

Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada: Report.

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An Interpretative Summary of this Risk Assessment, Appendixes, Answers to public comments and the Analytica® model are available at

<http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/>

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Abbreviations and Acronyms

a_w	Water activity
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CRC	Consolidated Regulations of Canada
cfu	Colony Forming Unit
d	Day
EGR	Exponential growth rate
FAO	Food and Agriculture Organization of the United Nations
FDA	U.S. DHHS Food and Drug Administration
FoodNet	Foodborne Diseases Active Surveillance Network
FSIS	USDA Food Safety Inspection Service
GT	Generation Time
HC – SC	Health Canada – Santé Canada
IC	Immunocompromised
IDFA	International Dairy Food Association
<i>Lm</i>	<i>Listeria monocytogenes</i>
MC	Monte-Carlo
m.l.e.	maximum likelihood estimator
MPN	Most Probable Number
PMC	Pasteurized-Milk Cheese
RMC	Raw-Milk Cheese
RTE	Ready-to-Eat or Ready-to-Eat food
UPC	Universal Product Code
USDA	United States Department of Agriculture
U.S. DHHS	United States Department of Health and Human Services
WHO	World Health Organization
w/w	weight in weight

Mathematical / Statistical Notations

$Beta(a, b)$	Beta distribution with shape parameters a and b
$Bernoulli(p)$	Bernoulli distribution with parameter p . Equivalent to $Binomial(1, p)$
$Binomial(n, p)$	Binomial distribution with number of trials n and probability p
CI95%	95% Confidence Interval or 95% Credible Interval
$E[x]$	Expected value of the random variable x
e^x or $\exp(x)$	Exponential of x
$Exponential(a)$	Exponential distribution with scale parameter a
$Gamma(a, s)$	Gamma distribution with shape parameter a and scale parameter s
$Laplace(a, b)$	Laplace distribution with location a and scale b
$Pr(x)$	Probability of x
$Pr(x y)$	Conditional probability of x given y
$Normal(\mu, \sigma)$ or $N(\mu, \sigma)$	Normal (Gaussian) distribution with mean μ and standard deviation σ
$\ln(x)$	Natural (base e) logarithm of x
$\logNormal(\mu, \sigma)$ or $LN(\mu, \sigma)$	Log normal distribution. $x \sim LN(\mu, \sigma)$ if $\ln(x) \sim N(\mu, \sigma)$
$\log(x)$ or $\log_{10}(x)$	Logarithm of x to base 10
$\log_b(x)$	Logarithm of x to base b
$\text{logit}(p)$	$\ln(p/(1-p))$
$Poisson(\lambda)$	Poisson distribution with mean λ
$\rho(x, y)$	Spearman rank correlation coefficient between x and y (Spearman's rho)
se	Standard error
$Triangular(a, b, c)$	Triangular distribution with minimum a , mode b and maximum c
$TruncatedNormal(\mu, \sigma, a, b)$	Truncated normal distribution <i>i.e.</i> $N(\mu, \sigma)$ restricted to the domain $[a, b]$
$Uniform(a, b)$	Uniform distribution from a to b
$Beta-Pert(a, b, c)$	Beta-Pert distribution with minimum a , most likely value b and maximum value c
$Weibull(a, b)$	Weibull distribution with shape a and scale b

Summary

Background

Listeria monocytogenes is a widely occurring pathogen that can be found in agricultural and food processing environments. Ingestion of *L. monocytogenes* can lead to the development of listeriosis, which may affect only in the gastrointestinal tract, but can invade other parts of the body (referred to as “invasive listeriosis”), with consequences that may include septicemia, meningitis, encephalitis, spontaneous abortion, and stillbirth. Epidemiological data show that listeriosis has one of the highest hospitalization rates and one of the highest case fatality rates among foodborne diseases in the United States (Mead et al. 1999; Scallan et al. 2011). Serious illness occurs preferentially in people considered as more susceptible, such as the elderly and those who have a pre-existing illness that reduces the effectiveness of their immune system, and in pregnant women (Rocourt 1996; Goulet et al. 2012).

The U.S. and Canada have experienced sporadic illnesses and outbreaks of listeriosis associated with the consumption of cheeses, including soft-ripened cheese. Both the U.S. Department of Health and Human Services / Food and Drug Administration (FDA) and Health Canada – Santé Canada (HC-SC) / Food Directorate continue to evaluate the safety of soft cheese, particularly soft cheese made from unpasteurized milk.

The *Listeria monocytogenes* in soft-ripened cheese risk assessment evaluates the effect of factors such as the microbiological status of milk, the impact of cheese manufacturing steps on *L. monocytogenes* levels, and conditions during distribution and storage on the overall risk of invasive listeriosis to the consumer, following the consumption of soft-ripened cheese in Canada and in the U.S. The risk assessment makes it possible to evaluate the effectiveness of some process changes and intervention strategies in reducing risk of illness.

A draft interpretive summary, draft report, draft appendices, and the draft Analytica[®] model were made available, on February 8, 2013, for public comments (Federal Register, Vol. 78, No. 28, pp 9701-9702). The comment period closed April 29, 2013. We received 96 comments. We

considered the comments that pertained directly to the risk assessment and have responded to them in a separate document.¹ Some of the revisions to the draft of the full risk assessment report were made in response to these comments.

Scope and General Approach

The *Listeria monocytogenes* soft-ripened cheese risk assessment focuses on the source(s) of *L. monocytogenes* contamination, the effects of individual manufacturing and/or processing steps, and the effectiveness of various intervention strategies on the levels of *L. monocytogenes* in the product as consumed and the associated risk of invasive listeriosis. The scope of this quantitative microbial risk assessment is:

- Pathogen of Concern: *Listeria monocytogenes*.
- Food(s) of Concern: Soft-ripened cheese.
- Populations of Interest: The general populations of the U.S. and Canada, and subpopulations identified as at-risk in both countries (*i.e.*, pregnant women, immunocompromised individuals and the elderly population).
- Endpoint(s) of concern: Invasive listeriosis.
- Risk metric: The probability of invasive listeriosis per soft-ripened cheese serving.

