
Route of LM: From soil to leaves (1) in soil until irrigation	f_{soil} (t, C_0, S)	Decay function: LM concentration in soil as a function of time t and season S	Log CFU/g soil	-	Data from Oliveira *et al.* (2011) in Table A 4.1
	C_{soil}	Concentration of LM in fertilized soil at sowing	Log CFU/g soil		Pre-calculation can be done from LM in manure. Alternatively: 4 MPN/g (95% CI: <1.0 – 28 MPN/g) (Dowe *et al.* 1997) 1964 CFU/g (range: 133 - 6233 CFU/g (Iwu *et al.*, 2022)
	$N_{soil\_bef\_irrig\_f}$	Numbers of LM in soil in autumn just before irrigation	CFU/g soil	IF (B_{fall}=1) IF (B_{soil+}=1) $10^{fsoil\ (tcult\text{-}tirrig,\ Csoil,\ Autumn)}$	-
	$N_{soil\_bef\_irrig\_s}$	Numbers of LM in soil in spring just before irrigation	CFU/g soil	IF (B_{spring}=1) IF (B_{soil+}=1) $10^{fsoil\ (tcult\text{-}tirrig,\ Csoil,\ Spring)}$	-

(2) Irrigation water to soil	W_{soil}	Volume of water remaining in soil after last irrigation	ml/g soil	-	EKE

	$Delta_{soil}$	Increase in LM in soil after last irrigation event	CFU	IF (B_{irrig+}=1)
				C_{soil} x W_{soil}
				ELSE
				0
					-

	$N_{soil\_aft\_irrig\_f}$	Numbers of LM in soil in autumn just after irrigation	Log CFU/g	IF (B_{fall}=1)
				IF (B_{soil+}=1)
				$N_{soil\_bef\_irrig\_f}$ + $Delta_{soil}$
					-

	$N_{soil\_aft\_irrig\_s}$	Numbers of LM in soil in spring just after irrigation	Log CFU/g	IF (B_{spring}=1
				IF (B_{soil+}=1)
				$N_{soil\_bef\_irrig\_s}$ + $Delta_{soil}$
					-

(3) Soil splash to leaves	TR_f	Transfer rate from soil to leaves due to soil splash after last irrigation in autumn	Log (CFU on leaves/CFU in soil)	-	Table A 4.2
	TR_s	Transfer rate from soil to leaves due to soil splash after last irrigation in spring	Log (CFU on leaves/CFU in soil)	-	Table A 4.2

	$N_{leaves\_splash\_f}$	Numbers of LM on lettuce leaves after irrigation following splash, in autumn	CFU/g leaves	IF (B_{fall}=1)
				IF (B_{soil+}=1)
				10^{TRf} x $N_{soil\_aft\_irrig\_f}$
					-

	$N_{leaves\_splash\_s}$	Numbers of LM on lettuce leaves after irrigation following splash in spring	CFU/g leaves	IF (B_{spring}=1)
				IF (B_{soil+}=1)
				10^{TRs} x $N_{soil\_aft\_irrig\_s}$
					-

Total LM on leaves	C_{leaves_f}	Total concentration on leaves (from irrigation and soil) after last irrigation in autumn	Log CFU/g leaves	IF (B_{fall}=1)
				IF (B_{soil+}=1) & (B_{irrig+}=1)
				Log($N_{leaves\_splash\_f}$ + $N_{leaves\_irrig\_0}$)
				IF (B_{soil+}=1) & (B_{irrig+}=0)
				Log($N_{leaves\_splash\_f}$ + 0)
				IF (B_{soil+}=0) & (B_{irrig+}=1)
				Log(0 + $N_{leaves\_irrig\_0}$)
					-

	C_{leaves_s}	Total concentration on leaves (from irrigation and soil) after last irrigation in spring	Log CFU/g leaves	IF (B_{spring}=1)
				IF (B_{soil+}=1) & (B_{irrig+}=1)
				Log($N_{leaves\_splash\_s}$ + $N_{leaves\_irrig\_0}$)
				IF (B_{soil+}=1) & (B_{irrig+}=0)
				Log($N_{leaves\_splash\_s}$ + 0)
				IF (B_{soil+}=0) & (B_{irrig+}=1)
				Log(0 + $N_{leaves\_irrig\_0}$)
					-

Decline in LM from irrigation to harvest	f_{leaves}(t, C_0, S)	Decay function: LM concentration on leaves as a function of time t and season S	Log CFU/g leaves	-	Data from Oliveira *et al.* (2011) in Table A 4.3. Combine results
	$C_{leaves\_irrig\_f}$	Concentration of LM on lettuce leaves at harvesting in autumn	Log CFU/g leaves	IF (B_{fall}=1) f_{leaves}(t_{irrig}, C_{leaves_f}, Autumn)	-

	$C_{leaves\_irrig\_s}$	Concentration of LM on lettuce leaves at harvesting in spring	Log CFU/g leaves	IF (B_{spring}=1) f_{leaves} (t_{irrig}, C_{leaves_s}, Spring)	-
	C_{leaves_prot}	Concentration of LM on lettuce leaves at harvesting in any season in protected cultivation	Log CFU/g leaves	IF (B_{fall}=1) $C_{leaves\_irrig\_f}$ IF (B_{spring}=1) $C_{leaves\_irrig\_s}$	-
Prevalences	F_{manure}	Impact of manure applied within a year before harvest on the prevalence of LM in soil	-	-	OR estimate (Table A 3.3)
	$B_{manure+}$	Field where manure was applied within one year of the cultivation of leafy greens	-	Bernoulli (P_{manure})	Assumption of frequency to be made
	P_{soil_manure}	Prevalence of LM in soil after correction for application of manure	-	IF ($B_{manure+}$=1) (F_{manure} x P_{soil})/(1-P_{soil}+(F_{manure} x P_{soil}) IF ($B_{manure+}$=0) P_{soil}	-
	P_{harv_prot}	Prevalence of lettuce with LM on lettuce leaves at the moment of harvest in protected production	-	1 – (1 – P_{irrig}) x (1 - P_{soil_manure})	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.9: Variables related to the primary production module of lettuce in hydroponic cultivation

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Cultivation	$P_{nutrient}$	Probability that the nutrient solution is contaminated with LM	-		Assumption to be made
	$C_{nutrient}$	Concentration of LM in nutrient solution	CFU/ml		Limited data
	$C_{internalized}$	Concentration of LM in hydroponic lettuce achieved by internalization	CFU/g leaves		<1.7 log CFU/leaf (Koseki *et al.*, 2011)
	$B_{nutrient+}$	Hydroponic solution feeding a batch contaminated with LM	-	Bernoulli ($P_{nutrient}$)	-
	$P_{internalize}$	Probability of internalization of LM from a contaminated nutrient solution		IF ($B_{nutrient+}$ = 1) logit(P_{int}) = - 4.793 + 0.759 $C_{nutrient}$	Equation (4.2)
	P_{harv_hp}	Prevalence of lettuce with LM on lettuce leaves at harvesting in hydroponic production	-	$P_{nutrient}$ x $P_{internalize}$	
	C_{leaves_hp}	Concentration of LM on lettuce leaves at harvesting in hydroponic production	Log CFU/g	Log ($C_{internalized}$/10)	Assuming 1 outer leaf of lettuce weighs 10 g on average

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.10: Variables related to the harvest module of lettuce (all types of cultivation)

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Population of harvested lettuces	P_{prod}	Probability that a lettuce originates from conventional (conv), protected (prot) or hydroponic cultivation (hp). All must add up to one.	-	Discrete (conv, prot, hp)	Assumption to be made
	C_{harv}	Concentration of LM in lettuce at harvest in any cultivation system	Log CFU/g	IF (Pprod=conv) C_{leaves_conv} IF (Pprod=prot) C_{leaves_prot} IF (Pprod=hp) C_{leaves_hp}	
	P_{harv}	Prevalence of LM in lettuce at harvest in any cultivation system	-	P_{harv_conv} x P_{prod}(conv) + P_{harv_prot} x P_{prod}(prot) + P_{harv_hp} x P_{prod}(hp)	-
Removal of outer leaves	R_{outer}	Fraction of LM population on lettuce reduced by removal of outer leaves	-		EKE, depends on the number of leaves removed
	C_{clean}	Concentration of LM in lettuce after removal of outer leaves	Log CFU/g	Log (10^{Charv} x (1 – R_{outer}))	

Cross-contamination	P_{cch}	Probability that crates or hands/ gloves are contaminated with LM	-	0.08; 0.17	*Listeria* spp. on plastic surfaces in contact with cantaloupe (Strawn and Danyluk, 2017)
	$TR_{plas\_lettuce}$	Transfer coefficient from food-contact surface to lettuce leaves	Log (CFU on produce/ CFU on surface)	Normal (-1.42, 0.52)	Hoelzer *et al.* (2012a)
	N_{plas}	Numbers of CFU on food-contact surface (harvesting equipment, crates, hands/gloves)	CFU	-	Assumption to be made
	$B_{lettuce+}$	Status of a lettuce being contaminated with LM	-	Bernoulli (P_{harv})	
	B_{cch+}	Status of cross-contamination taking place during accommodation in crates	-	Bernoulli (P_{cch})	-
	N_{trans}	Numbers of CFU transferred to a lettuce leaf in contact	CFU	ROUND(N_{plas} x $10^{TRplas\_lettuce}$)	-
	C_{post_harv}	Concentration of LM in lettuce post-harvest	Log CFU/g	IF ($B_{lettuce+}$=0) & (B_{cch+}=1) Log (0 + N_{trans}/10) IF ($B_{lettuce+}$=1) & (B_{cch+}=1) Log ($10^{C\ clean}$ + N_{trans}/10) IF ($B_{lettuce+}$=1) & (B_{cch+}=0) Log ($10^{C\ clean}$ + 0)	
	P_{post_harv}	Prevalence of lettuce with LM on the lettuce post-harvest	-	1 – (1 – P_{harv}) x (1 – P_{cch})	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.11: Variables related to the module for chilling of lettuce destined for formal retail and for further processing

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Properties	α_0	Initial physiological state of LM in lettuce	-	0.072	Koseki and Isobe (2005)
	$\mu_{whole}=f(T)$	Growth rate of LM in whole lettuce as a function of temperature T	h^{-1}		Data for lettuce from Table A 5.6.
	MPD	Maximum population density of LM in lettuce as a function of temperature T	Ln CFU/g	MPD=0.037 T+12.434	Koseki and Isobe (2005)
Brief chill storage	$Temp_{chill}$	Chilling temperature of lettuce	°C		Between 4–7 °C (Terry *et al.*, 2011)
	t_{chill}	Brief time lettuces are chill stored before dispatch for transport	h		
	μ_{chill}	Growth rate of LM at given temperature	h^{-1}	$\mu_{chill}=f(Temp_{chill})$	Chill temperatures
	MPD_{chill}	Maximum population density of LM at $Temp_{chill}$	Ln CFU/g	$0.037Temp_{chill}+12.434$	
	C_{chill}	Concentration of LM in the contaminated fraction of chilled lettuces before transport	Log CFU/g	$Q_0=\alpha_0/(1-\alpha_0)$ $N^0=10^{C_{post\text{-}harv}}$ $\frac{dN}{dt}=\mu_{chill}\frac{Q}{1+Q}\left(1-\frac{N}{e^{MPD_{chill}}}\right)N$ $\frac{dQ}{dt}=\mu_{chill}Q$	Equation must be integrated until t_{chill}
	$Q(t_{chill})$	Value of Q at the time t_{chill} used to initialize LM growth for next module for lettuce destined for formal retail and for further processing	-		

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.12: Variables related to the module of transportation of whole lettuce to processor, formal and informal retail

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Transport of whole lettuce to be sold in formal retail (b)	$Temp_{for}$	Temperature of transport from farm/ packinghouse to retail	°C		Consider chilled and ambient temperatures
	T_{for}	Duration of transport from farm/ packinghouse to retail	h		
		Growth rate of LM at given temperature	h^{-1}	$\mu_{PH} = f(Temp_{PH})$	-
	MPD_{for}	Maximum population density of LM at $Temp_{PH}$	Ln CFU/g	$0.037\ Temp_{PH} + 12.434$	
	C_{for}	Concentration of LM in the contaminated fraction of lettuces at the end of transport to formal retail	Log CFU/g	$Q_0 = Q(t_{chill})$ $N_0 = 10^{C_{chill}}$ $\frac{dN}{dt} = \mu_{for}\frac{Q}{1+Q}\left(1 - \frac{N}{e^{MPD_{for}}}\right) N$ $\frac{dQ}{dt} = \mu_{for} Q$	Equation must be integrated until t_{for}
	$Q(t_{trans\text{-}for})$	Value of Q at the end of transport (time t_{for}) used to initialize next module of formal retail			
Transport to processor for lettuce to be prepared as RTE (a)	$Temp_{Proc}$	Temperature of transport from farm to RTE processor	°C		Cold transportation is likely
	t_{Proc}	Duration of transport from farm to RTE processor	h		
	μ_{Proc}	Growth rate of LM at a given temperature	h^{-1}	$\mu_{Proc} = f(Temp_{Proc})$	

	MPD_{Proc}	Maximum population density of LM at $Temp_{Proc}$	Ln CFU/g	$0.037\ Temp_{Proc} + 12.434$	
	C_{Proc}	Concentration of LM in the contaminated fraction of lettuces at the end of transport	Log CFU/g	$Q_0 = Q\ (t_{chill})$ $N_0 = 10^{C_{chill}}$ $\frac{dN}{dt} = \mu_{Proc} \frac{Q}{1+Q} (1 - \frac{N}{e^{MPD_{Proc}}}) N$ $\frac{dQ}{dt} = \mu_{Proc} Q$	Equation must be integrated until t_{Proc}
	$Q(t_{trans\text{-}Proc})$	Value of Q at the end of transport (time t_{Proc}) used to initialize LM growth for next module for lettuce destined for processing			
Transport of lettuce to be sold at informal retail (c)	$Temp_{inf}$	Temperature of transport from farm to informal retail	°C		Consider ambient temperature only
	t_{inf}	Duration of transport from farm to informal retail	h		
		Growth rate of LM at a given $Temp_{inf}$	h^{-1}	$\mu_{inf} = f\ (Temp_{inf})$	
	MPD_{inf}	Maximum population density of LM at $Temp_{inf}$	Ln CFU/g	$0.037\ Temp_{inf} + 12.434$	
	C_{inf}	Concentration of LM in the contaminated fraction of lettuces at the end of transport	Log CFU/g	$Q_0 = \alpha_0/(1-\alpha_0)$ $N^0 = 10^{C_{chill}}$ $\frac{dN}{dt} = \mu_{inf} \frac{Q}{1+Q} (1 - \frac{N}{e^{MPD_{inf}}}) N$ $\frac{dQ}{dt} = \mu_{inf} Q$	Equation must be integrated until t_{inf}
	$Q(t_{trans\text{-}inf})$	Value of Q at the end of transport (time t_{inf}) used to initialize LM growth for next module for lettuce destined for informal retail			

Table A 4.13: Variables related to the module for processing of RTE pre-cut lettuce

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Batch	Lettuces	Number of lettuces being processed in a batch	-	-	Assumption to be made
	$W_{lettuce}$	Weight of a lettuce	g	-	Assumption to be made
	W_{pack}	Weight of a pack of pre-cut lettuce	g	-	Assumption to be made
	Packs	Number of packs produced in a batch	-	Lettuces x $W_{lettuce}$ / W_{pack}	-
Washing	R_{ww}	Reduction in LM due to washing with water	Log CFU/g		Reduction factors for water from Table A 4.4
	C_{wash}	Concentration of LM in lettuce after washing	Log CFU/g	C_{PH} - R_{ww}	
Sanitizing	P_{sanit}	Probability that a sampled batch sanitizes lettuce with ClO2, SAEW, H2O2, SH or does not sanitize at all	-		EKE
	B_{sanit}	Status of the batch being sanitized with sanitizer X	-	Discrete(P_{sanit})	-
	R_{ClO2}	Reduction in LM due to sanitizing with ClO2	Log CFU/g		Reduction factors from Table A 4.4
	R_{SAEW}	Reduction in LM due to sanitizing with SAEW	Log CFU/g		Reduction factors from Table A 4.4
	R_{H2O2}	Reduction in LM due to sanitizing with H2O2	Log CFU/g		Reduction factors from Table A 4.4

	R_{SH}	Reduction in LM due to sanitizing with SH	Log CFU/g		Reduction factors from Table A 4.4
	C_{sanit}	Concentration of LM in lettuce after sanitization	Log CFU/g	IF (B_{sanit} = ClO2) $C_{wash} - R_{ClO2}$ IF (B_{sanit} = SAEW) $C_{wash} - R_{H2O2}$ IF (B_{sanit} = H2O2) $C_{wash} - R_{SH}$ IF (B_{sanit} = SH) $C_{wash} - R_{SH}$ IF (B_{sanit} = None) C_{wash}	
Cross-contamination and partitioning	P_{cc}	Probability that dryers or conveyors in contact with lettuce are contaminated with LM	-	Beta()	45 positives out of 474 environmental elements sampled in RTE produce factories in Ireland (Leong *et al.*, 2017)
	$TR_{fac-lettuce}$	Cross-contamination transfer coefficient from dryer or conveyor surface to lettuce	Log (CFU in lettuce / CFU on surface)	Normal (-1.42, 0.52)	Hoelzer *et al.* (2012a)
	N_{fac}	Numbers of LM on dryers or conveyors in contact with shredded lettuce ready to be transferred	CFU		Assumption to be made
	b	Dispersion factor related to the heterogeneous distribution of LM in bulk shredded lettuce	-	-	Assumption to be 1 to represent moderate clustering

B_{cc+}	Status of dryers or conveyors in contact with lettuce being contaminated with LM	-	Bernoulli (P_{cc})	
$N_{lettuce+}$	Number of lettuces contaminated with LM in a batch	-	$N_{lettuce+}$ = Binomial (Lettuces, P_{post_harv})	
N_{bulk}	Numbers of LM in the mass of shredded lettuce in a batch	CFU	N_{bulk} = Poisson ($10^{Csanit} \times N_{lettuce+} \times W_{lettuce}$)	

N_{from_fac}	Numbers of LM transferred to the bulk of shredded lettuce from dryers or conveyors in a batch	CFU	IF (B_{cc+}=1)
			$N_{from\_fac} = 10^{TRfac\text{-}lettuce} \times N_{fac}$
			$N_{total} = N_{bulk} + N_{from\_fac}$
			ELSE
			$N_{total} = N_{bulk}$	

N_{total}	Total numbers of LM in the bulk of shredded lettuce in a batch	CFU	N_{pack} = Binomial (N_{total}, Beta (b, b x Packs – 1))	
N_{pack}	Total number of LM cells distributed in a pack of pre-cut lettuce	CFU	Pi_{pack} = (Packs – 1)/(N_{total} + Packs – 1)	
Pi_{pack}	Probability that a pack of pre-cut lettuce is LM-free	-		
P_{RTE}	Prevalence of RTE pre-cut lettuce packs contaminated with LM	-	1-Pi_{pack}	-
C_{RTE}	Concentration of LM in RTE diced cantaloupe packs	Log CFU/g	Log (N_{pack} / W_{pack})	-

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.14: Variables related to the cold chain module for RTE pre-cut lettuce from end of processing until transportation to home

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Functions	α_0	Initial physiological state of LM in lettuce	-	0.072	Koseki and Isobe (2005) for lettuce, will be assumed for MAP lettuce
	$\mu_{MAP}=f(T)$	Growth rate of LM in MAP lettuce as a function of temperature T	h^{-1}		Data for MAP lettuce from Table A 4.6.
	MPD	Maximum population density of LM in lettuce as a function of temperature T	Ln CFU/g	MPD=0.037 T+12.434	Koseki and Isobe (2005) for lettuce, will be assumed for MAP lettuce
End processing	$Temp_{end}$	Storage temperature of RTE pre-cut lettuce packs at the end of processing	°C		
	t_{end}	Time that RTE pre-cut lettuce packs are cold-stored at the end of processing	h		
Transport to retail	$Temp_{t-r}$	Temperature of RTE pre-cut lettuce packs from end processing to retail	°C	Pert (3.0, 5.0, 10.3)	
	t_{t-r}	Transportation time	h	Pert (2, 3, 10)	

Retail	$Temp_{ret}$	Temperature of RTE pre-cut lettuce packs kept at retail	°C	Pert (3.5, 5.6, 9.8)	
	t_{ret}	Time that RTE pre-cut lettuce packs are kept at retail until purchase	h		
Transportation to home	t_{r-h}	Transportation time of RTE pre-cut lettuce from retail to home	h	Normal (43, 19)/60	Nauta *et al.* (2003)
	ΔT_{r-h}	Mean change in temperature during transport from retail to home	°C	$-1.318t_{r-h}^2 + 5.870t_{r-h}$	Audits International (2000)
	$Temp_{r-h}$	Temperature during transport of RTE pre-cut lettuce from retail to home	°C	$Temp_{ret} + \Delta T_{r-h}$	
Growth during cold chain	C_{end}	Concentration of LM in RTE pre-cut lettuce at the end of cold storage in the processing facility	Log CFU/g	$Q_0 = \alpha_0/(1-\alpha_0)$ $N_0 = 10^{C_{RTE}}$ $\mu_{end} = f(Temp_{end})$ $MPD_{end} = 0.037\,Temp_{end} + 12.434$ $\frac{dN}{dt} = \mu_{end}\frac{Q}{1+Q}\left(1 - \frac{N}{e^{MPD_{end}}}\right)N$ $\frac{dQ}{dt} = \mu_{end}Q$	Integrate Baranyi-Roberts model until t_{end}

$C_{t\text{-}r}$	Concentration of LM in RTE pre-cut lettuce when arriving at retail	Log CFU/g	$Q_0 = Q(t_{end})$ $N_0 = 10^{C_{end}}$ $\mu_{t\text{-}r} = f(Temp_{t\text{-}r})$ $MPD_{t\text{-}r} = 0.037\ Temp_{t\text{-}r} + 12.434$ $\frac{dN}{dt} = \mu_{t\text{-}r}\frac{Q}{1+Q}(1 - \frac{N}{e^{MPD_{t\text{-}r}}})N$ $\frac{dQ}{dt} = \mu_{t\text{-}r}Q$	Integrate Baranyi-Roberts model until $t_{t\text{-}r}$
C_{ret}	Concentration of LM in RTE pre-cut lettuce at the end of retail	Log CFU/g	$Q_0 = Q(t_{t\text{-}r})$ $N_0 = 10^{C_{t\text{-}r}}$ $\mu_{ret} = f(Temp_{ret})$ $MPD_{ret} = 0.037\ Temp_{ret} + 12.434$ $\frac{dN}{dt} = \mu_{ret}\frac{Q}{1+Q}(1 - \frac{N}{e^{MPD_{ret}}})N$ $\frac{dQ}{dt} = \mu_{ret}Q$	Integrate Baranyi-Roberts model until t_{ret}
$C_{r\text{-}h}$	Concentration of LM in RTE pre-cut lettuce at the end of transportation to home (when arriving at home)	Log CFU/g	$Q_0 = Q(t_{ret})$ $N_0 = 10^{C_{ret}}$ $\mu_{r\text{-}h} = f(Temp_{r\text{-}h})$ $MPD_{r\text{-}h} = 0.037\ Temp_{r\text{-}h} + 12.434$ $\frac{dN}{dt} = \mu_{r\text{-}h}\frac{Q}{1+Q}(1 - \frac{N}{e^{MPD_{r\text{-}h}}})N$ $\frac{dQ}{dt} = \mu_{r\text{-}h}Q$	Integrate Baranyi-Roberts model until $t_{r\text{-}h}$

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.15: Variables related to the module of temperature chain of whole lettuce sold at formal retail

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Whole lettuce at retail	$Temp_{ret}$	Temperature of whole lettuce at retail	°C		Consider ambient and cold temperature
	t_{ret}	Time that whole lettuces are kept at retail until purchase	h		
Transportation from retail to home	$t_{r\text{-}h}$	Transportation time of whole lettuce from retail to home	h	Normal (43, 19)/60	Nauta *et al.* (2003)
		Mean change in temperature during transport from retail to home	°C	$-1.318t_{r\text{-}h}^2+5.870t_{r\text{-}h}$	Audits International (2000)
	$Temp_{r\text{-}h}$	Temperature during transport of lettuce from retail to home	°C	$Temp_{ret}+\Delta T_{r\text{-}h}$	
Growth	C_{ret}	Concentration of LM in whole lettuce at the end of retail	Log CFU/g	$Q_0 = Q(t_{trans\text{-}for})$ $N_0=10^{C_{for}}$ $\mu_{ret} = f(Temp_{ret})$ $MPD_{ret} = 0.037\,Temp_{ret} + 12.434$ $\frac{dN}{dt} = \mu_{ret}\frac{Q}{1+Q}\left(1-\frac{N}{e^{MPD_{ret}}}\right)N$ $\frac{dQ}{dt} = \mu_{ret}Q$	Use function for integrating Baranyi-Roberts model until t_{ret}
	$C_{r\text{-}h}$	Concentration of LM in whole lettuce at the end of transport to home (when arriving home)	Log CFU/g	$Q_0 = Q(t_{end})$ $N_0=10^{C_{end}}$ $\mu_{t\text{-}r} = f(Temp_{t\text{-}r})$ $MPD_{t\text{-}r} = 0.037\,Temp_{t\text{-}r} + 12.434$ $\frac{dN}{dt} = \mu_{t\text{-}r}\frac{Q}{1+Q}\left(1-\frac{N}{e^{MPD_{t\text{-}r}}}\right)N$ $\frac{dQ}{dt} = \mu_{t\text{-}r}Q$	Use function for integrating Baranyi-Roberts model until $t_{r\text{-}h}$

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.16: Variables and functions related to the merchandizing of whole lettuce in informal markets and transportation to home

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Cross-contamination	P_{ccm}	Probability that one lettuce gets contaminated with LM due to poor handling in informal markets	-		EKE
	$TR_{stall-lettuce}$	Cross-contamination transfer coefficient from any stall surface (crates, boxes, tables) or from hands to lettuce leaves in informal markets	Log	Normal(-1.720, 1.070)	Hands to produce, taken from Hoelzer *et al*. (2012a)
	N_{stall}	Numbers of LM cells on any stall surface (floor, boxes) or hands ready to be transferred to lettuce leaves in informal markets	CFU		
	$B_{lettuce+}$	Status of a lettuce being contaminated with LM	-	Bernoulli (P_{post_harv})	-
	B_{ccm+}	Status of cross-contamination taking place	-	Bernoulli (P_{ccm})	-
	N_{s-l}	Numbers of LM cells transferred to lettuce	CFU	ROUND(N_{stall} x $10^{TRstall-lettuce}$)	-

	C_{stall}	Concentration of LM in lettuce in informal markets	Log CFU/g	IF ($B_{lettuce+}$=0) & (B_{ccm+}=1)
				Log [(0 + N_{s-l}/$W_{lettuce}$]
				IF ($B_{lettuce+}$=1) & (B_{ccm+}=1)
				Log [(10^{Cinf}x $W_{lettuce}$ + N_{s-l})/ $W_{lettuce}$]
				IF ($B_{lettuce+}$=1) & (B_{ccm+}=0)
				C_{inf}
				ELSE
				Remove
					-

	P_{market}	Prevalence of lettuces contaminated with LM in informal markets	-	$1 - (1 - P_{post\_harv}) \times (1 - P_{cch})$	-
Growth	t_{market}	Time that whole lettuces are displayed in stalls until purchase	h	-	Assumption to be made
	$Temp_{market}$	Temperature at which whole lettuces are displayed on stalls until purchase	°C		Ambient temperature
	C_{market}	Concentration of LM in whole lettuce at the end of informal retail (contaminated fraction)	Log CFU/g	$Q_0 = Q(t_{trans\text{-}inf})$ $N_0 = 10^{C_{stall}}$ $\mu_{market} = f(Temp_{market})$ $MPD_{market} = 0.037\,Temp_{market} + 12.434$ $\frac{dN}{dt} = \mu_{market}\frac{Q}{1+Q}\left(1 - \frac{N}{e^{MPD_{market}}}\right)N$ $\frac{dQ}{dt} = \mu_{market}Q$	Use function for integrating Baranyi-Roberts model until t_{market}
Transportation to home	$t_{inf\text{-}h}$	Transportation time for whole lettuces from informal markets to home	h	-	Assumption to be made
Growth during transportation to home	$C_{inf\text{-}h}$	Concentration of LM in whole lettuce at the end of transport to home (when arriving home)	Log CFU/g	$Q_0 = Q(t_{market})$ $N_0 = 10^{C_{market}}$ $\mu_{inf\text{-}h} = f(Temp_{market})$ $MPD_{inf\text{-}h} = 0.037\,Temp_{market} + 12.434$ $\frac{dN}{dt} = \mu_{inf\text{-}h}\frac{Q}{1+Q}\left(1 - \frac{N}{e^{MPD_{inf\text{-}h}}}\right)N$ $\frac{dQ}{dt} = \mu_{inf\text{-}home}Q$	Use function for integrating Baranyi-Roberts model until $t_{inf\text{-}h}$

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.17: Variables and functions related to consumer handling of whole lettuce

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Lettuces from any retail type	P_{formal}	Probability that lettuce comes from formal retail (versus informal markets)	-		

	P_{whole}	Prevalence of whole lettuce contaminated with LM		IF (Bernoulli (P_{formal}) = 1)
				$P_{post\text{-}harv}$
				ELSE
				P_{market}	

	C_{whole}	Concentration of LM in whole lettuce		IF (Bernoulli (P_{formal}) = 1)
				$C_{r\text{-}h}$
				ELSE
				$C_{inf\text{-}h}$	

	Q_{whole}	Value of Q for Baranyi equation at the point of purchase		IF (Bernoulli (P_{formal}) = 1)
				$Q(t_{r\text{-}h})$
				ELSE
				$Q(t_{inf\text{-}h})$	

Remaining shelf-life	SL_{whole}	Maximum lifetime of whole lettuce after processing (in ideal conditions)	h		Assumption to be made
Storage before preparation	$Temp_{home}$	Storage temperature at home	°C		To be taken from published models for leafy greens
	t_{home}	Storage time before consumption	h		To be taken from published models for leafy greens

	R_{Temp-t}	Negative correlation value between $Temp_{home}$ and t_{home} used for copula sampling	-	0.25	FDA-FSIS (2003)

	C_{prep}	Concentration of LM in whole lettuce at the start of preparation (contaminated fraction)	Log CFU/g	IF (t_{home}<SL_{whole})
				$Q_0 = Q(t_{whole})$
				$N_0 = 10^{C_{whole}}$
				$\mu_{home} = f(Temp_{home})$
				$MPD_{home} = 0.037\ Temp_{home} + 12.434$
				$\frac{dN}{dt} = \mu_{home}\frac{Q}{1+Q}(1 - \frac{N}{e^{MPD_{home}}})N$
				$\frac{dQ}{dt} = \mu_{home}Q$
				ELSE
				Remove
					Use function for integrating Baranyi-Roberts model until t_{home}

Washing with tap water	t_{wash}	Time taken for washing with tap water	min		Assumption to be made
	R_{wash}	Reduction in LM concentration in leaves due to washing	Log CFU/g		Equation from Domenech *et al.* (2013)
	C_{wash}	Concentration of LM in lettuce after washing (contaminated fraction)	Log CFU/g	$C_{prep} - R_{wash}$	
Cross-contamination	P_{chopp}	Probability that chopping board is contaminated with LM	-		Data needed
	N_{chopp}	Number of cells on chopping board	CFU		Data to be taken from other models

$TR_{chopp\text{-}lettuce}$	Cross-contamination transfer coefficient of LM from chopping board to lettuce	%	Normal (31.7, 6.90)	Kuan *et al.* (2017b) from Table A 4.5
$N_{chopp\text{-}lettuce}$	Numbers of LM transferred from contaminated chopping board to lettuce being prepared for a serving	CFU	ROUND (N_{chopp} x $TR_{chopp\text{-}lettuce}$)	
SS_{whole}	Serving size of lettuce (from whole lettuce)	g		Assumption to be made
N_{bc}	Numbers of LM in a contaminated portion of lettuce	CFU	Poisson (10^{Cwash} x SS_{whole})	
$B_{lettuce+}$	Status of whole lettuce having LM	-	Bernoulli (P_{whole})	
B_{chopp+}	Status of chopping board being contaminated with LM	-	Bernoulli (P_{chopp})	

$N_{whole\text{-}serv}$	Numbers of LM in a serving prepared from a whole lettuce at the point of consumption	CFU	IF ($B_{lettuce+}$=1) & (B_{chopp+}=1) N_{bc} + $N_{chopp\text{-}lettuce}$
			IF ($B_{lettuce+}$=1) & (B_{chopp+}=0) N_{bc} + 0
			IF ($B_{lettuce+}$=0) & (B_{chopp+}=1) 0 + $N_{chopp\text{-}lettuce}$
			ELSE
			Remove	

$C_{whole\text{-}serv}$	Concentration of LM in a serving prepared from a whole lettuce at the point of consumption	Log CFU/g	Log ($N_{whole\text{-}serv}$ / SS_{whole})	
$P_{whole\text{-}serv}$	Prevalence of servings prepared from a whole lettuce with LM		1 – (1 - P_{whole}) x (1 - P_{chopp})	

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 4.18: Variables and functions related to the consumer handling of RTE pre-cut lettuce