The risk assessment follows *Codex alimentarius*, U.S. and Canadian recommendations (*Codex alimentarius* Commission 1999; Health Canada Decision Making Framework 2000; CFSAN Risk Analysis Working Group 2002). It comprises *hazard identification*, *hazard characterization*, *exposure assessment* and *risk characterization* components (*Codex alimentarius* Commission 1999).

The primary metric used in this report is the risk per serving of soft-ripened cheese. A fully quantitative approach is taken and mathematical / probabilistic modeling is employed to estimate

¹ Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada: Replies to Public Comments available at <http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/>

the risk per serving of soft-ripened cheese in both countries, as well as to test the effects of some alternatives on those risks, as requested in the management charge (see Appendix,² section “Charge developed by the Risk Manager Team”). A second-order (or two dimensional) Monte-Carlo simulation is used (Frey 1992). This framework lets one evaluate separately the variability (from serving to serving, from subpopulation to subpopulation, from country to country) in the risk estimates and the uncertainty about those estimates of variability in the risk that accrues from, particularly, data uncertainty.

The model structure is based on literature data, previous risk assessments (Bemrah et al. 1998; FDA/FSIS 2003; FAO/WHO 2004; Sanaa et al. 2004) and expert sources (Health Canada, Bureau Microbial Hazards; FDA CFSAN). Data were obtained from the literature (see section “References”), from government nutrition surveys (National Center for Health Statistics 2003-2004; Statistics Canada 2004), from a specific survey on home storage time and temperature practices (RTI International et al. 2005) and from specific expert elicitations (CFSAN 2008; IDFA 2008).

This summary provides an overview of the methods used and the main results of this risk assessment. The major reference remains the body of this report and its appendices. The reader should refer to the specific sections for details on the model, the results of the risk assessment and limitations on interpretations.

Risk Assessment

Hazard Identification

The biology, pathology, and ecology of *L. monocytogenes* and the epidemiology of *L. monocytogenes* as a foodborne hazard have been extensively described in previous risk assessments (FDA/FSIS 2003; FSIS 2003; FAO/WHO 2004) and in the microbiological literature (e.g. Swaminathan and Gerner-Smidt 2007). Only a summary of this information is presented in the report (see section 4, “Hazard Identification”).

² Available at <http://www.fda.gov/Food/FoodScienceResearch/RiskSafetyAssessment/>

Hazard Characterization

In this risk assessment, the “Elderly” population, the “Pregnant women” population, and the “Immunocompromised” population are considered as “susceptible,” following FAO/WHO (2004). The “Immunocompromised” population is deemed to include individuals like those in the “General” population except for a medical condition that makes the individuals more susceptible to invasive listeriosis.

The FAO/WHO (2004) models are used as dose-response models to evaluate the probability of invasive listeriosis following the ingestion of a given dose of *L. monocytogenes*. These models are exponential models (Haas et al. 1999, p. 264-266) and their parameterization uses the FAO/WHO (2004) inferences from epidemiological data (Mead et al. 1999) and the detailed exposure assessment developed in the U.S. (FDA/FSIS 2001). These models have a single parameter, r , which one commonly interprets as the probability that a single cell will cause invasive listeriosis in an individual at random. In this risk assessment, one value of the parameter r , point estimate 1.06×10^{-12} , is used for the Elderly, Immunocompromised and Pregnant women populations (*i.e.* the “susceptible population” (FAO/WHO 2004)), and another value of the parameter r , point estimate 2.37×10^{-14} , is used for the remaining “General” (“non-susceptible” (FAO/WHO 2004)) population (see Table 2.17, p. 56 and Table 2.20, p.58, FAO/WHO 2004). An uncertainty distribution is associated with the r parameters when uncertainty is considered in the model (see section 5, “Hazard Characterization”).

Exposure Assessment

A full “farm-to-fork” product pathway or process model is developed to address the questions posed by the management charge (see section 6, “Basic Processes” and section 7, “Exposure Assessment” and Appendix, section “Charge developed by the Risk Manager Team”). A pasteurized-milk soft-ripened cheese baseline model and a raw-milk soft-ripened cheese baseline model serve as reference cases to compare the risk under other alternative scenarios.

Pasteurized-Milk Cheese Baseline Model

The pasteurized-milk cheese baseline model considers the manufacture of a Camembert-like soft-ripened cheese, as a representative example of soft-ripened cheese, made from pasteurized milk, using the stabilized cheese process (Kosikowski and Mistry 1987; Lawrence et al. 1987). From this point onwards, if a reference is made in this report to “pasteurized-milk cheese” or PMC in the context of the risk assessment model structure or its outputs, the reference is made to include soft-ripened cheese.

It is assumed that all bacteria present in the milk are definitively inactivated during the pasteurization step. In this baseline scenario, contamination with *L. monocytogenes* happens from environmental *L. monocytogenes* in the processing plant and contamination occurs after the ripening phase and before packaging. No other contamination (*e.g.* at store or at home) is considered in this baseline scenario or in other scenarios. The frequency and level of *L. monocytogenes* in-plant contamination is inferred from detection and enumeration data obtained by Gombas *et al.* (2003) on soft-ripened cheeses at retail in two U.S. FoodNet sites and a “back-calculation” procedure to derive the distribution of the level of in-plant contamination.

Bacterial growth is modeled from the point of contamination to the point of consumption. The full growth model includes the lag time in the growth when bacterial contamination comes from the environment and includes a specific model for growth in a solid medium such as cheese. Growth parameters –lag time, growth rate and maximum population density- are inferred from meta-analyses of literature data (see section 6.1, “Growth”). Bacterial growth is affected by the temperature and the storage time during aging, during transport and marketing, at retail and at home. Time and temperature profiles are derived from specific studies and from expert elicitation (RTI International et al. 2005; CFSAN 2008; IDFA 2008). Due to a lack of specific national data, cheese processing, time and temperature during aging, transport and marketing and at retail are considered to be the same in both countries.

Soft-ripened cheese serving size distributions are inferred from data from government nutrition surveys in Canada and the U.S. (National Center for Health Statistics 2003-2004; Statistics Canada 2004).