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Remaining shelf-life	SL_{RTE}	Maximum life time of RTE pre-cut lettuce after processing (in ideal conditions)	h		Assumption to be made
Storage before preparation	$Temp_{RTE\text{-}home}$	Home storage temperature of RTE pre-cut lettuce	°C		To be taken from published models for leafy greens
	$t_{RTE\text{-}home}$	Storage time of RTE pre-cut lettuce before consumption	h		To be taken from published models for leafy greens
	$R_{Temp\text{-}t}$	Negative correlation value between $Temp_{home}$ and t_{home} used for copula sampling	-	0.25	FDA-FSIS (2003)

	$C_{RTE\text{-}prep}$	Concentration of LM in whole lettuce at the start of preparation (contaminated fraction)	Log CFU/g	IF ($t_{RTE\text{-}home} < SL_{RTE}$)
				$Q_0 = Q(t_{r\text{-}h})$
				$N_0 = 10^{C_{r\text{-}h}}$
				$\mu_{RTE\text{-}home} = f(Temp_{RTE\text{-}home})$
				$MPD_{RTE\text{-}home} = 0.037\ Temp_{RTE\text{-}home} + 12.434$
				$\frac{dN}{dt} = \mu_{RTE\text{-}home} \frac{Q}{1+Q} \left(1 - \frac{N}{e^{MPD_{RTE\text{-}home}}}\right) N$
				$\frac{dQ}{dt} = \mu_{RTE\text{-}home} Q$
				ELSE
				Remove
					Use function for integrating Baranyi-Roberts model until $t_{RTE\text{-}home}$

Washing with tap water	$P_{RTE\text{-}wash}$	Probability of washing RTE pre-cut lettuce with water	-		Assumption to be made
	$t_{RTE\text{-}wash}$	Time taken for washing with tap water	min		Assumption to be made
	$R_{RTE\text{-}wash}$	Reduction in LM concentration in leaves due to washing	Log CFU/g		Equation from Domenech *et al.* (2013)
	$B_{RTE\text{-}wash+}$	Status of RTE lettuce being washed	-	Bernoulli ($P_{RTE\text{-}wash}$)	

	$C_{RTE\text{-}wash}$	Concentration of LM in lettuce after washing	Log CFU/g	IF ($B_{RTE\text{-}wash+}$=1)
				$C_{RTE\text{-}prep} - R_{RTE\text{-}wash}$
				ELSE
				$C_{RTE\text{-}prep}$	

	SS_{RTE}	Serving size of lettuce (from RTE pre-cut lettuce)	g		Assumption to be made
	$N_{RTE\text{-}serv}$	Numbers of LM in a serving of lettuce	CFU/g	Poisson ($10^{CRTE\text{-}wash} \times SS_{RTE}$)	
	$Pi_{RTE\text{-}serv}$	Probability that the serving is LM free (despite lettuce being contaminated initially)	-	Prob(Poisson($10^{CRTE\text{-}wash} \times SS_{RTE}$)=0)	

	$C_{RTE\text{-}serv}$	Concentration of LM in a serving prepared from an RTE pre-cut lettuce pack	Log CFU/g	IF ($N_{RTE\text{-}serv}$>0)
				Log ($N_{RTE\text{-}serv}$/ SS_{RTE})
				ELSE
				Remove	

	$P_{RTE\text{-}serv}$	Prevalence of servings prepared from RTE pre-cut lettuce packs with LM		$P_{RTE} \times (1 - Pi_{RTE\text{-}serv})$	

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Annex 5

Technical description of the exposure assessment model for *L. monocytogenes* in non-RTE frozen corn/peas

SECTION 1: ASSUMPTIONS AND DATA

Module 1. Processing

Preparation

Table A 5.1 compiles data on the occurrence of *L. monocytogenes* in both minimally-processed and fresh whole vegetables extracted from the Pathogens-In-Foods database. In addition, the table presents occurrence data from Magdovitz *et al.* (2021) on raw vegetables arriving at frozen food facilities. Such data can be assumed to represent contamination prevalence in vegetables before the blanching stage. Counts of *L. monocytogenes* at this point of the chain are very low. Table A 5.2 presents counts data in vegetables that can be used to approach the distribution of the initial concentration.

Blanching

Mazzotta (2001) carried out challenge studies mimicking the effect of blanching to determine the heat resistance parameters (D- and z-values) of a cocktail of *L. monocytogenes* (serotypes 1/2a, 1/2b, and 4b) in selected vegetables. They suggested that blanching, conducted as a process to inactivate catalase or peroxidase activity, can also be considered as an inactivation treatment for *L. monocytogenes* that reaches a reduction of 5 $\log_{10}$, if vegetables are treated for at least 10 s at 75 °C or 1 s at 82 °C. Heat resistance parameters are shown in Table A 5.3. In a more recent study, Ceylan *et al.* (2017) assessed the thermal inactivation of a cocktail of *L. monocytogenes* in peas, broccoli and carrots that were exposed to hot water or steam under specific time and temperature combinations. Reduction values for two separate experiments are shown in Table 5.3.

Table A 5.1: *L. monocytogenes* prevalence in minimally-processed and fresh whole vegetables sampled at packinghouse or at retail

Country	Product	Sample size	Positive enrichment	Prevalence (%)	LoQ	<LoQ	Source[1]
Minimally-processed							
Italy	RTE vegetables[2]	699	2	0.28	-	-	De Giusti *et al.* (2010)
Austria	RTE produce[3]	143	0	0.00	-	-	Wagner *et al.* (2007)
Spain	Whole vegetables[3]	28			2	28	Abadias *et al.* (2008)
Italy	RTE vegetable salads[3]	56	0	0.00	-	-	Pianetti *et al.* (2008)
Italy	RTE packed vegetables[3]	1160	4	0.34	-	-	Losio *et al.* (2015)
Spain	Broccoli fresh-cut[3]	16	1	6.25	-	-	Moreno *et al.* (2012)
Italy	Minimally processed pumpkins[3]	33	1	3.03	-	-	Cardamone *et al.* (2015)
Fresh whole vegetables							
Italy	Whole vegetables[2]	265	3	1.13	-	-	De Giusti *et al.* (2010)
Greece	Peppers[2]	60	12	20	-	-	Kokkinakis *et al.* (2007)
Romania	Fresh onions[2]	10	0	0	-	-	Carp-Carare *et al.* (2013)
Greece	Peppers[2]	60	8	13.3	-	-	Kokkinakis *et al.* (2007)
Italy	Vegetables[3]	738	33	4.47	-	-	Gianfranceschi *et al.* (2003)
the United Kingdom	Organic vegetables[3]	3198	-	-	1.3	3198	Sagoo *et al.* (2001)
Spain	Raw whole vegetables[3]	141	2	1.41	-	-	Badosa *et al.* (2008)
Türkiye	Raw vegetables[3]	44	6	13.6	-	-	Cetinkaya *et al.* (2014)
Albania	Onions[3]	16	0	0	-	-	Lika *et al.* (2014)
Albania	Broccoli[3]	21	0	0.00	-		Lika *et al.* (2014)
Spain	Fresh broccoli[3]	17	2	11.7	-	-	Moreno *et al.* (2012)

Czech Republic	Vegetables[3]	249	16	6.42	-	-	Vojkovská *et al.* (2017)
Vegetables arriving at frozen food facility							
United States	Corn	59	8	13.6	-	-	Magdovitz *et al.* (2021)
	Carrots	54	0	0.00	-	-	
	Green beans	72	3	4.17	-	-	
	Peas	96	6	6.25	-	-	

Sources: **Source** column cites the corresponding studies. See References section for further details.

[1]Extracted from the Pathogens-in-Foods database (https://pif.esa.ipb.pt/) and also from Magdovitz *et al.* (2021).
[2]Sampled at the packing house.
[3]Sampled at retail.

Table A 5.2: *L. monocytogenes* concentration in positive samples of vegetables sampled on farm, at packinghouse or at retail

Country	Product	Sample size	Positive enrichment	Counts (MPN/g)	Source[1]
Freshly-harvested					
Malaysia	Carrots	10	1	3.0	Jeyaletchumi *et al.* (2011)
	Yardlong beans	10	1	3.6	
Vegetables arriving at frozen food facility					
United States	Corn	59	1 7	10-100 >100	Magdovitz *et al.* (2021)
	Green beans	72	3	>100	
	Peas	96	2 4	10-100 >100	
Retail					
Malaysia	Bulk carrots	13	1	<3.0	Kuan *et al.* (2017a)

Sources: **Source** column cites the corresponding studies. See References section for further details.

Table A 5.3: Data on the effect of blanching temperatures on *L. monocytogenes* inactivation

Source	**Characteristics**	**Outcomes**			
Mazzotta (2001)	Inoculated cocktail N-7004 (Scott A, serotype 4b), N-7285 (serotype 1/2a), N-7298 (serotype 1/2b), and N-7017 (Murray B, serotype 4b)		Temperature (°C)	D value (min)	Z value (°C)
		Broccoli	56 60 62	2.3 ± 0.21 0.62 ± 0.03 0.39 ± 0.05	7.8
		Pepper	56 60 62	3.9 ± 0.35 0.92 ± 0.08 0.31 ± 0.01	5.5
		Peas	56 60 62	5.2 ± 0.28 1.04 ± 0.14 0.41 ± 0.08	5.5
Ceylan *et al.* (2017)	Inoculated cocktail FSC-CC 1838, FSC-CC 1720, FSC-CC2454, FSC-CC 2450, Scott A FSC-CC 2452		Temperature (°C) / Time(min)	LM reduction (log CFU/g) Trial 1	LM reduction (log CFU/g) Trial 2
		Treated with hot water			
		Broccoli	85.0 / 0.5 87.8 / 0.5	>8.79 >8.79	>8.72 >8.72
		Carrots	85.0 / 0.5 87.8 / 0.5	>8.23 >8.18	>8.81 >8.99
		Peas	85.0 / 0.5 87.8 / 0.5	>7.59 >8.18	7.49 >8.29
		Treated with steam			

Broccoli	85.0 / 1.0	2.77	1.07
	85.0 / 2.0	5.15	>4.43
	96.7 / 1.0	>8.71	>6.67
	96.7 / 2.0	>8.71	>7.63
Carrots	85.0 / 1.0	2.66	3.26
	85.0 / 2.0	>9.03	8.20
	96.7 / 1.0	>9.03	>8.83
	96.7 / 2.0	>9.03	>8.95
Peas	85.0 / 1.0	4.01	0.67
	85.0 / 2.0	6.04	4.57
	96.7 / 1.0	>8.89	>8.36
	96.7 / 2.0	>9.12	>9.20

Sources: **Source** column cites the corresponding studies. See References section for further details.

Post-blanching recontamination

Recontamination can occur post-blanching. Table A 5.4 compiles the frequencies of recovery of *L. monocytogenes* in swabs from both food and non-food contact surfaces in frozen food processing plants.

Table A 5.4: Data on *L. monocytogenes* contamination of food and non-food contact surfaces in frozen food processing plants

Site swabbed	Total samples	Positive samples	Prevalence (%)	Source
Food contact surfaces				
Conveyor belts	4	0	0.00	Truchado *et al.* (2022)
Freezing tunnels	8	2	25.0	
Packaging	4	0	0.00	
Food and non-food contact surfaces				
Conveyor belts after blanching, freezing tunnels	88	36	41.3	Pappelbaum *et al.* (2008)

Sources: **Source** column cites the corresponding studies. See References section for further details.

End of processing

For validating the outputs of the processing module, simulated prevalence and counts can be compared with published surveys of *L. monocytogenes* prevalence and counts in non-RTE frozen vegetables (Table A 5.5). In terms of quantitative analysis, Moravkova *et al.* (2017), Willis *et al.* (2020) and FSAI (2022) found that all *L. monocytogenes*-positive samples presented counts below 100 CFU/g (Table A 4.5).

Module 2. Consumer handling

Defrosting and storage after defrosting.

L. monocytogenes growth after defrosting, in uncooked vegetables or in slightly cooked vegetables, will be modelled using data from Kataoka *et al.* (2017) or PROFEL (2020). Kataoka *et al.* (2017) proposed an equation to predict the exponential growth rate (EGR, [log CFU/g/h]) in defrosted corn and green peas, respectively, as a function of temperature (T, [°C])

$$\sqrt{EGR} = 0.0168\ T + 0.0623 \quad (5.1)$$

$$\sqrt{EGR} = 0.0225\ T + 0.0178 \quad (5.2)$$

PROFEL (2020) determined the growth potential of *L. monocytogenes* in three batches of selected vegetables by inoculating a cocktail of four strains, including the ST6 multi-country outbreak strain. Samples were kept frozen for 2 weeks, and then transferred to a refrigerator at 9 °C to have the vegetables thawed and stored for 24 or 48 h. Results are summarized in Table A 5.6. Green peas and sweet corn presented the highest growth potential estimates at 0.62-0.73 and 0.69-1.28 log CFU/g, respectively, after 24 h storage; and 0.85-2.16 and 1.37-2.35 log CFU/g, respectively, after 48 h storage. Overall, except for one batch of tested corn, all of the frozen vegetables in the experiments presented growth potential values lower than 1.0 log CFU/g when thawed and stored in a refrigerator at the temperature abuse conditions of 9 °C.

Cooking vegetables

Data from Willis *et al.* (2020) and FSAI (2022) will be used to work out the probability of not cooking non-RTE frozen vegetables. According to a survey at retail in England, 77 percent of frozen vegetables packages recommended cooking before consumption, 4 percent specified that they were RTE, and on 19 percent of the packages there were no indications for cooking (Willis *et al.*, 2020).

Table A 5.5: *L. monocytogenes* prevalence and counts (when available) in non-RTE frozen vegetables sampled at the end of processing and at retail

Country	Product	Sample size	Positive enrichment	Preva-lence (%)	LoQ	>LoQ	Source[1]
End of processing							
Spain	Frozen vegetables	906	11	1.21	-	-	Aguado *et al.* (2004)
Poland	Frozen mixed vegetables	248	113	45.6	-	-	Pappelbaum *et al.* (2008)
	Frozen leeks	29	0	0.00	-	-	
	Frozen onions	45	0	0.00	-	-	
	Frozen vegetables (tomatoes, celery, parley, paprika and Brussels sprouts)	73	17	23.3	-	-	
	Frozen corn	12	1	8.33	-	-	
	Frozen green peas	110	22	20.0	-	-	
Retail							
Türkiye	Frozen peppers	216	0	0.00	-	-	Lee *et al.* (2007)

Poland	Frozen vegetable mix (broccoli, carrots, green beans, peas, corn, red beans, onions, peppers, potatoes)	9100	504	5.54	-	-	Skowron *et al.* (2019)
Spain	Frozen vegetables	1750	31	1.77	-	-	Vitas *et al.* (2004)
Portugal	Frozen sliced green peppers	31	7	22.6	-	-	Mena *et al.* (2004)
	Frozen sliced red peppers	33	0	0.00	-	-	
	Frozen peas	27	4	14.8	-	-	
Czech Republic	Frozen vegetables (carrots, broccoli, peas, mix, sprouts)	66	0	0.00	-	-	Vojkovská *et al.* (2017)
Multiple	Frozen vegetables (peas, carrots, corn)	43	9	20.9	1.7	0	Moravkova *et al.* (2017)
Multiple	Frozen vegetables	673	69	10.3	1.0	3	Willis *et al.* (2019)
Ireland	Frozen vegetables	366	21	5.73	2.0	0	FSAI (2022)[2]

Sources: **Source** column cites the corresponding studies. See References section for further details.

[1]Extracted from the Pathogens-in-Foods database (https://pif.esa.ipb.pt/)

[2]Not extracted from the Pathogens-in-Foods database

In Ireland (FSAI, 2022), 9.3 percent of the frozen vegetable samples did not have any non-RTE label or cooking instructions printed on the packaging and indicating that frozen vegetables must be fully cooked. The survey participants were asked about their preparation habits, and a high proportion (84-89 percent) recognized that vegetables such as sweet corn, peas, mushrooms and mixed vegetables need to be cooked prior to consumption. Of the total number of people who said they consume a specific frozen food, when asked about cooking, 2.9 percent responded that they consumed frozen peas without cooking, 2.0 percent mixed vegetables without cooking, 8.4 percent carrots without cooking, 2.6 percent broccoli without cooking and 10.3 percent sweet corn without cooking. In relation to the reduction attained by cooking, the assumption of the EFSA model (EFSA, 2020) will be used. That work reports a Pert distribution, assuming a reduction of between 1 and 9 log units, and the most likely reduction of 5 log units.

Table A 5.6: *L. monocytogenes* growth potential (log CFU/g) after 24 or 48 hours defrosting in a refrigerator at 9 °C, tested in three different batches

Vegetable	Batch	After 24 h	After 48 h
Garden peas	1	0.62	1.26
	2	0.70	0.85
	3	0.73	2.16
Parsnips	1	0.13	0.96
	2	0.85	1.71
	3	0.33	1.73
Sweetcorn	1	0.69	1.37
	2	1.10	2.35
	3	1.28	1.87
White cabbage	1	0.44	-
	2	0.59	1.79
	3	0.50	0.80

SECTION 2: THE EXPOSURE ASSESSMENT MODEL

The exposure assessment model for frozen vegetables focuses on non-RTE blanched frozen peas and corn. The models begin with the processing module, specifically the blanching step (Table A 5.7). A batch of pre-blanching vegetables has a probability of contamination with *L. monocytogenes* of P_0 and concentration C_0. The concentration of *L. monocytogenes* after blanching can be estimated by applying the log-linear decay primary model and the Bigelow secondary model, with known kinetic parameters D_{ref} at T_{ref} and z. After blanching, conveyors, freezing or packaging equipment in contact with the blanched vegetables may be contaminated with *L. monocytogenes*, at a probability P_{cc}. A fraction (TR_{equip_veg}) of the number of cells on the contaminated equipment surface (N_{equip}) is transferred to the bulk of vegetables (N_{from_equip}). Thus, a consignment of vegetables for processing, if *L. monocytogenes*-free, may become contaminated from equipment; and if already positive for *L. monocytogenes*, the concentration may increase or remain the same depending on the cross-contamination event happening or not, respectively. The packaging stage is represented by a beta-binomial process of partitioning, in order to determine the number of *L. monocytogenes* cells in a pack of frozen vegetables (C_{pack}). The prevalence of packs of frozen vegetables with *L. monocytogenes* decrease (P_{pack}) when taking into account that a proportion of packs (Pi_{pack}) even from a contaminated batch will not contain *L. monocytogenes* cells. One stage of post-packaging treatment is considered as a "generic" intervention that decreases contamination by a factor R_{post}.

The consumer handling module encompasses the partitioning of the bag into n_{serv} servings of size SS. Within a frozen bag, cells are assumed to be heterogeneously distributed with a dispersion factor b. Thus, the number of *L. monocytogenes* cells in a serving (N_{serv}) from a contaminated pack is sampled from a beta-binomial distribution. The prevalence of servings containing *L. monocytogenes* (P_{serv}) and the concentration (C_{serv}) are determined from this. The portion of vegetables is defrosted at the temperature T_{def}, and kept either in the fridge or on the countertop for t_{def} hours until consumption. During defrosting, the growth of *L. monocytogenes* will be modelled using a log-linear model and a growth rate for defrosted vegetables (EGR_{def}), assuming that there is no lag phase. There is a probability ($1-p_{cook}$) that frozen vegetables will not be cooked by the consumer. If cooked, *L. monocytogenes* concentration in contaminated servings will decrease in R_{cook} logs. At the point of consumption, the prevalence of servings containing *L. monocytogenes* is still P_{serv} and the concentration in contaminated servings is $C_{consumption}$.

Table A 5.7: Variables related to the processing module of non-RTE frozen corn/peas

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Pre-blanching	P_0	Probability that a batch of pre-blanching vegetables is contaminated with LM	-	-	Table A 5.1 (According to this table, experts suggest setting mean prevalence at 0.058)
	C_0	Concentration of LM in a batch of vegetables pre-blanching	Log CFU/g	-	Table A 5.2 (experts suggest setting Normal (1,0))
Blanching	D_{ref}	Time to reach a decimal reduction of LM in vegetables at the temperature T_{ref}	min	-	Estimate from Table A 5.3 (Mazzota, 2001)
	T_{ref}	Reference temperature	°C	-	Set by Table A 5.3 (Mazzotta, 2001)
	z	Number of temperature degree units required for a one-log reduction in D value	°C	-	Estimate from Table A 5.3 (Mazzotta, 2001)
	T_{blanch}	Temperature for blanching	°C	-	To be determined from the literature
	t_{blanch}	Time taken for blanching	min	-	To be determined from the literature
	C_{blanch}	Concentration of LM in vegetables after blanching	Log CFU/g	$C_0 - \frac{t_{blanch} \times 10^{\frac{T_{blanch} - T_{ref}}{z}}}{D_{ref}}$	Decline in LM can be compared with data from Ceylan *et al.* (2017) for higher T_{blanch}

Cross-contamination	P_{cc}	Probability that conveyors, freezing or packaging equipment in contact with blanched vegetables are contaminated with LM	-		Table A 5.4
	TR_{equip_veg}	Cross-contamination transfer coefficient from food-contact surface to blanched vegetables	-	$10^{Normal\ (-0.400,\ 0.40)}$	Hoelzer *et al*. (2012a), TR from stainless steel to vegetables
	W_{batch}	Weight of batch of bulk frozen vegetables	g	-	Assumption to be made
	W_{pack}	Weight of pack of frozen vegetables	g	-	Assumption to be made
	b	Dispersion factor related to the heterogeneous distribution of LM in the mass of frozen vegetables	-	1	Moderate clustering
	N_{equip}	Numbers of LM on conveyor/equipment ready to be transferred	CFU		Assumption to be made
	B_{bulk+}	Status of a batch of frozen bulk vegetables being contaminated with LM	-	Bernoulli (P_0)	-
	B_{cc+}	Status of conveyor/ equipment being contaminated	-	Bernoulli (P_{cc})	-

Packs	Number of packs produced in a batch	-	ROUND (W_{batch}/W_{pack})	-
N_{bulk}	Numbers of LM in the bulk of frozen vegetables	CFU	IF (B_{bulk+}=1) & (B_{cc+}=1) $N_{bulk} = 10^{Cblanch} \times W_{batch}$	
N_{from_equip}	Numbers of LM transferred to the bulk of frozen vegetables from contaminated equipment	CFU	$N_{from\_equip} = Binomial (N_{equip}, TR_{equip\_veg})$ $N_{total} = N_{bulk} + N_{from\_equip}$ $N_{pack} = Binomial (N_{total}, Beta (b, b \times Packs - 1))$	
N_{total}	Total numbers of LM in the bulk of frozen vegetables	CFU	$Pi_{pack} = (Packs - 1)/(N_{total} + Packs - 1)$	
N_{pack}	Total number of LM cells distributed in a pack of frozen vegetables	CFU	IF (B_{bulk+}=1) & (B_{cc+}=0) $N_{bulk} = 10^{Cblanch} \times W_{batch}$ $N_{pack} = Binomial (N_{bulk}, Beta (b, b \times Packs - 1))$	

Pi_{pack}	Probability that a pack of frozen vegetables is LM-free		$Pi_{pack} = (Packs - 1)/(N_{bulk} + Packs - 1)$
			IF (B_{bulk+}=0) & (B_{cc+}=1)
			$N_{from\_equip} = Binomial (N_{equip}, TR_{equip\_veg})$
			$N_{pack} = Binomial (N_{from\_equip}, Beta (b, b \times Packs - 1))$
			$Pi_{pack} = (Packs - 1)/(N_{from\_equip} + Packs - 1)$
			ELSE
			Remove	

	P_{pack}	Prevalence of packs of frozen vegetables contaminated with LM	-	$[1-Pi_{pack}] \times [1-(1-P_0)\times(1-P_{cc})]$	
	C_{pack}	Concentration of LM in packs of frozen vegetables	Log CFU/g	$Log (N_{pack} / W_{pack})$	
Post-packaging treatment	R_{post}	Reduction in LM in packs	Log CFU/g		Assumed for generic post-processing treatment, or for specific treatment based on literature
	C_{post}	Concentration of LM in packs of frozen vegetables at the end of processing	Log CFU/g	$C_{pack} - R_{post}$	
	P_{post}	Prevalence of packs of frozen vegetables contaminated with LM at the end of processing	-	P_{pack}	Compare data from Table A 5.5, assuming there is no post-packaging treatment

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 5.8: Variables related to the consumer handling module for non-RTE frozen corn/peas

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Partitioning into a serving	W_{pack}	Weight of a pack of frozen vegetables	g		Assumption to be made
	SS	Serving size	g		Assumption to be made
	n_{serv}	Number of servings from a pack of frozen vegetables	-	W_{pack}/SS	
	b	Dispersion parameter of LM in a contaminated pack	-	1	Moderate dispersion
	N_{pack}	LM cells in a contaminated pack	CFU	$10^{Cpost} \times W_{pack}$	
	N_{serv}	LM cells in a serving from a contaminated pack	CFU	Binomial (N_{pack}, Beta(b, b × (n_{serv} -1)))	
	Pi_0	Probability that the serving originating from a contaminated pack is free of LM	-	$Pi_0 = (n_{serv} - 1)/(N_{pack} + n_{serv} - 1)$	
	C_{serv}	Concentration of LM in a serving	Log CFU/g	Log (N_{serv}/ SS)	
	p_{serv}	Prevalence of servings containing LM	-	$P_{post} \times (1 - Pi_0)$	

Defrosting	T_{def}	Temperature at which the frozen vegetables are defrosted	°C		Scenarios to be tested if defrosted in the fridge or at room temperature
	t_{def}	Time between taking frozen portions and preparing them	h		
	EGR_{def}	Exponential growth rate of LM in defrosted vegetables	Log CFU/g/h	Equations 7.1 and/or 7.2	
	C_{def}	Mean concentration of LM in defrosted vegetables just before utilization/preparation	Log CFU/g	MIN (8, $C_{serv} + EGR_{def} \times t_{def}$)	Lag phase is not considered, log-linear growth assumed; and maximum concentration is 8 log CFU/g
Cooking	p_{cook}	Probability of cooking	-		To be determined from Willis *et al.* (2019) and FSAI (2022)
	R_{cook}	Reduction in LM population due to the various ways of cooking	Log CFU/g	Pert (1; 5; 9)	Taken from EFSA (2020)
	B_{cook+}	Status of cooking of the portion of vegetables	-	Bernoulli (p_{cook})	

	$C_{consumption}$	Mean concentration of LM in vegetables (from contaminated packs) at the point of consumption	Log CFU/g	IF (B_{cook+}=1)
				$C_{def} - R_{cook}$
				ELSE
				C_{def}	

	$p_{consumption}$	Prevalence of servings containing LM at the point of consumption	-	P_{serv}	

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Annex 6

Technical description of the exposure assessment model for *L. monocytogenes* in cold-/hot-smoked fish and gravad fish

SECTION 1: ASSUMPTIONS AND DATA

Module 1. Primary processing

In the processing line, raw fish are typically received and stored in fish holding tanks at temperatures around 0 °C but can go up to a maximum of 3 °C (Tocmo *et al.*, 2014), which can lead to growth, even if slow, of *L. monocytogenes* at temperatures above the minimum for growth (~0.5 °C; Tocmo *et al.*, 2014). Table A 6.1 compiles estimates of prevalence of *L. monocytogenes* in raw fish sampled at slaughter and in processing facilities (before processing), which vary across studies between 0 and 41 percent. From the sources retrieved, the overall prevalence of *L. monocytogenes* in raw fish in processing facilities at the beginning of primary processing (19.8 percent; 95 percent CI: 17.2–22.6 percent; 162/819) appears greater than that of raw fish at slaughtering (5.89 percent; 95 percent CI: 4.47–6.95 percent; 91/1545). No current information on the populations of *L. monocytogenes* in fish has been found.

Table A 6.1: *L. monocytogenes* prevalence in raw fish sampled at slaughtering and at primary processing facilities

Country	Product	Sample size	Positive enrichment	Prevalence (%)	Source
Slaughtering					
Spain	Sea bass skin	50	0	0.00	Correia *et al.* (2020)[1]
Spain	Gilthead sea bream skin	45	0	0.00	
Norway	Fish	450	6	1.30	Svanevik *et al.* (2015)[1]
Latvia	Freshly caught freshwater fish	129	2	1.60	Terentjeva *et al.* (2015)[1]
Iran	Fresh fish	220	17	7.72	Yadollahi *et al.* (2013)
Finland	Rainbow trout	223	41	18.4	Miettinen and Wirtanen (2006)[1]
Finland	Chilled rainbow trout	5	0	0.00	Katzav *et al.* (2006)
Finland	Fresh raw fish	140	7	5.00	Markkula *et al.* (2005)[1]
Finland	Rainbow trout	103	15	14.6	Miettinen and Wirtanen (2005)[1]
Italy	Fish	15	2	13.3	Caruso *et al.* (2004)[1]
Finland	Rainbow trout heads	60	1	1.67	Autio *et al.* (1999)
Finland	Slaughtered rainbow trout	55	0	0.00	Johansson *et al.* (1999)
Norway	Slaughtered salmon	50	0	0.00	Rorvik *et al.* (1995)

Primary processing					
Italy	Raw salmon	21	5	23.8	Di Ciccio *et al.* (2012)[1]
Ireland	Raw salmon	60	17	28.3	Dass (2011)
Brazil	Raw salmon	255	105	41.2	Cruz *et al.* (2008)
Finland	Raw fish	45	2	4.40	Markkula *et al.* (2005)[1]
Finland	Fish before processing	212	9	4.20	
Poland	Incoming salmon	46	2	4.34	Medrala *et al.* (2003)
Poland	Incoming sea-trout	26	4	15.4	
Finland	Raw fish	18	2	11.1	Miettinen *et al.* (2001)[1]
Denmark	Raw fish	12	0	0.00	Vogel *et al.* (2001)[1]
Denmark	Raw fish	18	0	0.00	
Finland	Raw rainbow trout	35	0	0.00	Autio *et al.* (1999)
Norway	Salmon before filleting	24	4	16.6	Rorvik *et al.* (1995)
Norway	Salmon after filleting	47	12	25.5	

Sources: **Source** column cites the corresponding studies. See References section for further details.

[1]Extracted from the Pathogens-in-Foods database (https://pif.esa.ipb.pt/)

Filleting

During processing, there are many opportunities for *L. monocytogenes* to be transferred from the exterior of fish to cut surfaces of fillets or sides (Eklund *et al.*, 1995). Rorvik *et al.* (1995) observed that the filleting stage was able to increase the prevalence of *L. monocytogenes* in raw salmon, from 16.6 percent (4/24) of frequency of recovery in raw salmon pre-filleting to 25.5 percent (12/47) in filleted salmon (Table A 6.1). At this stage of processing, flesh areas of fish can be inoculated by contact with contaminated skin sides of fillets, filleting tables and knives and gloves of personnel. For instance, Dass (2011) detected *L. monocytogenes* types c and b on filleting boards, deboning pins, conveyor belts and knives. In another study, Chen *et al.* (2010) monitored contamination of *L. monocytogenes* in catfish fillets and in environmental samples collected from various areas of the processing plant. They isolated serotype 1/2b (47 percent) from trimming boards, conveyor belts and holding tables, and found that conveyors were contaminated with *L. monocytogenes* in 16.6 percent of the sampling times (6/36). Likewise, sampling on different occasions from a processing facility for cold-smoked fishery products, Eklund *et al.* (1995) determined a frequency of contamination of *L. monocytogenes* on filleting tables of 11.1 percent (1/9). In a processing facility for gravlax salmon in Brazil, Cruz *et al.* (2008) detected 80 percent of positive swabs of *L. monocytogenes* from handlers, and 25 percent from knives and tables. Lundén *et al.* (2000) explained that the contamination on tables and cutting surfaces can adhere strongly within a short period of time. This means that filleted fish may become contaminated during the first stages of processing.

Chilling and cold storage

Table A 6.2 brings together estimates of growth rates of *L. monocytogenes* from inoculation experiments in raw fish. Such data can be meta-analysed to obtain an overall growth rate estimate (Figure A 6.1), which can be used to model the growth of this pathogen before the processes of smoking (production of cold-smoked fish) or maceration (production of gravad fish). Jia *et al.* (2020) proved that, in raw salmon, the presence of native microflora extends the lag phase of *L. monocytogenes* by nearly 3 times, in comparison to that of salmon subjected to irradiation prior to inoculation with the pathogen. The following equation can be used to model the lag phase (h) of *L. monocytogenes* in raw salmon, in the presence of native microbiota:

$$\lambda=\exp(A)\times[B(T-T_{min})^{0.75}]^{2\times m} \quad (6.1)$$

where the minimum temperature for growth (T_{min}) is 1.3 °C (SE=0.3 °C), the coefficient A is 0.84 [SE=0.30], the coefficient B is 0.0581 [SE=0.0014], and the coefficient m is 1.11 [SE=0.15].

Table A 6.2: Growth kinetics data for *L. monocytogenes* in raw fish at different constant temperatures

Study	Medium	Strain	Stressed	Temperature (°C)	Specific GR (h^{-1})
FSA-Torry[1] (ComBase)	Raw salmon fillet	Scott A	Not stressed	5	0.0070
				5	0.0039
				5	0.0029
				5	0.0065
				5	0.0053
				5	0.0061
CSIC[2] (ComBase)	Raw salmon	P3	Not stressed	8	0.0469
				8	0.1615
Le Marc (2001)	Raw salmon	17765	Not stressed	12.8	0.0750
				2.9	0.0220
Jia *et al.* (2020)	Raw salmon flesh	Cocktail: CICC21632, CICC21633, CICC21635, CICC21639	Stressed (Rifampicim)	4	0.0190
				8	0.0744
				12	0.1502
				16	0.2418
				20	0.3469
				25	0.4950
				30	0.6596
				35	0.8393

[1] FSA-Torry. Data generated by the Food Standards Agency, Torry, Aberdeen, the United Kingdom.

[2] CSIC (Spanish National Research Council). Data generated by Instituto del Frio, Spain.