Table 1 summarizes. In it, we show how much the model specification differentiates among subpopulations and countries:

- Bulk milk prevalence is specific to Canada and U.S.;
- *L. monocytogenes* levels in contaminated milk, growth in milk and growth in cheese are common to Canada and U.S.;
- Milk and cheese processing is common to Canada and U.S.;
- Transport and marketing, and retail storage data are from U.S. sources and are extrapolated to Canada;
- Home storage time and temperature data are from U.S. sources and are extrapolated to Canada;
- Serving size distributions are inferred from countries' national nutrition surveys;
- Dose response function parameterizations are common to Canada and U.S. and distinguish susceptible and non-susceptible populations.

Table 1: Level of variability considered in terms of process, data and estimates, according to subpopulations and country.

The same letter and case indicates that the same data and distribution model are used for the considered subpopulations. Variability specifications also include a full or at least a partial model of data uncertainty.

Process / Data / Estimates	Canada: Elderly	Canada: Pregnant women	Canada: IC*	Canada: General	United States: Elderly	United States: Pregnant women	United States: IC*	United States: General
<i>L. monocytogenes</i> contamination in farm	A	A	A	A	B	B	B	B
<i>L. monocytogenes</i> contamination during processing	C	C	C	C	C	C	C	C
Milk and Cheese processing, transport and marketing, from farm to retail (inclusive)	D	D	D	D	D	D	D	D
<i>L. monocytogenes</i> growth model and parameters	E	E	E	E	E	E	E	E
Storage time at home	F	G	H	H	I	J	K	K
Storage temperature at home	L	M	N	N	L	M	N	N
Serving size	O	P	Q	Q	R	R	R	R
Resulting exposure assessment	S	T	U	U	V	W	X	X
Dose Response	Y	Y	Y	Z	Y	Y	Y	Z
Resulting Risk Assessment	a	b	c	d	e	f	g	h

* Immunocompromised.

Raw-Milk Cheese Baseline Model

An alternative baseline deals with the manufacture of a Camembert-like soft-ripened cheese made from raw milk, using the traditional process (Sanaa et al. 2004). From this point onwards, if a reference is made in this report to “raw-milk cheese” or RMC in the context of the risk assessment model structure or its outputs, the reference is made to include soft-ripened cheese.

Following others (Bemrah et al. 1998; Sanaa et al. 2004), this model for raw-milk soft-ripened cheese includes a farm model considering two sources of contamination: environmental contamination on farm, and contamination from mastitic cows. Distributions for bulk tank prevalence are inferred from meta-analyses of farm bulk tank surveys done in Canada and in the U.S. A distribution for the levels of contamination in contaminated bulk tank milk is inferred

from the scientific literature (see section 7.1, “On Farm”). Growth in milk during farm tank storage, tanker truck transport and dairy silo storage is modeled using growth parameters in milk from the literature. Specific bacterial growth and inactivation during the cheese processing (ripening) are inferred from the literature on that subject (Ryser and Marth 1987; Back et al. 1993; Sanaa et al. 2004; Ryser 2007; Liu and Puri 2008; Liu et al. 2009). Growth in cheese during ripening is modeled using environmental parameters of “traditional process” (Sanaa et al. 2004), in contrast to the “stabilized process” used for industrialized cheeses in the pasteurized-milk cheese baseline (Kosikowski and Mistry 1987; Lawrence et al. 1987). The post-ripening process for raw-milk cheeses is the same as for pasteurized-milk cheeses except that raw-milk cheeses are stored for a minimum of 60 days from the date of the beginning of the manufacturing process at a temperature of at least 2°C (35°F), according to Canadian and U.S. regulations (Food and Drugs Act B.08.030, B.08.043, B.08.044³ and 21 CFR 133.182(a)⁴, respectively). In the raw-milk cheese baseline, milk is collected for cheese-making from a single herd, on the farm where the cheese-making operation resides (farmstead-scale operations). Artisanal-scale operations, where milk for cheese-making is collected from 2 farms and pooled, is also evaluated, but is not used as a baseline for purposes of this risk assessment.

Alternative Scenarios

For raw-milk cheeses, other alternatives are evaluated and compared to the baseline, pasteurized-milk cheese case and to the baseline raw-milk cheese case. These alternatives are:

- Apply an unspecified procedure that reduces the bacterial load in milk by 3 log₁₀, 4 log₁₀, 5 log₁₀ or 6 log₁₀ (i.e. an average 1,000-fold; 10,000-fold; 100,000-fold or 1,000,000-fold reduction in the concentration, respectively);
- Apply an unspecified antimicrobial substance that reduces the surface *L. monocytogenes* contamination by 2 log₁₀ (i.e. an average 100-fold reduction in the concentration);

³ http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._870/index.html

⁴ <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?cfrpart=133>

- Remove the 60-day aging regulation in place in Canada and in the U.S. for soft-ripened cheese; in that alternative, raw-milk soft-ripened cheeses are aged for the same lengths of time as pasteurized-milk soft-ripened cheeses;
- Test 25 ml of raw milk from the farm tank, from the tanker truck or from the dairy silo, and remove the detected *L. monocytogenes* positive units;
- Test a sample of 25 g from 5 cheeses of every cheese lot⁵, and remove the detected positive lots.

Another evaluated alternative is the implementation of a testing procedure for lots of pasteurized-milk cheeses.

Following *Codex alimentarius*, U.S. and Canadian recommendations, evaluations of the availability, feasibility and cost of mitigations is done, not as part of the risk assessment (this report), but externally to the risk assessment, as part of the risk management that the risk assessment would inform. For example, the risk assessment does not consider the availability of a specific milk mitigation alternative that achieves a 3-6 log₁₀ reduction in *L. monocytogenes* concentration in bulk milk, nor the feasibility of testing some or all bulk milk prior to cheese making, nor the availability of an antimicrobial agent, nor the cost of testing some or all cheese lots.