Sources: **Study** column cites the corresponding studies. See References section for further details.

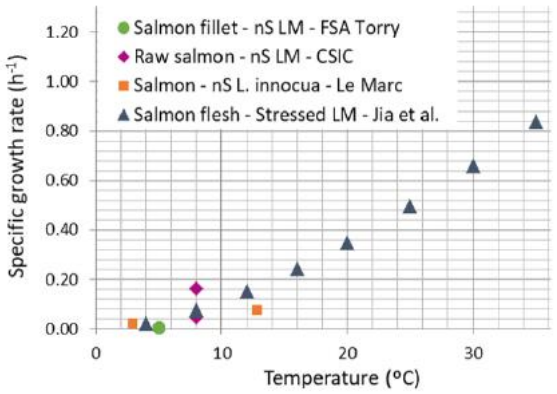

Figure A 6.1: Specific growth rate estimates for *L. monocytogenes* (LM) in raw salmon against temperature, extracted from the literature. nS: non-stressed.

Sources: See References section.

Cold transport to secondary processing

The growth of *L. monocytogenes* in chilled fish during transport can also be estimated with the growth data presented in Table A 6.2. The time-temperature profiles for refrigerated transport of cold-smoked salmon modelled by Afchain *et al.* (2005) can be used in the model.

Module 2. Secondary processing

Salting/Brining

Salting can be done in two ways: by dry-salting or by injection of a saturated NaCl solution. During salting, *L. monocytogenes* can survive in the filleted fish, given its halotolerance. Recirculating brine is another source for contamination since *L. monocytogenes* can grow in NaCl solutions of up to 10 percent (Peiris *et al.*, 2009). Gudbjornsdottir *et al.* (2005) found *L. monocytogenes* at a frequency of 21.4 percent in injection brines (3/14), which supported the fact that brine containers and the brine itself may serve as reservoirs for *L. monocytogenes*.

The extent of reduction due to salting and brining can be represented using the results from Porsby *et al.* (2008) and Neunlist *et al.* (2005), respectively. Porsby *et al.* (2008) investigated the overall effects of the individual processing steps for the production of cold-smoked salmon and, through a challenge study conducted

at a pilot plant, determined that brining on its own marginally slowed down the growth of *L. monocytogenes*, at a mean of 1.9 log reduction after 24 h. Neunlist *et al.* (2005) noted a reduction in *L. monocytogenes* due to dry-salting for 2 h at 12 °C of 0.6 log CFU/g. In terms of reducing *L. monocytogenes*, Niedziela *et al.* (1998) pointed out that dry-salting is more effective than brine injection.

Smoking and maturation (relevant to smoked fish)

After drying fillets for approximately 1–4 h at 20–25 °C, cold-smoking is undertaken in a room where fish fillets are subjected to 4–8.5 h of liquid smoke at <30 °C (Tocmo *et al.*, 2014). Although the temperature during smoking is too low to inhibit or kill *L. monocytogenes*, the phenolic compounds from smoke at a concentration of 20 ppm can inhibit *L. monocytogenes* (Membré *et al.*, 1997). Thus, the inactivation often observed in this microorganism by the joint processes of salting, drying and smoking essentially results from the combined effect of the reduction in the water activity (the increase in salt content) and the antimicrobial activity of the phenolic compounds. In the work of Neunlist *et al.* (2005), described earlier, the salting step only had a weak effect on *L. monocytogenes*' culturability of 0.6 log CFU/g. Then, smoking salmon presented a similar effect, reducing *L. monocytogenes* counts in 0.5 log CFU/g; however, during the subsequent maturation, the effect of salt and smoke continued, and the mean number of *L. monocytogenes* was further reduced by 0.5 log CFU/g. Thus, the combination of the three steps significantly reduced the concentration of *L. monocytogenes* by 1.6 log CFU/g from an initial inoculation level of 6.3 log CFU/g to 4.7 log CFU/g after maturation. Table A 6.3 compiles the available information on the combined effect of cold-smoking and maturation on *L. monocytogenes* in fish that could be retrieved from the literature.

In each of those challenge studies (Table A 6.3), the conditions of smoking and the treatment prior to smoking were different. Eklund *et al.* (1995) inoculated *L. monocytogenes* in two different ways: on the surface to simulate external contamination and internally to simulate contamination through brine injection, and cold-smoking was carried out at two distinct ranges of temperature: a lower temperature at 17–21 °C and a higher temperature at 22–30 °C. In Porsby *et al.* (2008), the effect of smoking and maturation was carried out in salmon fillets that were salted either by brine injection or dry-salting and in fresh unsalted salmon fillets.

Table A 6.3: Available data to build log reduction factors due to smoking

Source[1]	**Conditions of smoking** Treatment prior to smoking	**Before smoking**	**After smoking**	**Log reduction (Before-After)**
Porsby *et al.* (2008)[1] Product: **salmon**	**Cold-smoking (24 °C in cycles of 6 h)**	(log CFU/cm^2)	(log CFU/cm^2)	(log CFU/cm^2)
	Brine injection	3.0 ± 0.3	2.5 ± 1.1	0.5
		3.3 ± 0.4	1.8 ± 0.9	1.5
		2.9 ± 0.2	1.0 ± 0.0	1.9
		3.3 ± 0.1	1.8 ± 0.6	1.5
		3.0 ± 0.1	1.1 ± 0.3	1.9
	Dry-salting	3.3 ± 0.2	1.3 ± 0.7	1.8
		3.1 ± 0.2	1.4 ± 0.7	1.7
	Fresh, non-salted	3.3 ± 0.1	1.9 ± 0.9	1.4
		3.5 ± 0.2	1.2 ± 0.9	2.3
		3.1 ± 0.3	2.4 ± 0.3	0.7
Neunlist *et al.* (2005) Product: **salmon**	**Liquid-smoking (0.6% v/w) and maturing at 4 °C x 24 h**	(log CFU/g)	(log CFU/g)	(log CFU/g)
	Dry-salting and resting at 12 °C x 2 h	5.70	4.70	1.0
Suñen *et al.* (2003) Product: **rainbow trout**	**Liquid-smoking and maturing at 4 °C x 24 h**	(log CFU/g)	(log CFU/g)	(log CFU/g)
	Dry-salting (4.5% salt)	2.7 ± 0.3	<1.7 ± 0.0	>1.0
Eklund *et al.* (1995) Product: **salmon**	**Cold-smoking (smoke first 8 h), Type of LM inoculation**	(log CFU/g)	(log CFU/g)	(log CFU/g)
	External contamination simulating dry-salting			
	Cold-smoking	2.04	0.83	1.21
	17–21 °C x 18h	2.56	1.11	1.45
	Cold-smoking 22–30 °C x 18 h	0.11	-0.39	0.50
		1.04	0.54	0.50
		2.42	1.84	0.58
	Through brine-injection			
	Cold-smoking	2.36	1.41	0.95
	17–21 °C x 18 h	3.34	2.63	0.71
	Cold-smoking 22–30 °C x 18 h	2.11	2.34	-0.23
		3.28	3.38	-0.10
		4.57	4.49	0.08

Sources: **Source** column cites the corresponding studies. See References section for further details.

[1]The determination of *L. monocytogenes* in salmon after the cold-smoking process was carried out after vacuum-packaging and storage at 5 °C.

According to the data gathered, only in the experiment where smoking was conducted at the higher temperatures of 22–30 °C on salmon samples that were inoculated (internally) via brine injection, did smoking and maturation appear to have any effect on *L. monocytogenes*. In all of the other experiments, smoking and maturation reduced the level of *L. monocytogenes* concentration from 0.5 to 2.3 log.

Maceration (relevant to gravad fish)

According to Wiernasz (2019), for gravlax production in France, salmon fillets are cured with a mix of salt, sugar, pepper and dill for 14 h at 6 °C. Afterwards, fillets are rinsed, sliced and vacuum-packed. Peiris *et al.* (2009) explain that gravad fish in Sweden is prepared from raw salmon or rainbow trout, and they must be rubbed with a mixture of sugar and salt and then peppered and covered with dill and stored in plastic bags at a low temperature for 48 h. Gravad fish can then be packaged, sliced or whole, under vacuum or a modified atmosphere. The preparation of gravad fish by a Brazilian processor described in Cruz *et al.* (2008) completely differed from the procedures described above. According to Cruz *et al.* (2008), salmon fillets are hand-rubbed with a commercial mixture of NaCl, sodium nitrate and sodium nitrite, and stored at 4 °C for 24 h in high density polypropylene boxes. After the excess of salt has been washed out with chlorinated water and drained, a mixture of sugar, NaCl, ground white pepper and dried dill is hand-rubbed into the flesh side of the fillets and stored for 24 h at 4 °C in the boxes. Fillets are then layered on stainless steel supports, sprayed with sweet wine and ripened for 48 h at 4 °C. Skins are then manually removed; skinless fillets are sliced, and the slices are vacuum-packed. The packages can either be stored at 4 °C or frozen at -20 °C.

As with cold-smoked fish processing lines, the processing environment for gravad fish has been found to be contaminated. In a processing line for gravlax, Cruz *et al.* (2008) found that non-food contact surface samples from reception of salmon and salt-removal areas were frequently contaminated with *L. monocytogenes* (80 percent). In addition, amongst the food contact surfaces, the pathogen was present in 40 percent of the samples from salting boxes, on the ripening trolley after 48 h in the cold room and on weighting trays, whereas a lower frequency of positive samples was found on the salting table (30 percent). However, no *L. monocytogene*s was detected in samples collected in the ripening cold room. The final composition of gravad salmon fillets is approximately 60.0 percent moisture, 19.7 percent protein, 16.1 percent fat, 3.4 percent salt, and 0.5 percent sugar (Aarnisalo *et al.*, 2007), with a pH of 6.0–6.2 and water activity of 0.93–0.97 (Orozco, 2000).

Slicing

Aarnisalo *et al.* (2007) carried out a study to investigate the transfer of *L. monocytogenes* from an inoculated slicing blade (slicer) to slices of gravad salmon, and from inoculated salmon fillets to the slicing machine and subsequently to slices of uninoculated fillets. A marked reduction in the counts from the slicing blade (5.9–9.0 log CFU/blade) to the fillets (1.6 log CFU/g) was observed after 39 slices; nonetheless, the first slices contained higher counts. It is recommended that these transfer data (i.e. slice concentration versus slice number) be digitized in order to model a distribution for the transfer coefficient values from the slicer to the slices of the whole fillet, as carried out in Pérez-Rodríguez *et al.* (2007). The slicer-to-slices transfer coefficient can then be used to model contamination at this stage.

Cross-contamination

Rorvik *et al.* (1997) found that *L. monocytogenes* in drains is a good predictor for finding *L. monocytogenes* in smoked salmon, with an estimated relative risk (RR) of 3.3 (95% CI: 0.84 – 12.9). In addition, they determined that well-maintained facilities and suitably clean equipment during production have a protective effect, with an estimate relative risk of 0.31 (95% CI: 0.09 -1.00). It can be interpreted that poorly maintained and less hygienic processing facilities increase the probability of retrieving *L. monocytogenes* in final products by 3.22 (95% CI: 1.00 – 11.1). The model proposed takes into account the RR for poorly maintained and less hygienic processing facilities estimated in Rorvik *et al.* (1997), and will be applied at a certain (low) probability that a batch of products is produced in facilities with poor hygiene status. In this way, the RR can modify the prevalence of *L. monocytogenes* in RTE seafood at the end of secondary processing.

Module 3. Cold chain

After production of packed smoked or gravad fish, products can be frozen or refrigerated. The nominal shelf-life for vacuum-packed smoked fish is in the range of 3 to 6 weeks at a storage temperature of 4–5 °C, although consumers can keep these products for up to 9 weeks until the onset of spoilage (FAO/WHO, 2004b). In order to predict *L. monocytogenes* growth during cold storage and further along the distribution chain, it is necessary to identify the intrinsic characteristics of both cold-smoked fish and gravad fish. Table A 6.4 presents values of important intrinsic characteristics of both products that can be used in the definition of variability distributions.

Table A 6.4: Physicochemical characteristics of cold-smoked and gravad salmon

Product	Study	pH	NaCl water phase (%)	Phenolic compound (ppm)	Other physicochemical properties
Cold-smoked salmon	Hwang and Sheen (2009)	6.5	-	4	a_w=0.970
	Porsby *et al.* (2008)	6.1±0.2	3.2±0.5	22±0.2	-
	Mejlholm and Dalgaard (2007a)	5.8, 5.9, 5.9, 6.0, 6.0, 6.1	5.26±0.18 3.58±0.50	8.9±0.9 13.5±1.5	Water phase lactate: 0.98±0.36 0.90±0.08 0.77±0.07 2.84±0.29 0.67±0.02 0.60±0.00 Water phase diacetate: 0.14±0.04 0.19±0.04 0.05±0.00 0.17±0.01 $\%CO_2$: 30.0±1.7 25.3±0.8
	FAO/WHO (2004b)	-	1.5 - 4.0	2.7 - 10.8, mean=5.5±1.5	a_w=0.977 - 0.990
	Leblanc *et al.* (2000)	-	-	5.0 - 10	-
	Orozco (2000)	6.2	2.5 – 4.0		a_w=0.970
	Eklund *et al.* (1995)	-	-	8 - 13	-
Gravad salmon	Mejlholm and Dalgaard (2007a)	6.1, 6.3	3.03±0.11	0.0 4.9±0.8	Water phase lactate: 0.64±0.01 0.62±0.02 Water phase diacetate: 0.12±0.01 $\%CO_2$: 24.1±1.1
	Aarnisalo *et al.* (2007)	-	3.4	-	a_w=0.930
	Orozco (2000)	6.1-6.3	-	-	a_w=0.974 - 0.984

Sources: **Study** column cites the corresponding studies. See References section for further details.

The growth of *L. monocytogenes* and the inhibitory effect of lactic acid bacteria will be estimated by making use of published models.

Growth rate of *L. monocytogenes*

There is a possibility that after processing and packaging, the factors determining the growth of *L. monocytogenes* are such that, when they are combined, they preclude growth. The growth model of Mejlholm and Dalgaard (2007a) for *L. monocytogenes* in lightly preserved seafood (validated for cold-smoked salmon and gravad fish) that has been expanded to predict also the occurrence of no-growth responses will be used. The specific growth rate (μ_{LM}) of *L. monocytogenes* in packed processed fish is defined by:

$$\mu_{LM} = 0.419\left[\frac{T+2.83}{T_{ref}+2.83}\right]^2\left[\frac{a_w-0.923}{0.077}\right]\left[1-10^{(4.97-pH)}\right]\left[1-\frac{[LAC_U]}{3.79}\right]\left[\frac{32.0-P}{32.0}\right].$$
$$\left[\frac{3.14-CO_{2\,eq}}{3.14}\right]\left[1-\sqrt{\frac{[DAC_U]}{4.8}}\right].\xi \qquad (6.2)$$

where: T is storage temperature (°C), T_{ref} is the reference temperature at 25 °C, a_w the water activity of the product, pH is the pH of the product, $[LAC_U]$ the concentration in millimoles of undissociated lactic acid, P the concentration of phenols in ppm, $CO_{2\,eq}$ the concentration of dissolved CO_2 expressed in ppm at equilibrium, $[DAC_U]$ the concentration in millimoles of undissociated diacetate, and a term ξ for the effect of interaction between environmental parameters, which has contributions from the different environmental parameters calculated. The term ξ is defined as:

$$\xi\{\varphi(T, a_w, pH, [LAC_U], P, CO_{2\,eq}, [DAC_U])\} \qquad (6.3)$$
$$= \begin{cases} 1, & \psi \le 0.5 \\ 2(1-\psi), & 0.5 < \psi < 1.0 \\ 0, & \psi \ge 1.0 \end{cases}$$

where $\xi\{\varphi(T, a_w, pH, [LAC_U], P, CO_{2\,eq}, [DAC_U])\}$ describes the effect of interactions between environmental parameters on μLM. It is assumed that: (1) if ψ is lower than 0.5, then no interactive effect between environmental parameters occurs (ξ=1); (2) if ψ is higher than 0.5 and lower than 1.0, then the growth rate is reduced, depending on the value of ψ; and (3) if ψ is higher than 1.0, then a no-growth response occurs (ξ=0). The parameter ψ is calculated as:

$$\psi = \sum_i \frac{\varphi_{e\,i}}{2\prod_{j\neq i}(1-\varphi_{e\,i})} \qquad (6.4)$$

where

$$\varphi_T = \left\{1 - \left[\frac{T+2.83}{T_{ref}+2.83}\right]\right\}^2$$

$$\varphi_{aw} = \left\{1 - \sqrt{\frac{a_w - 0.923}{0.077}}\right\}^2$$

$$\varphi_{pH} = \left\{1 - \sqrt{1 - 10^{(4.97-pH)}}\right\}^2$$

$$\varphi_{[LAC][DAC]} = \left\{1 - \left[1 - \sqrt{\frac{[LAC_U]}{3.79}}\right]\left[1 - \sqrt{\frac{[DAC_U]}{4.80}}\right]\right\}^2$$

$$\varphi_P = \left\{1 - \sqrt{\frac{32.0 - P}{32.0}}\right\}^2$$

$$\varphi_{CO_2} = \left\{1 - \left[3.140 - \sqrt{\frac{CO_{2\,eq}}{3.140}}\right]\right\}^2 \quad (6.5)$$

The application of Equation (6.5) requires the intrinsic and extrinsic characteristics of the products to be known. They should be defined based on outcomes of product analyses. Table A 6.4 compiles these product characteristics as extracted from published articles.

Lag phase duration of *L. monocytogenes*

In the Baranyi model, $q_{0\,LM}$ defines the initial concentration of the hypothetical substrate needed for starting *L. monocytogenes* growth.

$$q_{0\,LM} = \frac{1}{exp(\mu\lambda) - 1} \quad (6.6)$$

The parameter $q_{0\,LM}$ of *L. monocytogenes* after the processing of fillets can be estimated using the equations, fitted by Hwang and Sheen (2009), for lag time (λ_{smoked} [h]) and the specific growth rate (μ_{smoked} [h^{-1}]) of the pathogen in smoked salmon as a function of temperature (T [°C]).

$$\lambda_{smoked} = 444.63 - 58.26\,T + 2.09T^2 \quad (6.7)$$

$$\mu_{smoked} = \ln(10)\,.\,(0.0529 + 0.0110\,T) \quad (6.8)$$

The parameter $q_{0\,LM}$ obtained in this way will initialize the logistic function α(t) of the Baranyi equation describing lag time.

Maximum population density of *L. monocytogenes*

The background microflora in the cold-smoked and gravad fish can have a significant effect on the reduction of the MPD_{LM} that can be reached by *L. monocytogenes*. Considering this effect is very important for the accurate estimation of the ultimate listeriosis risk. Mejlholm and Dalgaard (2007a), for different conditions of pH (5.8-6.1), water phase lactate (0.60–2.84 percent), water phase diacetate (0.05–0.19 percent), water phase salt (3.58–5.26 percent), phenol (8.9–13.5 ppm), temperature (7.8–14.3 °C) and CO_2 (25.3–30.0 percent), estimated MPD values between 3.6 and 8.4 log CFU/g in cold-smoked salmon, whereas in gravad fish, the MPD_{LM} values were estimated at 8.6 and 8.9 log CFU/g, for two different experimental conditions of pH (6.3, 6.1), water phase lactate (0.64 percent, 0.62 percent), water phase diacetate (0, 0.12 percent), water phase salt (3.03 percent), phenol (4.9 ppm), temperature (14.3 °C) and CO_2 (24.1 percent). In another study, Hwang and Sheen (2009) determined MPD_{LM} values of *L. monocytogenes* in vacuum-packed cold-smoked salmon stored at 4, 8, 12 and 16 °C to be 5.2, 5.4, 5.5 and 5.6 log CFU/g, respectively, using a low mean inoculum of the pathogen of $10^{1.5}$ CFU/g. Using a higher *L. monocytogenes* inoculum of $10^{2.5}$ CFU/g, the respective MPD_{LM} values were 4.9, 5.8, 5.9 and 6.9 log CFU/g. In this study, salmon samples were not irradiated prior to inoculation, so these MPD_{LM} values reflect the inhibitory effect of the indigenous lactic acid bacteria.

The aforementioned MPD_{LM} values can be used to build a distribution of MPD_{LM} for the model. Furthermore, in challenge studies, MPD has been observed to be affected by both temperature and inoculum size. Assessing data from published literature, Pérez-Rodríguez *et al.* (2017) estimated Pearson correlations of 0.45/0.52 and Spearman's rank correlations of 0.41/0.55 between storage temperature and MPD of *L. monocytogenes* in smoked/gravad fish. Pearson correlation coefficients between the MPD of *L. monocytogenes* of smoked salmon and the initial concentration were estimated at 0.88, 0.96 and 0.52 using data from Hwang and Sheen (2009), Gimenez and Dalgaard (2004) and Besse *et al.* (2006). Taking these correlations into consideration, the MPD should be sampled in copula with temperature and with the initial *L. monocytogenes* concentration.

Growth rate of lactic acid bacteria

The growth model of Mejlholm and Dalgaard (2007b) of LAB in lightly preserved seafood will be used in the proposed QRA model. The specific growth rate (μ_{LAB}) of LAB in packed processed fish is defined by:

$$\mu_{LAB} = 0.659\left[\frac{T+3.05}{T_{ref}+3.05}\right]^2\left[\frac{a_w-0.928}{1-0.928}\right]\left[1-10^{(4.24-pH}\right]\left[1-\frac{[LAC_U]}{12.0}\right]\left[\frac{40.3-P}{40.3}\right].$$
$$\left[\frac{6.691-CO_{2\,eq}}{6.691}\right]\left[1-\sqrt{\frac{[DAC_U]}{33.3}}\right].\xi \qquad (6.9)$$

where: T is storage temperature (°C), T_{ref} is the reference temperature at 25 °C, a_w the water activity of the product, pH is the pH of the product, $[LAC_U]$ the concentration in millimoles of undissociated lactic acid, P the concentration of phenols in ppm, $CO_{2\ eq}$ the concentration of dissolved CO_2 expressed in ppm at equilibrium, $[DAC_U]$ the concentration in millimoles of undissociated diacetate; and a term ξ for the effect of interaction between environmental parameters, which has contributions from the different environmental parameters calculated. The term ξ is defined as:

$$\xi\{\varphi(T, a_w, pH, [LAC_U], P, CO_{2\ eq}, [DAC_U])\} = \begin{cases} 1, & \psi \le 0.5 \\ 2(1-\psi), & 0.5 < \psi < 1.0 \\ 0, & \psi \ge 1.0 \end{cases} \quad (6.10)$$

where $\xi\{\varphi(T, a_w, pH, [LAC_U], P, CO_{2\ eq}, [DAC_U])\}$ describes the effect of interactions between environmental parameters on μ_{LM}. It is assumed that: (1) if ψ is lower than 0.5, then no interactive effect between environmental parameters occurs (ξ=1); (2) if ψ is higher than 0.5 and lower than 1.0, then the growth rate is reduced, depending on the value of ψ; and (3) if ψ is higher than 1.0, then a no-growth response occurs (ξ=0). The parameter ψ is calculated as:

$$\psi = \sum_i \frac{\varphi_{e\,i}}{2\prod_{j\neq i}(1-\varphi_{e\,i})} \quad (6.11)$$

where

$$\varphi_T = \left\{1 - \left[\frac{T+3.05}{T_{ref}+3.05}\right]\right\}^2$$

$$\varphi_{aw} = \left\{1 - \sqrt{\frac{a_w - 0.928}{1-0.928}}\right\}^2$$

$$\varphi_{pH} = \left\{1 - \sqrt{1 - 10^{(4.24-pH)}}\right\}^2$$

$$\varphi_{[LAC][DAC]} = \left\{1 - \left[1 - \sqrt{\frac{[LAC_U]}{12.0}}\right]\left[1 - \sqrt{\frac{[DAC_U]}{33.3}}\right]\right\}^2$$

$$\varphi_P = \left\{1 - \sqrt{\frac{40.3 - P}{40.3}}\right\}^2$$

$$\varphi_{CO_2} = \left\{1 - \left[6.691 - \sqrt{\frac{CO_{2\ eq}}{6.691}}\right]\right\}^2 \quad (6.12)$$

Growth of *L. monocytogenes* as inhibited by lactic acid bacteria

The differential form of the Baranyi model with the Jameson effect will be employed to predict the growth of *L. monocytogenes* as inhibited by LAB. The presence of a lag phase will be assumed for LAB through a distribution of the parameter $q_{0\ LAB}$ of lactic acid bacteria. It will be modelled using data from the literature as well as the maximum population density for LAB (MPD_{LAB}). The growth model to be solved is:

$$\frac{1}{N_{LM}(t)}\frac{dN_{LM}(t)}{dt} = \left(\frac{Q_{LM}(t)}{1+Q_{LM}(t)}\right)\mu_{LM}\left(1-\frac{N_{LM}}{10^{MPD_{LM}}}\right)\left(1-\frac{N_{LAB}}{10^{MPD_{LAB}}}\right)$$

$$\frac{dQ_{LM}(t)}{dt} = \mu_{LM}Q_{LM}(t)$$

$$\frac{1}{N_{LAB}(t)}\frac{dN_{LAB}(t)}{dt} = \left(\frac{Q_{LAB}(t)}{1+Q_{LAB}(t)}\right)\mu_{LAB}\left(1-\frac{N_{LM}}{10^{MPD_{LM}}}\right)\left(1-\frac{N_{LAB}}{10^{MPD_{LAB}}}\right)$$

$$\frac{dQ_{LAB}(t)}{dt} = \mu_{LAB}Q_{LAB}(t) \qquad (6.13)$$

The initial concentration of *L. monocytogenes* (N_{LM} (0)) is taken from the concentration at the end of slicing (in smoked fish) or at the graving process (in gravad fish). The initial concentration of N_{LAB} (0) will be obtained from that modelled by Pérez-Rodríguez *et al.* (2017) (minimum $10^{0.95}$ CFU/g, mean $10^{2.90}$ CFU/g and maximum $10^{6.0}$ CFU/g) for smoked and gravad fish.

Temperatures and times for transport, retail and home storage

The expected temperatures from processed product to consumers for RTE foods are defined in the European guidelines for performing challenge studies on *Listeria monocytogenes* (Bergis *et al.*, 2021).

A cold-smoked salmon risk model (Chen *et al.*, 2022) used a single paper (Afchain *et al.*, 2005) as the basis for the time-temperature profiles for cold-smoked salmon from refrigerated transport, to retail, through consumer storage and display for eating. The risk assessment also used expert opinions to define the numbers of days in storage at the processing facility, retail, and consumer homes as 10, 30 and 20 days (suggesting a total 60 post-processing shelf life). Data on cold chain temperatures (times and temperatures for transportation to retail, retail storage and retail display) could be borrowed from risk assessments of other products, for instance fresh-cut leafy greens (Zeng *et al.*, 2014; Tsironi *et al.*, 2017; Sampedro *et al.*, 2022).

A 2011 study of shelf-life prediction for smoked salmon included a cumulative

frequency distribution of temperatures in 123 Belgian retail display counters for this product. Around 4 °C was the approximate 75th percentile, though the graph could be digitized in order to fit a statistical distribution (Vermeulen *et al.*, 2011). Temperature profile data for home refrigerators useful for this model can be taken from Dumitrascu *et al.* (2020).

SECTION 2: THE EXPOSURE ASSESSMENT MODEL

The exposure assessment models for smoked and gravad fish are designed to encompass primary processing, transportation to secondary processing, secondary processing and cold chain/temperature chain. The primary processing module is intended to begin before filleting, with data on the prevalence (P_{fish}) and counts (C_{fish}) of *L. monocytogenes* in gutted, cleaned fish (Table A 6.5). The module allows for a short holding time before filleting. In this lapse of time (t_{hold}), *L. monocytogenes* can grow. Such growth is estimated using a growth rate and a lag phase duration model for *L. monocytogenes* in raw fish as a function of temperature. During filleting, *L. monocytogenes* can enter the fillets through contaminated utensils, at a probability P_{ccf}. If the sampled fish to be filleted already contains *L. monocytogenes*, the numbers are added to those of cross-contamination transfer (TR_{fillet}). The prevalence (P_{fillet}) and concentration of *L. monocytogenes* (C_{fillet}) in fish fillets are then determined. Afterwards, fillets are cold-stored in the primary processing facility and then transported to secondary processing. The same growth kinetic parameters for *L. monocytogenes* in fresh fish are applied at this combined stage to compute the concentration of *L. monocytogenes* in fillets arriving at the secondary processing facility. The lag time duration will be carried over from the previous stage. Fish fillets are then processed, smoked (Table A 6.6) or macerated (Table A 6.7).

Smoked fish

For the processing of smoked fish, fish fillets first undergo the step of salting (Table A 6.6). Salting can be done either by brining (salt injection) or by dry-salting. During salting, it is assumed that cross-contamination can occur. If salting is carried out by brining, the probability that cross-contamination occurs is the probability that the brine solution is contaminated (P_{ccb}), whereas if salting is carried out by dry-salting, the probability of cross-contamination is the probability that gloves or table surfaces are contaminated with *L. monocytogenes* (P_{ccs}). In the first case of cross-contamination, the numbers of *L. monocytogenes* introduced into the fillet depend on the number of cells in the brine solution (N_{brine}) and

the volume injected per fillet ($V_{injected}$). In the second case, the numbers of *L. monocytogenes* transferred to the fillet surface depend on the transfer coefficient ($TR_{surf\text{-}fillet}$) and the cells of the donor surface ($N_{surface}$). Considering therefore the type of salting (brining), the occurrence of cross-contamination conditional on the type of salting (P_{ccb} or P_{ccs}) and the fact of a fillet being contaminated or not, the concentration of *L. monocytogenes* on/in a fillet right after salting (C_{salted}) is determined. Smoking and maturation are known to decrease the concentration of *L. monocytogenes* in fillets, which is represented in the model by applying a reduction factor $R_{smoking}$ to the contaminated fraction of salted fillets (P_{salted}).

The prevalence of fillets contaminated with *L. monocytogenes* entering the processing line can increase if there is poor overall hygiene in the processing of the batch, at a probability of occurrence $P_{poorHyg}$. In that case, there will be an increase in prevalence using the relative risk $F_{poorHyg}$, and the prevalence prior to slicing will become $P_{salted\text{-}cc}$. To properly model the cross-contamination that can occur during slicing, the data from Aarnisalo *et al.* (2007) should be digitized and characterized in a transfer coefficient (TR). $TR_{slicing}$ must represent the extent of transfer from a contaminated slicer blade to the whole fillet being sliced, and not to only one slice. Modelling $TR_{slicing}$ in this way will meet the needs of the present module.

The probability that the slicing machine is contaminated with *L. monocytogenes* is P_{slicer}, and its realization generates two statuses: cross-contamination from slicer to slices from one fillet will occur ($B_{slicer+}$) and cross-contamination from slicer to slices from one fillet will not occur ($B_{slicer-}$). Furthermore, in combination with the contamination status of the fillet, the sampled fillet contains *L. monocytogenes* ($B_{fillet+}$) and the sampled fillet does not contain *L. monocytogenes* ($B_{fillet-}$), and three contamination scenarios are created for the slicing and partitioning step:

(i) If $B_{fillet-}$ and $B_{slicer+}$, the number of *L. monocytogenes* cells transferred to the slices of one fillet ($N_{slicedFillet}$) is determined in a binomial process by knowing TR and the number of *L. monocytogenes* cells on the blade (N_{slicer}). Then, the number of *L. monocytogenes* cells randomly distributed in a pack of cold-smoked salmon (N_{dist_CSpack}) is determined by a beta-binomial process, assuming a parameter b for cell clustering and that $Packs_{CSfillet}$ (number of packs) can be produced from one fillet.

(ii) If $B_{fillet+}$ and $B_{slicer+}$, the number of *L. monocytogenes* cells in the contaminated fillet after smoking (Nfillet) is to be estimated using a Poisson distribution with the concentration of *L. monocytogenes* in smoked fish (C_{smoked}) and the weight of a fillet (W_{fillet}) as parameters. Since contamination from the slicing blade also takes place, the number of *L. monocytogenes* transferred to the

slices of the fillet ($N_{slicedFillet}$) is to be computed as in the previous point (i). The total number of *L. monocytogenes* cells transferred to all the slices of one fillet (N_{total}) is then the sum of N_{fillet} and $N_{slicedFillet}$. Then, partitioning by the beta-binomial process is applied as in (i) to determine N_{dist_CSpack}.

(iii) If $B_{fillet+}$ and $B_{slicer-}$, the number of *L. monocytogenes* cells in the contaminated fillet after smoking (N_{fillet}) is to be estimated using a Poisson distribution with the concentration of *L. monocytogenes* in smoked fish (C_{smoked}) and the weight of a fillet (W_{fillet}) as parameters. Then, to determine N_{dist_CSpack}, partitioning by the beta-binomial process is applied as in (i).

The prevalence of smoked fish packs contaminated with *L. monocytogenes* (P_{css}) is determined from the prevalences $P_{salted\text{-}cc}$, P_{slicer}, and the probability that a pack of smoked fish originating from an *L. monocytogenes*-positive fillet is not contaminated (Pi_{pack}). The concentration of *L. monocytogenes* in contaminated packs (C_{css}) is therefore obtained from N_{dist_CSpack}.