For the baseline models and the alternatives, the exposure assessment outputs are the distribution of the frequency of contaminated servings and the distribution of the number of *L. monocytogenes* per contaminated soft-ripened cheese serving. These distributions vary among

⁵ This protocol is considered for risk assessment purposes as representative of a typical protocol likely to be used by manufacturers. It is more sensitive (less false negative results) than sampling a single analytical portion of 25 g from the same lot. Nevertheless, it is different from, and less sensitive than the composite protocol (*i.e.* 50 g from each of 5 cheeses for each composite and two composites from the lot) recommended in the FDA Bacteriological Analytical Manual (BAM) (FDA 2011) for the detection of *Listeria monocytogenes* in cheese products that is typically used for regulatory purposes.

subpopulations and between countries, as a result of the propagation of different serving size, home storage time and home storage temperature distributions.

The exposure assessment results suggest very high variability in the number of *L. monocytogenes* at the time of consumption amongst contaminated servings⁶:

- the prevalence of contaminated servings is predicted to be about 0.6-0.7% (6-7 per 1000 servings) for pasteurized-milk cheese, all from environmental contamination, and the prevalence of contaminated servings is predicted to be 3.2% for raw-milk cheese made in Canada and 4.7% for raw-milk cheese made in the U.S.;
- for the Canadian Elderly population, 50% of contaminated servings of pasteurized-milk cheese are predicted to have 17 or less cfu/serving; 90% of contaminated servings are predicted to have less than 5,135 cfu/serving;
- for pasteurized-milk cheese and raw-milk cheese, few servings are predicted to be heavily contaminated, for example, at levels that reach the maximum population density of *L. monocytogenes*.

Risk Characterization

The outputs from the exposure assessment are combined with the dose-response model to develop the risk characterization outputs (see section 8, “Risk Characterization (Method)”). Combined with the dose-response models used for the “susceptible” and the “non susceptible” populations, risk estimates differ among the Elderly, the Pregnant women, the Immunocompromised and the General populations and between the two countries (Table 1, Table 2). The major outputs of the baseline models are expressed as the risk of invasive listeriosis per soft-ripened cheese serving at random, in a specified population (Canada or U.S.; Elderly, Immunocompromised, or Pregnant women population and General population). The risk outputs for alternative scenarios are described also by the ratio of the mean risk of invasive listeriosis per serving for the considered alternative scenario to the mean risk of invasive

⁶ Results provided in this summary are for the pasteurized-milk cheese and raw milk, farmstead operation scale only; additional results for the artisanal-scale operation are provided in the report, notably section 7.1 “On farm” and section 10, “Results of the Model Application Alternatives”.

listeriosis per serving for the baseline, pasteurized-milk cheese case or the baseline, raw-milk cheese case. A sensitivity analysis of the pasteurized-milk cheese baseline model is performed by changing one parameter at a time or using classical Spearman's rank correlation coefficients.

Pasteurized-milk cheese baseline model (see section 9, "Results of the Model Application Examples")

If no data uncertainty is considered, the predicted mean risk of invasive listeriosis from consumption of a serving of pasteurized soft-ripened cheese per serving varies as

- 7.2×10^{-9} , 1.8×10^{-8} , 6.1×10^{-9} among the susceptible populations (Elderly, Pregnant women and Immunocompromised, respectively) in Canada and 1.4×10^{-10} in the non-susceptible population (General) in Canada; and,
- 7.3×10^{-9} , 1.8×10^{-8} , 5.2×10^{-9} among the susceptible populations (Elderly, Pregnant women and Immunocompromised, respectively) in the U.S. and 1.2×10^{-10} in the non-susceptible population (General) in the U.S.

These mean values correspond to one case of invasive listeriosis per

- 138 million servings in the Elderly population, 56 million servings in the Pregnant women population, 163 Million servings in the Immunocompromised population and 7,290 Million servings in the General population, in Canada; and,
- 136 Million servings for the Elderly population, 55 Million servings for the Pregnant women population, 193 Million servings for the Immunocompromised population and 8,644 Million for the General population, in the U.S.

Differences among subpopulations and between Canada and U.S. come from differences in the characteristics that influence the risk: serving sizes, home storage characteristics and dose-response.

Results from the second-order Monte-Carlo simulation for the pasteurized-milk cheese baseline case suggest that the serving-to-serving *variability* in the risk largely overwhelms the data *uncertainty*, as considered in this report. The sensitivity analysis suggests that the main factors that influence the variability in the risk per serving among servings within the same

subpopulation are the prevalence and the level of the initial environmental contamination during cheese manufacture, and the amount of bacterial growth in cheese, particularly during home storage. The main influential factor for our uncertainty about that variability is the dose-response r parameter.

Alternative Scenarios (see section 10, “Results of the Model Application Alternatives”)

For populations in Canada, predicted mean risk per raw-milk soft-ripened cheese serving at random varies as 3.8×10^{-7} , 9.2×10^{-7} , 4.2×10^{-7} among the susceptible populations (Elderly, Pregnant women, Immunocompromised, respectively) and 9.5×10^{-9} in the non-susceptible population (General). These values correspond to one case of invasive listeriosis per 2,600,000 servings eaten by individuals in the Elderly population, 1,100,000 servings in the Pregnant women population, 2,400,000 servings in the Immunocompromised population and 105 Million servings in the General population. For the Elderly population in Canada, the predicted mean risk of invasive listeriosis from consuming a raw-milk soft-ripened cheese serving at random is 53 times higher than the mean risk for pasteurized-milk cheese and the mean risk is 52, 69 and 69 times higher for the Pregnant women, the Immunocompromised and the General populations in Canada, respectively.

For populations in the U.S., the predicted mean risk per raw-milk soft-ripened cheese serving at random varies as 8.2×10^{-7} , 1.8×10^{-6} , 8.1×10^{-7} among the susceptible Elderly, Pregnant women and Immunocompromised populations, respectively, and 1.8×10^{-8} in the non-susceptible General population. These values correspond to one case of invasive listeriosis per 1,200,000 servings eaten by individuals in the Elderly population, 570,000 servings in the Pregnant women population, 1,200,000 servings in the Immunocompromised population and 55 Million servings in the General population in the U.S. This predicted mean risk of invasive listeriosis from consuming a raw-milk soft-ripened cheese serving at random is 112, 96, 157 and 157 times higher than the mean risk for pasteurized-milk cheese for the Elderly, Pregnant women, Immunocompromised and General populations in the U.S., respectively.