Gravad fish

The module for gravad fish (Table A 6.7) is very similar to that for smoked fish, with the exception that instead of undergoing the stages of salting and smoking/maturation, the processing of gravad fish involves mixing with salt/sugar and maceration. During maceration, two phenomena can occur: cross-contamination from surfaces in contact with the fillet, at a probability P_{ccm}, and microbial growth during maceration time. Growth kinetic parameters for slightly preserved fish are used to compute the concentration in gravad fillets at the end of maceration by means of a Baranyi-Roberts-based Jameson-effect model. From this stage, the lag phases for both *L. monocytogenes* and lactic acid bacteria are initialized and the values of Q at the end of maceration ($Q(t_{mac})$) are retained for both bacteria to carry over the lag phase on to the next module. Slicing, cross-contamination and packaging (partitioning) are modelled exactly as for smoked fish. The prevalence of gravad fish packs contaminated with *L. monocytogenes* (P_{gs}) and the concentration of *L. monocytogenes* in contaminated packs (C_{gs}) are determined from this module

The cold chain involves time-temperature histories of the smoked and gravad fish from end of processing (after packaging in the processing module) until home storage (Table A 6.8). In addition to the time spans and temperatures defined for simulating the cold chain, the lifetimes that consumers give to cold-smoked fish (SLcss) and gravad fish are needed (SL_{gs}). It is expected that SL_{gs} is lower than SL_{css}. In the first place, the intrinsic characteristics of smoked and gravad fish as well as the atmosphere composition of the package must be known. This will

determine the growth rates of *L. monocytogenes* and lactic acid bacteria.

To evaluate the growth of *L. monocytogenes* in the contaminated packs of smoked fish along the cold chain, a Baranyi-based Jameson-effect primary model will be used, but it is important to determine the parameter Q_0 of *L. monocytogenes* in fillets after smoking ($Q_{0\ LM\text{-}CSS}$). A separate simulation will be performed to build a distribution for the parameter $Q_{0\ LM\text{-}CSS}$ using the λ and μ values generated by Equations 6.7 and 6.8) (Hwang and Sheen, 2009) at a sampled temperature $Temp_{endp}$. Such a distribution of $Q_{0\ LM\text{-}CSS}$ will be used to calculate the values for every contaminated pack of smoked fish.

The growth rates for *L. monocytogenes* and lactic acid bacteria ($\mu_{LM\text{-}CSS}$ and $\mu_{LAB\text{-}CSS}$) at a given temperature and the intrinsic conditions of the smoked fish will be estimated using the validated models of Mejlholm and Dalgaard (2007a) and Mejlholm and Dalgaard (2007b), respectively. Other parameters, such as maximum population densities of *L. monocytogenes* and lactic acid bacteria in smoked fish ($MPD_{LM\text{-}CSS}$, $MPD_{LAB\text{-}CSS}$), initial number of LAB ($N_{0\ LAB\text{-}CSS}$) in smoked fish, and the parameter Q_0 for the lactic acid bacteria ($q_{0\ LAB\text{-}CSS}$), are defined. For sampling accurate $MPD_{LM\text{-}CSS}$, a copula must be constructed for MPD with storage temperature, and with the initial concentration of *L. monocytogenes*. With all of those values, the Jameson-effect model can be solved as a differential equation considering the temperature changes along the cold chain. At the point of consumption, the prevalence of smoked fish packs contaminated with *L. monocytogenes* ($P_{css\text{-}cons}$) will be the same as the prevalence in packs leaving processing (P_{css}). The concentration of *L. monocytogenes* in cold-smoked fish packs at the time of consumption is C_{css_cons}, and will not consider eventual iterations where time of consumption is beyond the lifetime of the product.

The procedure for evaluating the growth of *L. monocytogenes* in contaminated packs of gravad fish along the cold chain is exactly the same as that described for smoked fish, but with different values (distributions) for the input parameters that are specific to gravad fish. These are related to the intrinsic characteristics of the product (pH, a_w, [LAC], P, $CO_{2\ eq}$), the initial concentration of lactic acid bacteria ($N_{0\ LAB\text{-}GS}$), the parameter Q_0 of lactic acid bacteria ($q_{0\ LAB\text{-}GS}$), the maximum population densities of *L. monocytogenes* and lactic acid bacteria ($MPD_{LM\text{-}GS}$, $MPD_{LAB\text{-}GS}$) and the time to consumption (t_{home_gs}). The Baranyi-based Jameson-effect estimations will only consider gravad fish packs that are consumed within the maximum lifetime of the product. At the point of consumption, the prevalence of gravad fish packs contaminated with *L. monocytogenes* (P_{gs_cons}) will be the same as the prevalence in packs leaving processing (P_{gs}). The concentration of *L. monocytogenes* in gravad fish packs at the time of consumption is C_{gs_cons}.

Table A 6.5: Variables related to the primary processing module of fish fillets and transportation to secondary processing

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Pre-filleting	P_{fish}	Prevalence of gutted, cleaned fish contaminated with LM	-		Data from Table A 6.1
	C_{fish}	Concentration of LM in gutted, cleaned fish	Log CFU/g		Frequently said to be low, but data still needed.
Transport to filleting or brief chill storage	$Temp_{hold}$	Temperature at which cleaned fish is kept before filleting			Very low temperatures, typically between 0 °C and 3 °C (Tocmo *et al.*, 2014)
	t_{hold}	Holding time for cleaned fish before filleting, which can be due to transport to another facility or brief storage	h		Consider that if filleting is carried out in the same establishment as slaughter, transport time is zero
	μ_{fish}	Growth rate of LM in fresh fish as a function of temperature T	h^{-1}	$\mu_{fish}=f_{SQRT}(T)$	Adjust square-root model to growth rate data from Table A 6.1
	λ_{fish}	Lag phase duration of LM in fresh fish as a function of temperature T	h	$\lambda_{fish}=\exp(0.84)\times[0.0581(T-1.3)^{0.75}]^{2.22}$	Equation (6.1) from Jia *et al.* (2020)

	C_{hold}	Concentration of LM in gutted, cleaned fish after the holding time	Log CFU/g	$\mu_{fish}=f_{SQRT}(Temp_{hold})$
				$\lambda_{fish}=\exp(0.84)\times[0.0581(Temp_{hold}-1.3)^{0.75}]^{2.22}$
				IF ($t_{hold} > \lambda_{fish}$)
				$C_{hold}=C_{fish}+\log(\exp(\mu_{fish}\times(t_{hold}-\lambda_{fish})))$
				$RL_{hold}=0$
				ELSE
				C_{fish}
				$RL_{hold}=\lambda_{fish}-t_{hold}$
					RL_{hold} is the remaining lag time at the temperature t_{hold}

Filleting	P_{ccf}	Probability that any utensil for filleting is contaminated with LM	-		Assume results from deheader, skinner and trimming board from Abdallah-Ruíz *et al.* (2022): 3 positive out of 18
	TR_{fillet}	Cross-contamination transfer coefficient from utensils used during filleting to fish fillets	%		Data can be approximated from Aarnisalo *et al.* (2007)
	$N_{utensils}$	Numbers of LM on utensil/ surface used for filleting	CFU		Assumption to be made
	B_{fish+}	Status of the sampled fish being contaminated with LM	-	Bernoulli (P_{fish})	
	B_{ccf+}	Status of the sampled fish being contaminated with LM	-	Bernoulli (P_{ccf})	
	W_{fish}	Weight of fish	g		To be determined
	W_{fillet}	Weight of a fish fillet	g		To be determined

	N_{fillet}	Numbers of LM in fish fillets	CFU/g	IF (B_{fish+}=1) & (C_{fillet} =1)
				[Poisson (10^{Chold} x W_{fish}) + Binomial ($N_{utensils}$, TR_{fillet})] / W_{fillet}
				IF (B_{fish+}=1) & (C_{fillet} =0)
				[Poisson (10^{Chold} x W_{fish}) + 0]/ W_{fillet}
				IF (B_{fish+}=0) & (C_{fillet} =1)
				[0 + Binomial ($N_{utensils}$, TR_{fillet})]/ W_{fillet}
				ELSE
				Remove	

	C_{fillet}	Concentration of LM in fish fillets	Log CFU/g	$\log (1 + N_{fillet})$	
	P_{fillet}	Prevalence of fillets contaminated with LM		$1 - [(1 - P_{fish})x(1 - P_{ccf})]$	
Cold storage in primary processing and transportation to secondary processing	$Temp_{store}$	Temperature at which fillets are cold-stored until arriving at secondary processing			Very low temperatures, typically between 0 °C and 3 °C (Tocmo *et al.*, 2014)
	t_{store}	Time elapsed between end of filleting and start of secondary processing	h		
	C_{fillet}	Concentration of LM in fish fillets after cold storage in primary processing	Log CFU/g	$\mu_{fillet}=f_{SQRT}(Temp_{store})$ IF $(RL_{hold}=0)$ $C_{store}=C_{hold}+\log(\exp(\mu_{fillet}\times t_{store}))$ IF $(RL_{hold}>0)$ $\lambda_{fish\text{-}eqT}=\frac{\lambda_{fish}(Temp_{store}-1.33)^{1.66}}{(Temp_{hold}-1.3)^{1.66}}$ IF $(t_{store} > \lambda_{fish\text{-}eqT})$ $C_{store}=C_{hold}+\log(\exp(\mu_{fillet}\times(t_{store}-\lambda_{fish\text{-}eqT})))$ $RL_{store}=0$ IF $(t_{store} < \lambda_{fish\text{-}eqT})$ $C_{store}=C_{hold}$ $RL_{store}=\lambda_{fish\text{-}eqT}-t_{store}$	RL_{store} is the remaining lag time at temperature t_{store}

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 6.6: Variables related to the secondary processing of smoked fish

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Weights	W_{fillet}	Weight of one fish fillet	g		Assumed
	W_{css_pack}	Content of one pack of smoked fish	g		Assumed
	W_{slice}	Weight of one slice of cold-smoked fish	g		To be determined
	$Packs_{CSfillet}$	Number of packs that can be produced from one smoked fillet	-	ROUND (W_{fillet}/ W_{css_pack})	
Brining/ Salting	Brining	Probability that salting is done via brine injection (1 minus the probability of dry-salting)	-		EKE
	P_{ccb}	Probability that cross-contamination occurs during brining (brine solution is contaminated with LM)	-		3/14 according to Gudbjornsdottir *et al.* (2005); and 2/23 according to Gudbjornsdottir *et al.* (2004)
	$V_{injected}$	Volume of brine solution injected per fillet	ml		EKE

N_{brine}	Numbers of LM in brine solution	CFU/ml		To be obtained from the literature
P_{ccs}	Probability that cross-contamination occurs during dry salting (gloves, table surfaces)	-		To be obtained from the literature
$TR_{surf\text{-}fillet}$	Cross-contamination transfer coefficient from gloves, table or surface to the surface of fillet being dry-salted	%		To be obtained from the literature
$N_{surface}$	Numbers of LM on gloves, table or surfaces when fillet is being dry-salted	CFU		EKE
B_{fillet}	Status that the sampled fillet is contaminated with LM		Bernoulli (P_{fillet})	
B_{brine}	Status that the salting is carried out through brining	-	Bernoulli (Brining)	-
B_{dry}	Status that the salting is carried out through dry-salting		1 - Bernoulli (Brining)	-
B_{ccb+}	Status that brining solution is contaminated		Bernoulli (P_{ccb})	
B_{ccs+}	Status that cross-contamination occurred during dry-salting		Bernoulli (P_{ccs})	

	N_{salted}	Numbers of LM in a fillet right after salting (brining or dry salting)	CFU per fillet	IF (B_{brine}=1)
				IF (B_{fillet}=1) & (B_{ccb+}=1)
				Poisson ($10^{Cfillet}$ x W_{fillet}) +
				Poisson ($V_{injected}$ x N_{brine})
				IF (B_{fillet}=1) & (B_{ccb+}=0)
				Poisson ($10^{Cfillet}$ x W_{fillet}) + 0
				IF (B_{fillet}=0) & (B_{ccb+}=1)
				0 + Poisson ($V_{injected}$ x N_{brine})
				ELSE
				Remove
				IF (B_{dry}=1)
				IF (B_{fillet}=1) & (B_{ccs+}=1)
				Poisson ($10^{Cfillet}$ x W_{fillet}) +
				Binomial ($N_{surface}$,$TR_{surf-fillet}$)
				IF (B_{fillet}=1) & (B_{ccs+}=0)
				Poisson ($10^{Cfillet}$ x W_{fillet}) + 0
				IF (B_{fillet}=0) & (B_{ccs+}=1)
				0 + Binomial ($N_{surface}$,$TR_{surf-fillet}$)
				ELSE
				Remove	

	C_{salted}	Concentration of LM in fish fillets after salting	Log CFU/g	Log (1 + N_{salted})/ W_{fillet}	
	P_{salted}	Prevalence of salted fish fillets contaminated with LM	-		
Smoking and maturation	$R_{smoking}$	Reduction in LM concentration due to the joint effects of salting, smoking and maturation	Log CFU/g		Data from Neunlist *et al.* (2005) and Eklund *et al.* (1995) from Table A 6.3
	C_{smoked}	Concentration of LM in smoked fillets	Log CFU/g	C_{salted} – $R_{smoking}$	

Overall cross-contamination	$P_{poorHyg}$	Probability that the secondary processing facility has unsatisfactory hygiene	-		Probability of LM in drains can be used as interpreted in Rorvik *et al.* (1997)
	$F_{poorHyg}$	Log of the relative risk of the impact of poor hygiene on the prevalence of LM in smoked fillets	-		RR= 3.3 (95% CI: 0.84 – 12.9), according to Rorvik *et al.* (1997)
	$B_{poorHyg+}$	Status of poor hygiene in the facility	-	Bernoulli ($P_{poorHyg}$)	-

	$P_{salted-cc}$	Prevalence of smoked fish fillets contaminated with LM considering potential cross-contamination		IF ($B_{poorHyg+}$=1)
				($F_{poorHyg}$ x P_{salted}) / (1 – P_{salted} + $F_{poorHyg}$ x P_{salted})
				ELSE
				P_{salted}
					-

Slicing, cross-contamination and partitioning	P_{slicer}	Probability that the slicing machine is contaminated with LM	-		Slicing machines: 0/6 (Eklund *et al.*, 1995) Slicing machines: 35/95 (Di Ciccio *et al.*, 2012) Skinning, slicing and blending machine: 7/45 (Chen *et al.*, 2010) Slicing and skinning machine: 2/36 (Dass, 2011)
	$TR_{slicing}$	Cross-contamination transfer coefficient from slicing machine to slicer	%		Data from Aarnisalo *et al.* (2007)
	N_{slicer}	Numbers of LM on a blade	CFU		Data needed

b	Dispersion factor related to the heterogeneous distribution of LM on slices from a fillet	-	1	Moderate clustering
$B_{fillet+}$	Status of the smoked fillet being contaminated with LM	-	Bernoulli ($P_{salted\text{-}cc}$)	
$B_{slicer+}$	Status of slicing machine (blades) being contaminated with LM	-	Bernoulli (P_{slicer})	
N_{fillet}	LM cells in a contaminated fillet after smoking	CFU	IF ($B_{fillet+}$=1) & ($B_{slicer+}$=1) N_{fillet} = Poisson ($10^{Csmoked}$ x W_{fillet})	
$N_{slicedFillet}$	Total number of LM cells transferred to all slices from one fillet	CFU	$N_{slicedFillet}$ = Binomial (N_{slicer}, $TR_{slicing}$) N_{total} = N_{fillet} + $N_{slicedFillet}$	
N_{dist_CSpack}	LM cells distributed in a pack of smoked fish	CFU	N_{dist_CSpack} = Binomial (N_{total}, Beta (b, b x $Packs_{CSfillet}$ – 1) Pi_{pack} = ($Packs_{CSfillet}$ – 1)/(N_{total} + $Packs_{CSfillet}$ – 1)	
N_{total}	Total number of LM transferred to all slices of one fillet plus previous contamination	CFU	IF ($B_{fillet+}$=0) & ($B_{slicer+}$=1) $N_{slicedFillet}$ = Binomial (N_{slicer}, $TR_{slicing}$) N_{dist_CSpack} = Binomial ($N_{slicedFillet}$, Beta (b, b x $Packs_{CSfillet}$ – 1) Pi_{pack} = ($Packs_{CSfillet}$ – 1)/($N_{slicedFillet}$ + $Packs_{CSfillet}$ – 1)	

Pi_{pack}	Probability that a pack of smoked fish is not contaminated (despite having come from a contaminated fillet)	-	IF ($B_{fillet+}$=1) & ($B_{slicer+}$=0)
			N_{fillet} = Poisson ($10^{Csmoked}$ x W_{fillet})
			N_{dist_CSpack} = Binomial (N_{fillet}, Beta (b, b x $Packs_{CSfillet}$ – 1)
			Pi_{pack} = ($Packs_{CSfillet}$ – 1)/(N_{Fillet} + $Packs_{CSfillet}$ – 1)
			ELSE
			Remove	

P_{css}	Prevalence of smoked fish packs contaminated with LM	-	[1-Pi_{pack}] x [1 – (1 – $P_{salted\text{-}cc}$)x(1 – P_{slicer})]	
C_{css}	Concentration of LM in smoked fish packs	Log CFU/g	Log (N_{dist_CSpack} /W_{css_pack})	

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 6.7: Variables related to the secondary processing of gravad fish

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Weights	W_{fillet}	Weight of one fish fillet	g		Assumed
	W_{gs_pack}	Content of a pack of gravad fish	g		Assumed
	W_{slice}	Weight of one slice of gravad fish	g		To be determined
	$Packs_{GSfillet}$	Number of packs that can be produced from one gravad fillet	-	ROUND (W_{fillet}/ W_{gs_pack})	
Mixing and maceration	P_{ccm}	Probability that cross-contamination occurs during mixing with salt/sugar and maceration	-		4/12 on tables and trays according to Abdallah-Ruiz *et al.* (2022)
	$TR_{surf-fillet}$	Cross-contamination transfer coefficient from tables, trays or gloves to fillets being mixed with salt/sugar	%		To be obtained from the literature
	$N_{surface}$	Numbers of LM on surfaces in contact with the fillet being mixed with sugar and salt	CFU		EKE
	B_{fillet}	Status that the sampled fillet is contaminated with LM		Bernoulli (P_{fillet})	
	B_{ccm+}	Status that cross-contamination occurred during mixing/ maceration		Bernoulli (P_{ccm})	

	N_{mixing}	Numbers of LM in a fillet just before maturation but after mixing	CFU per fillet	IF (B_{fillet}=1) & (B_{ccm+}=1)
				Poisson ($10^{Cfillet}$ x W_{fillet}) +
				Binomial ($N_{surface}$,$TR_{surf-fillet}$)
				IF (B_{fillet}=1) & (B_{ccm+}=0)
				Poisson ($10^{Cfillet}$ x W_{fillet}) + 0
				IF (B_{fillet}=0) & (B_{ccm+}=1)
				0 + Binomial ($N_{surface}$,$TR_{surf-fillet}$)
				ELSE
				Remove	

	C_{mixed}	Concentration of LM in fish fillets after salting	Log CFU/g	Log (1 + N_{mixing})/ W_{fillet}	
	P_{mixed}	Prevalence of salted fish fillets contaminated with LM	-	1 – (1 – P_{fillet}) x (1 – P_{ccm})	
Intrinsic characteristics of gravad fish (as fillets being treated before packaging	pH, a_w, [LAC]	pH, water activity and undissociated lactic acid concentration of gravad fish (without packaging)	-, -, millimoles		Table A 6.4
Growth kinetics data for LM in gravad fish	MPD_{LM-GS}	Maximum population of LM in gravad fish	Log CFU/g	Uniform (8.6, 8.9)	From Mejlholm and Dalgaard (2007a)
	MPD_{LAB-GS}	Maximum population of LAB in gravad fish	Log CFU/g		Pérez-Rodríguez *et al.* (2017)
	$N_{0\ LAB-GS}$	Initial numbers of LAB in gravad fish	CFU/g	$10^{Pert(0.95, 2.90, 6.0)}$	Pérez-Rodríguez *et al.* (2017)
	$Q_{0\ LM-GS}$	Parameter Q_0 of LM in gravad fish	-		Construct a distribution using Equations 6.7 and 6.8
	$Q_{0\ LAB-GS}$	Parameter Q_0 of LAB in gravad fish	-		Pérez-Rodríguez *et al.* (2017)

Growth during maceration time	$Temp_{mac}$	Temperature of gravad fish during maceration	°C		Around 4 °C
	t_{mac}	Time for maceration	h		16 – 24 h
		Growth rate of LM in gravad fish	h^{-1}	Equations (6.2), (6.3), (6.4) and (6.5)	Evaluated at $Temp_{mac}$ and intrinsic properties pH, a_w, [LAC]
		Growth rate of LAB in gravad fish	h^{-1}	Equations (6.9), (6.10), (6.11) and (6.12)	Evaluated at $Temp_{mac}$ and intrinsic properties pH, a_w, [LAC]
	C_{mac}	Concentration of LM in gravad fish at the end of maceration	Log CFU/g	Equation (6.13)	Evaluated at 10^{Cmixed}, $N_{0\ LAB-GS}$, $Q_{0\ LM-GS}$, $Q_{0\ LAB-GS}$, MPD_{LM-GS}, MPD_{LAB-GS}
	Q_{LM-GS} (t_{mac})	Parameter Q for LM in gravad fish at time t_{mac}	-		Keep the value to initialize the cold-chain module
	Q_{LAB-GS} (t_{mac})	Parameter Q for LAB in gravad fish at the end of maceration time t_{mac}	-		Keep the value to initialize the cold-chain module
	N_{LAB-GS} (t_{mac})	Numbers of LAB in gravad fish at the end of maceration time t_{mac}	CFU/g		Keep the value to initialize the cold-chain module
Slicing, cross-contamination and partitioning	P_{slicer}	Probability that the slicing machine is contaminated with LM	-		Slicing machines: 0/6 (Eklund *et al.*, 1995) Slicing machines: 35/95 (Di Ciccio *et al.*, 2012) Skinning, slicing and blending machine: 7/45 (Chen *et al.*, 2010) Slicing and skinning machine: 2/36 (Dass, 2011)
	$TR_{slicing}$	Cross-contamination transfer coefficient from slicing machine to slicer	%		Data from Aarnisalo *et al.* (2007)

N_{slicer}	Numbers of LM on a blade	CFU		Data needed
b	Dispersion factor related to the heterogeneous distribution of LM on slices from a fillet	-	1	Moderate clustering
$B_{fillet+}$	Status of the macerated fillet being contaminated with LM	-	Bernoulli (P_{mixed})	
$B_{slicer+}$	Status of slicing machine (blades) being contaminated with LM	-	Bernoulli (P_{slicer})	

N_{fillet}
$N_{slicedFillet}$
N_{dist_GSpack}
N_{total}
Pi_{pack}
	LM cells in a contaminated fillet after maceration
	Total number of LM cells transferred to all slices from one fillet
	LM cells distributed in a pack of gravad fish
	Total number of LM transferred to all slices of one fillet plus previous contamination
	Probability that a pack of gravad fish is not contaminated (despite having come from a contaminated fillet)
		CFU
		
		CFU
		
		CFU
		
		CFU
		
		-
			IF ($B_{fillet+}$=1) & ($B_{slicer+}$=1)
			N_{fillet} = Poisson (10^{Cmac} x W_{fillet})
			$N_{slicedFillet}$ = Binomial (N_{slicer}, $TR_{slicing}$)
			N_{total} = N_{fillet} + $N_{slicedFillet}$
			N_{dist_GSpack} = Binomial (N_{total}, Beta (b, b x $Packs_{GSfillet}$ − 1)
			Pi_{pack} = ($Packs_{GSfillet}$ − 1)/(N_{total} + $Packs_{GSfillet}$ − 1)
			IF ($B_{fillet+}$=0) & ($B_{slicer+}$=1)
			$N_{slicedFillet}$ = Binomial (N_{slicer}, $TR_{slicing}$)
			N_{dist_CSpack} = Binomial ($N_{slicedFillet}$, Beta (b, b x $Packs_{GSfillet}$ − 1)
			Pi_{pack} = ($Packs_{GSfillet}$ − 1)/($N_{slicedFillet}$ + $Packs_{GSfillet}$ − 1)
			IF ($B_{fillet+}$=1) & ($B_{slicer+}$=0)
			N_{fillet} = Poisson (10^{Cmac} x W_{fillet})
			N_{dist_GSpack} = Binomial (N_{fillet}, Beta (b, b x $Packs_{GSfillet}$ − 1)
			Pi_{pack} = ($Packs_{GSfillet}$ − 1)/(N_{Fillet} + $Packs_{GSfillet}$ − 1)
			ELSE
			Remove	

P_{gs}	Prevalence of gravad fish packs contaminated with LM	-	[1-Pi_{pack}] x [1 − (1 − P_{mixed})x(1 − P_{slicer})]	
C_{gs}	Concentration of LM in gravad fish packs	Log CFU/g	Log (N_{dist_GSpack} /W_{gs_pack})	

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Table A 6.8: Variables related to the cold chain for smoked fish and gravad fish

Domain	Variable name	Description	Unit	Equations	Data/Assumptions
Intrinsic characteristics of smoked fish (after packaging)	pH, a_w, [LAC], P, CO_{2eq}, [DAC]	pH, water activity, undissociated lactic acid concentration, concentration of phenols, concentration of dissolved CO_2 at equilibrium, and concentration of undissociated diacetate in smoked fish (after packaging)	-, -, millimoles, ppm, ppm, millimoles		Table A 6.4
Intrinsic characteristics of gravad fish (after packaging)	pH, a_w, [LAC], CO_{2eq}, [DAC]	pH, water activity, undissociated lactic acid concentration, concentration of dissolved CO_2 at equilibrium, and concentration of undissociated diacetate in gravad fish (after packaging)	-, -, millimoles, ppm, millimoles		Table A 6.4
Growth kinetics data for smoked salmon	$MPD_{LM\text{-}CSS}$	Maximum population of LM in smoked fish	Log CFU/g	Uniform (8.6, 8.9)	From Mejlholm and Dalgaard (2007a)
	$MPD_{LAB\text{-}CSS}$	Maximum population of LAB in smoked fish	Log CFU/g		Pérez-Rodríguez *et al*. (2017)
	$N_{0\ LAB\text{-}CSS}$	Initial numbers of LAB in smoked fish	CFU/g	$10^{\ Pert(0.95,\ 2.90,\ 6.0)}$	Pérez-Rodríguez *et al*. (2017)
	$Q_{0\ LM\text{-}CSS}$	Parameter Q_0 of LM in smoked fish	-		Construct a distribution using Equations 6.7 and 6.8
	$Q_{0\ LAB\text{-}CSS}$	Parameter Q_0 of LAB in smoked fish	-		Pérez-Rodríguez *et al*. (2017)

Growth kinetics data for smoked salmon	$MPD_{LM\text{-}GS}$	Maximum population of LM in gravad fish	Log CFU/g	Uniform (8.6, 8.9)	From Mejlholm and Dalgaard (2007a)
	$MPD_{LAB\text{-}GS}$	Maximum population of LAB in gravad fish	Log CFU/g		Pérez-Rodríguez *et al.* (2017)
	$N_{0\,LAB\text{-}GS}$	Initial numbers of LAB in gravad fish	CFU/g	$10^{\,Pert(0.95,\,2.90,\,6.0)}$	Pérez-Rodríguez *et al.* (2017)
	$Q_{0\,LAB\text{-}CSS}$	Parameter Q_0 of LAB in gravad fish	-		Pérez-Rodríguez *et al.* (2017)
Shelf-life	SL_{css}	Lifetime of smoked fish, considering extra time given by the consumer	h		Data needed
	SL_{gs}	Lifetime of gravad fish, considering extra time given by the consumer	h		Data needed
Cold chain for both products	$Temp_{endp}$	Temperature after packaging	°C		To be assumed
	t_{endp}	Storage time before dispatch	h		To be assumed
	$Temp_{p\text{-}r}$	Temperature during transportation from end of processing to retail	°C		To be assumed
	$t_{p\text{-}r}$	Transportation time from end of processing to retail	h		To be assumed
	$Temp_r$	Temperature at retail	°C		To be assumed
	t_r	Time at retail	h		To be assumed
	$Temp_{r\text{-}h}$	Temperature during transportation from retail to home	°C		To be assumed

	t_{r-h}	Transportation time from retail to home	h		To be assumed
	$Temp_{home}$	Storage temperature at home	°C		To be assumed
Copula	R_{MPD-T}	Correlation coefficient between MPD of LM and storage temperature, used to build a copula for sampling MPD_{LM-CSS} and MPD_{LM-GS}	-	Uniform (0.45 – 0.52)	Pérez-Rodríguez *et al*. (2017)
	R_{MPD-N0}	Correlation coefficient between MPD and initial concentration of LM used to build a copula for sampling MPD_{LM-CSS} and MPD_{LM-GS}	-	Uniform (0.52 – 0.88)	Hwang and Sheen (2009) and Besse *et al.* (2006).
Time to consumption	t_{home_css}	Time to consumption of cold-smoked fish	h		To be assumed
	t_{home_gs}	Time to consumption of gravad fish	h		To be assumed
Growth during cold-chain for smoked fish		A vector of 5 growth rates of LM in smoked salmon at the different storage/display temperatures	h^{-1}	Equations (6.2), (6.3), (6.4) and (6.5)	Evaluated at intrinsic properties pH, a_w, [LAC], P, CO_{2eq}, [DAC] and at temperatures $Temp_{endp}$, $Temp_{p-r}$, $Temp_r$, $Temp_{r-h}$, $Temp_{home}$
		A vector of 5 growth rates of LAB in smoked salmon at the different storage/display temperatures	h^{-1}	Equations (6.9), (6.10), (6.11) and (6.12)	Evaluated at intrinsic properties pH, a_w, [LAC], P, CO_{2eq}, [DAC] and at temperatures $Temp_{endp}$, $Temp_{p-r}$, $Temp_r$, $Temp_{r-h}$, $Temp_{home}$

	$C_{css\text{-}cons}$	Concentration of LM in smoked fish at the point of consumption	Log CFU/g	IF $(t_{endp} + t_{p\text{-}r} + t_r + t_{r\text{-}h} + t_{home}) < SL_{css}$ Equation (5.13) Integrated for the (T,t) profile: Temp: $Temp_{endp}$, $Temp_{p\text{-}r}$, $Temp_r$, $Temp_{r\text{-}h}$, $Temp_{home}$ Time: t_{endp}, $t_{p\text{-}r}$, t_r, $t_{r\text{-}h}$, t_{home}	Evaluated at 10^{Ccss}, $N_{0\ LAB\text{-}CSS}$, $Q_{0\ LM\text{-}CSS}$, $Q_{0\ LAB\text{-}CSS}$, $MPD_{LM\text{-}CSS}$, $MPD_{LAB\text{-}CSS}$
	$P_{css\text{-}cons}$	Prevalence of smoked fish contaminated with LM at the point of consumption	-	P_{css}	
Growth during cold-chain for gravad fish		A vector of 5 growth rates of LM in gravad salmon at the different storage/display temperatures	h^{-1}	Equations (6.2), (6.3), (6.4) and (6.5)	Evaluated at intrinsic properties pH, a_w, [LAC], CO_{2eq}, [DAC] and at temperatures $Temp_{endp}$, $Temp_{p\text{-}r}$, $Temp_r$, $Temp_{r\text{-}h}$, $Temp_{home}$
		A vector of 5 growth rates of LAB in gravad salmon at the different storage/display temperatures	h^{-1}	Equations (6.9), (6.10), (6.11) and (6.12)	Evaluated at intrinsic properties pH, a_w, [LAC], CO_{2eq}, [DAC] and at temperatures $Temp_{endp}$, $Temp_{p\text{-}r}$, $Temp_r$, $Temp_{r\text{-}h}$, $Temp_{home}$
	$C_{gs\text{-}cons}$	Concentration of LM in packs of gravad fish at the point of consumption	Log CFU/g	IF $(t_{endp} + t_{p\text{-}r} + t_r + t_{r\text{-}h} + t_{home}) < SL_{gs}$ Equation (6.13) Integrated for the (T,t) profile: Temp: $Temp_{endp}$, $Temp_{p\text{-}r}$, $Temp_r$, $Temp_{r\text{-}h}$, $Temp_{home}$ Time: t_{endp}, $t_{p\text{-}r}$, t_r, $t_{r\text{-}h}$, t_{home}	Evaluated at 10^{Cmac}, $N_{LAB\text{-}GS}(t_{mac})$, $Q_{LM\text{-}GS}(t_{mac})$, $Q_{LAB\text{-}GS}(t_{mac})$, $MPD_{LM\text{-}GS}$, $MPD_{LAB\text{-}GS}$
	$P_{gs\text{-}cons}$	Prevalence of gravad fish packs contaminated with LM at the point of consumption	-	P_{gs}	

Sources: **Data/Assumptions** column cites the corresponding studies. See References section for further details.

Annex 7 Virulence groups for *L. monocytogenes*

Table A 7.1: Classes of virulence and associated CC/STs proposed by Fritsch *et al.* (2018) based on data from Maury *et al.* (2016)

More Virulent	Virulent	Less virulent
CC1, CC101, CC2, CC220, CC224, CC4, CC451, CC54, CC6, CC7, CC87	CC14, CC155, CC177, CC18, CC20, CC21, CC26, CC3, CC37, CC379, C388, CC398, CC5, CC59, CC8, CC403, any other CCs	CC121, CC204, CC31, CC9, CC193, CC19, ST214

Sources: See References section.

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In response to a request from the 52nd Session of the Codex Committee on Food Hygiene (CCFH), the Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) convened a meeting, with the objective of developing formal full risk assessment models for *Listeria monocytogenes* in lettuce, cantaloupe, frozen vegetables and ready-to-eat fish.

In the light of the available data and the current risk assessment approaches, the expert group aimed to collectively ascertain the stages from primary production to consumption to be represented in the model, including approaches that accommodate the testing of scenarios, interventions and sampling schemes that could reduce the risk of listeriosis.

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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