Amongst all the evaluated alternatives for raw-milk cheeses, applying a procedure that reduces the bacterial load in raw milk by 6 log₁₀ and testing every raw-milk cheese lot are the only alternatives that lead to a predicted mean risk per raw-milk soft-ripened cheese serving at random lower than the one obtained in the pasteurized-milk cheese baseline scenario (Table 2). The impact of testing raw-milk cheese lots is nevertheless very sensitive to the proportion of cheese lots that are tested. The other alternatives are less efficient. Removing the 60 days regulation reduces the predicted risk of invasive listeriosis following the consumption of raw-milk soft-ripened cheese by a factor of approximately 1.5-2 for Canada and for the U.S. compared to the baseline raw-milk cheese case. A 3 log₁₀, a 4 log₁₀ or a 5 log₁₀ reduction of milk contamination before the cheese processing would reduce the predicted mean risk by a factor of approximately 7.2 (Canada) or 10 (U.S.), 35 or 50, and 56 or 95 compared to the baseline raw-milk cheese scenario, respectively, but would still lead to a higher mean risk than the one estimated in the pasteurized-milk cheese baseline. The impact of a hypothetical substance (an antimicrobial voluntarily added during the manufacture of the raw-milk cheese) that would reduce the *L. monocytogenes* concentration present at the surface of the cheese by 2 log₁₀ cfu would provide a lower mean risk of invasive listeriosis per serving than estimated in the raw-milk cheese baseline, but this risk would still be 50 and 86 times higher than the mean risk per serving of pasteurized-milk cheese, as estimated in the pasteurized-milk cheese baseline, in Canada and the U.S., respectively. For raw-milk cheeses, testing milk is less efficient than testing cheese lots. Testing milk in the farm tank at every milking reduces the predicted mean risk by a factor of approximately 24 in Canada and 37 in the U.S. compared to the baseline raw-milk cheese scenario, which includes no bulk milk testing, and remains still more risky than the pasteurized-milk cheese baseline case.

Finally, testing pasteurized-milk cheese lots has no, or little, impact on the predicted mean risk for pasteurized-milk cheeses.

Table 2: Impact of various alternatives on the predicted mean risk of invasive listeriosis per soft-ripened cheese serving relative to the risk per serving of baseline cases for Elderly population in Canada and in the U.S.

See table footnotes. See the report for all details and limitations.

Alternative	Baseline: Pasteurized- milk cheese, Canada	Baseline: Pasteurized-milk cheese, United States	Baseline: Raw-milk cheese, Canada	Baseline: Raw-milk cheese, United States
Pasteurized-milk cheese, Baseline	1 (Reference)	1 (Reference)	0.019** = 1/53	0.009 = 1/112
Raw-milk cheese, Baseline	53*	112	1 (Reference)	1 (Reference)
Raw-milk cheese, no 60 day aging condition	36	62	0.67 = 1/1.5	0.56 = 1/1.8
Raw-milk cheese if a process that leads to a 3 log ₁₀ reduction of the <i>L. monocytogenes</i> contamination in incoming milk is applied	7.4	11	0.14 = 1/7.2	0.10 = 1/10
Raw-milk cheese if a process that leads to a 4 log ₁₀ reduction of the <i>L. monocytogenes</i> contamination in incoming milk is applied	1.7	2.0	0.028 = 1/35	0.020 = 1/50
Raw-milk cheese if a process that leads to a 5 log ₁₀ reduction of the <i>L. monocytogenes</i> contamination in incoming milk is applied	1.1	1.2	0.018 = 1/56	0.011 = 1/95
Raw-milk cheese if a process that leads to a 6 log ₁₀ reduction of the <i>L. monocytogenes</i> contamination in incoming milk is applied	0.84 = 1/1.2	0.80 = 1/1.25	0.014 = 1/71	0.008 = 1/125
Raw-milk cheese if a substance that reduces the surface contamination by 2 log ₁₀ is applied	50	86	0.83 = 1/1.2	0.82 = 1/1.2
Raw-milk cheese if the milk is tested in farm bulk tank, at every milking***	2.2	3.0	0.042 = 1/24	0.027 = 1/37
Raw-milk cheese if the cheese lots are tested***	0.080 = 1/12	0.134 = 1/7.4	0.002 = 1/658	0.001 = 1/832

* Interpretation: “The mean risk of invasive listeriosis from a serving of soft-ripened cheese made from raw milk is 53 times the mean risk of invasive listeriosis from a serving of soft-ripened cheese made from pasteurized milk for servings consumed by individuals from the Elderly population in Canada”.

** Ratios <1: the mean risk of the alternative is smaller than the reference; this example, which is the same as the preceding one, could read: “The mean risk of invasive listeriosis from a serving of soft-ripened cheese made from pasteurized milk is 0.019 times the mean risk of invasive listeriosis from a serving of soft-ripened cheese made from raw milk for servings consumed by individuals from the Elderly population in Canada”.

*** Volume tested: 25 ml for milk, 25 g made of 5 g from each of 5 cheeses at random for cheese lot; single *L. monocytogenes* detection probability: 0.75, test frequency: 100% of farms, tankers, dairy silos, cheese lots, respectively. Detected positive units are removed from production.

Limitation, Caveats and Data Gaps (see section 11)

The model and, as a consequence, the results and conclusions are limited to the considered pathogen and the considered type of cheese. These results do not apply to other type of cheese with different growth characteristics, such as hard cheese.

The inferences about prevalence and level of in-plant environmental *L. monocytogenes* contamination in Canada and in the U.S. rely on a single study (Gombas et al. 2003). That study

may provide incomplete information about the prevalence of contaminated lots and contaminated cheeses within contaminated lots.⁷

There is a considerable uncertainty in the dose-response model. The sensitivity analysis shows that, within the small part of the overall uncertainty that is considered here, the uncertainty surrounding the r parameter of the dose-response model dominates all other sources of uncertainty in the risk results. A part of this uncertainty is naturally discarded within this risk assessment, when alternatives are compared to the baseline models. Nevertheless, the absolute values obtained in this risk assessment should not be compared with other results obtained using a different dose-response model without some caution.

Only a small part of the overall uncertainty is considered in this study, while it is recognized that there are many other types of uncertainty in risk assessments. Total uncertainty includes parameter uncertainty (measurement errors, sampling errors, systematic errors), model uncertainty (uncertainty due to necessary simplification of real-world processes, mis-specification of the model structure, model misuse, use of inappropriate surrogate variables), and scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgment, incomplete analysis) (US EPA 1997). While our results suggest that the considered uncertainty is less important than variability, absolute values should be considered with some caution.

Results rely also on extrapolations –over time, for example, from bulk tank surveys carried out in the 1990s to current day farm bulk tank characteristics and from nutrition surveys done in the early 2000s to present day; from a sampling population to the reference population of interest, for example, from U.S. home storage data to Canada, from U.S. retail-level contamination levels and frequency to Canada, from women of child-bearing age to pregnant women; and, from laboratory to production-- from study populations to populations appropriate as a reference for this study. Biases and uncertainty that those extrapolations introduce are unknown.

⁷ We tested the impact of an (hypothetical) lower environmental contamination prevalence in the response to public comments document.

Sensitivity analyses to some key parameters are provided in the body of this document. Despite the limitations identified above, we concluded that no critical data gaps impair the conclusions of this risk assessment. For example, the exact processing may differ from one soft-ripened cheese to another. However, the general conclusions - *i.e.* that the risk of listeriosis from consumption of soft-ripened cheese made from raw milk is substantially larger than that for consumption of soft-ripened cheese made from pasteurized milk, that the 60-day aging regulation actually increases the risk of listeriosis for consumption of raw-milk cheeses, that a 6 log reduction (or equivalent) of the contamination in milk is necessary to make raw-milk cheeses as safe from listeriosis as pasteurized-milk cheeses, and that most other strategies are not sufficient by themselves - apply to any soft-ripened cheeses in or on which *Listeria monocytogenes* can grow substantially. That said, as with any risk assessments, additional data would likely decrease the uncertainty in the risk estimates.

This risk assessment answers the management charge (see Appendix, section “Charge developed by the Risk Manager Team”) set by the FDA and the Health Canada risk managers for soft-ripened cheese. The results inform risk managers about managing risk of invasive listeriosis from the consumption of soft-ripened cheese.

1. Introduction

Listeria monocytogenes is a widely occurring pathogen that is frequently present in soil, sewage, freshwater sediment and effluents; it can be found in agricultural food processing plants. Ingestion of *L. monocytogenes* can lead to the development of listeriosis, with consequences that may include septicemia, meningitis, encephalitis, spontaneous abortion, and stillbirth. Epidemiological data show that listeriosis has one of the highest hospitalization rate and case fatality among foodborne diseases (Mead et al. 1999; CDC 2008; Scallan et al. 2011).

The United States and Canada continue to experience sporadic illnesses and outbreaks of listeriosis associated with the consumption of cheese, particularly soft and soft-ripened cheese. Both the U.S. DHHS - Food and Drug Administration (FDA) and Health Canada - Santé Canada (HC) continue to evaluate the safety of these cheeses, particularly cheese made from unpasteurized milk. As part of this effort, FDA and HC carried out a quantitative risk assessment to evaluate the effectiveness of and public health impact of processing and intervention strategies to reduce or prevent *L. monocytogenes* contamination in soft-ripened cheeses. These cheeses are of interest because of the large number of factors that affect risk (*e.g.*, microbiological quality of the source material, scale of operation, manufacturing practices), interest in international distribution of cheese made under different conditions, and increasing interest in applying alternative risk mitigation technology. In addition, outbreaks and recalls associated with cheese have prompted a need to evaluate current and potential risk management strategies.

Specifically, the risk assessment considered the public health impact of:

- variations in *L. monocytogenes* levels in the raw materials used to produce cheese;
- changes in *L. monocytogenes* levels (*i.e.*, growth, inactivation, or contamination) at each step of the manufacturing process, between final packaging and sale at retail, and between retail sale and consumption; and
- currently available and possible future intervention and control strategies.

The public health issues considered included:

- changes in the level of risk associated with the use of raw milk, pasteurized milk, or milk treated by a process that achieves a 3 log₁₀ reduction in *L. monocytogenes* in cheese-making;
- changes in the level of risk from modifications of, or deviations from, existing manufacturing processes (including sanitation);
- changes in the level of risk associated with the use of new or additional interventions;
- changes in the level of risk associated with different conditions during transport, distribution, and home storage.

In addition, the level of risk was evaluated for susceptible populations including the Elderly, Pregnant women, and the Immunocompromised.

2. Background

2.1. Recalls, Outbreaks and Sporadic Cases Associated with L. monocytogenes in Soft-Ripened Cheese

Listeria has been the most common microbial cause of recalls for cheese products in both the U.S. and Canada. In the U.S. from 1986 to 2008 there were a total of 137 recalls of various types of cheeses, of which 108 (79%) were *Listeria*-related. In Canada from 2004 through mid-2009 there were 15 cheese recalls, of which 11 (73%) were *Listeria*-related. A wide variety of cheeses were involved in these recalls (Table 3). The three most common types of cheeses involved in these recalls were fresh soft cheeses, which have previously been shown to be at high risk for *L. monocytogenes* contamination (FDA/FSIS 2003), hard cheeses (which represent the largest market share), and the soft-ripened cheeses.

Table 3: U.S. and Canadian *L. monocytogenes* related cheese recalls.

Cheese Type	U.S. (1986-2008)	Canada (2004-2009)
Hard	25	1
Fresh soft	24	5
Soft-ripened	22	1
Unknown / Undefined / Multiple	15	1
Semi-soft	13	2
Soft-unripened	3	0
Processed	6	1

Sources: Canadian Food Inspection Agency, U.S. Food and Drug Administration.

Table 4: Cheese associated *Listeria* outbreaks.

Year	Location	Implicated Cheese	No. of Illnesses (Deaths) ¹	Raw Milk	Reference
1983-1987	Switzerland	Vacherin Mont d'Or	122 (34)	No	(Bula et al. 1995; Norton and Braden 2007)
1985	U.S. (CA)	Queso fresco and queso cotija	142 (48)	Likely (or cross contamination)	(CDC 1985; Norton and Braden 2007)
1989-1990	Denmark	Multiple	26 (6)	NS ²	(Jensen et al. 1994; Norton and Braden 2007)
1995	France	Brie de Meaux	37 (11)	Yes	(Goulet et al. 1995; Norton and Braden 2007)
1997	France	Livarot	14	Yes	(Jacquet et al. 1998)
1999	France	"Epoisses" like	3	Yes	(AFSSA 2000, page 50)
2000	U.S. (NC)	Queso fresco	13 (5 stillbirths)	Yes	(MacDonald et al. 2005; Norton and Braden 2007)
2001	Sweden	Fresh cheese	>120	Yes	(Danielsson-Tham et al. 2004)
2001	Japan	Washed cheese	86	No	(Makino et al. 2005)
2002	Canada (QC)	Multiple types	17	Y	(Gaulin et al. 2003; Norton and Braden 2007)
2003	U.S. (TX)	Queso fresco	13 (2)	Yes	(Norton and Braden 2007; Swaminathan and Gerner-Smidt 2007)
2005	U.S. (TX)	Queso fresco	12	Yes	(CDC 2005)
2005	Switzerland	Tomme	10 (3 + 2 miscarriages)	Yes	(Bille et al. 2006)
2006	U.S. (OR)	Unspecified	3	No	(CDC 2013a)
2006-2007	Germany	Harzer Käse	189 (26)	No	(Koch et al. 2010)
2007	Norway	Camembert	17 (3)	No	(Johnsen et al. 2010)
2008	Canada (QC)	Multiple	38 (5)	No	(MAPAQ 2010; Gaulin et al. 2012)
2008	Chile	Brie	91(5)	NS	(Promed 2008)
2008	US (multi State)	Mexican-style asadero cheese	8 (0)	No	(Jackson et al. 2011) (CDC 2013c) }
2009	US (multi-state)	Mexican-style	8 (3)	No	{CDC, 2013 #1958
2009-2010	Austria,Germany,Czech Republic	Quargel acid-cured cheese	34 (8)	No	(Fretz et al. 2010a; Fretz et al. 2010b; Schoder et al. 2012)
2009	US (multi-State)	Mexican-style cheese	18 (0)	No	(CDC 2013a; CDC 2013c)
2010	US (multi-State)	Mexican-style cheese	6 (1)	No	(CDC 2013a; CDC 2013c)
2011	Belgium	Hard cheese (Pave du Nord)	12 (4)	No	(Yde et al. 2012)
2011	US (MI)	Chive Cheese, Ackawi cheese	2 (1)	No	(CDC 2013a; CDC 2013c)
2011	US (NJ)	Mexican-style cheese	2 (0)	No	(CDC 2013a; CDC 2013c)
2011	US (multi-State)	Blue veined aged cheese	15 (1)	Yes	(CDC 2013a; CDC 2013c)
2012	US (multi State)	Ricotta	22 (4)	No	(CDC 2012)
2012	Spain	Latin – style fresh cheese	2 (0)	Unclear	(de Castro et al. 2012)
2013	US (multi-State)	Soft-ripened cheese	6 (1)	No	(CDC 2013b)

1. The number of cases associated with a particular food is not always clearly stated in the publications. 2. NS – Not Stated

There have been listeriosis outbreaks linked to cheese consumption worldwide (Table 4). The majority of these outbreaks were associated with fresh-soft or soft-ripened cheeses.

These data show that, while listeriosis may be associated with the consumption of any type of cheese, fresh-soft and soft-ripened cheeses could be of significant public health concern.

Importantly, the majority of listeriosis cases are sporadic cases, *i.e.* not linked to outbreaks (86% of the listeriosis cases reported to CDC (2013c)). There is very little information about the origin of these sporadic cases (Varma et al. 2007). For multiple reasons (small batches, extreme heterogeneity of individual susceptibility), we expect to see primarily sporadic cases of listeriosis linked to small-scale cheese producers. Note that a French case-control study carried out on 120 sporadic cases of listeriosis observed in 1997 indicated that some cases could be associated with consumption of soft cheeses ((De Walk et al. 1998) cited by (De Buyser et al. 2001)).

2.2. Overview of Cheese Regulation

The overall production process is similar for all cheeses. Changes at specific points in the process lead to production of different types of cheese. In general, the process consists of receiving and holding milk, possible pre-treatment (*e.g.*, pasteurization) of the milk, addition of starter cultures and enzymes, coagulation and cutting of the coagulum, draining and molding of curd, ripening, and packaging. The incoming milk may be from one of more herds or farms depending on the nature and scale of the production facility, and milk from different sources may be combined on the farm, during transport, or at the manufacturer. After production and packaging, the products may follow very different pathways from the manufacturer to consumption depending on the nature of the product and the manufacturer (artisanal or large scale) or if the cheese is intended for further repackaging or processing.

Cheeses are generally classified or labeled based on the production process and the properties of the cheese. Standards of identity have been established for a number of cheeses in the U.S. (21 CFR Part 133). These standards describe the major steps of the production process for each type of cheese as well as properties such as a minimum fat content (w/w) and a maximum moisture

content in the final product. Similarly, Canadian Food and Drug Regulations define the properties of a number of types of cheeses (CRC, c870). U.S. regulations have a standard for soft-ripened cheeses not otherwise standardized that specifies the production process and final milk fat content (21 CFR 133.182). Canadian regulations are not as specific regarding the production processes for cheeses and soft-ripened cheeses in particular, but do define a soft-ripened cheese such as Camembert as having less than 56% moisture and more than 22% milk fat and soft-ripened cheese such as Brie as having less than 54% moisture and more than 23% milk fat (B.08.033).

Both U.S. and Canadian regulations also contain provisions related to cheese safety. These include regulatory definitions of the times and temperatures needed for milk pasteurization. U.S. regulations define “pasteurized” to mean that milk has been heated in properly designed and operating equipment to one of several temperatures for defined times (Table 5) as well as other time-temperature combinations that have been “demonstrated to be equivalent thereto in microbial destruction” (21 CFR 133.3(d)). In addition, for soft-ripened cheeses in the U.S., “[m]ilk shall be deemed to have been pasteurized if it has been held at a temperature of not less than 143°F for a period of not less than 30 minutes, or for a time and at a temperature equivalent thereto in phosphatase destruction” (21 CFR 133.182(c)(2)).

Table 5: Time temperature combinations for milk pasteurization as defined in 21 CFR 133.3(d).

Temperature	Time
145°F	30 min.
161°F	15 s.
191°F	1 s.
204°F	0.05 s.
212°F	0.01 s.

Canadian cheese regulations define pasteurization conditions as “being held at a temperature of not less than 61.6°C for a period of not less than 30 minutes” or “for a time and a temperature that is equivalent thereto in phosphatase destruction” (B.08.030) (61.6°C = 142.9°F). The U.S. definition of soft-ripened cheese also states that “[i]f the milk used is not pasteurized, the cheese so made is cured at a temperature of not less than 35°F for not less than 60 days” (21 CFR 133.182(a)). In Canada, Regulation B08.043 of the Food and Drugs Act and Regulations requires that any cheese made from milk from an unpasteurized source be stored and B.08.030 defines

“stored” as to have been kept or held at a temperature of 2°C (36°F) or more for a period of 60 days or more from the date of the beginning of the manufacturing process.

In both the U.S. and Canada, cheese that is produced and distributed purely intrastate or intraprovince is still subject to regulation at the state or provincial level. In the U.S., there are significant state-to-state differences in the regulations governing the sale of raw milk. In Canada, as of September 2009, the province of Québec allows the manufacture and sale of soft and semi-soft cheeses made from raw milk that have not been aged for 60 days if the manufacturer meets requirements prescribed in the provincial regulation respecting food.

2.3. Overview of Cheese Production

As the baseline models for this risk assessment use a representative example of soft-ripened cheese, the following overview of cheese production refers specifically to Brie and Camembert, in which both microbial and fungal activities (primarily from *Penicillium candida* and *Penicillium Camemberti*) determine the physical characteristics and flavor of the cheese. Because fungal growth and activity occurs primarily near the surface, both have distinct inner cores and external rinds. The entire production and aging process takes approximately 14 days to 5 weeks. The generic terms Brie and Camembert are used to describe types of soft-ripened cheeses made with generally similar processes. The specific terms “Brie de Meaux”, “Brie de Melun” and “Camembert de Normandie” are controlled French designation of origination (“*Appellation d'origine contrôlée*”) that indicate both the place of production and the specific process used.

The cheese-making process for Brie and Camembert is outlined in Figure 1, and described in more detail in the description of the exposure assessment component of the model. Although various producers might use slightly different versions of this process for Brie and Camembert, the final products are highly similar except for size. Nevertheless, in some commercial cheese production, a uniformly smooth texture is assured by use of thermophilic starters at a temperature that is well below that of their optimum growth. This process is known as “stabilization”. Ripening of stabilized cheeses occurs uniformly throughout. Cutting such

cheeses in two reveals a smooth, glistening, plastic-like appearance of the entire cut surfaces without a center curd core.

Several factors determine whether and at what level *L. monocytogenes* could become introduced to contaminate the final product. Extrinsic factors include the microflora of the incoming milk, the possible use of a microbial control treatment, potential cross-contamination during manufacturing, and the temperature at each step. The most significant intrinsic factors are the water activity (a_w) and pH of the milk and nascent cheese as the process progresses.

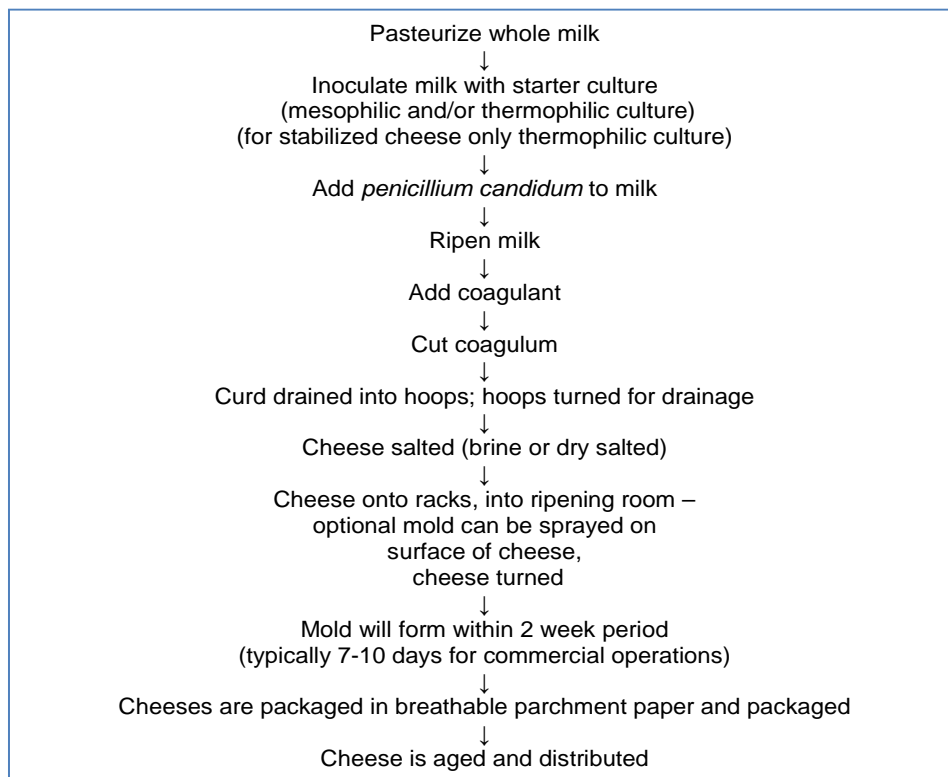


Figure 1: General flow chart for commercial production of Camembert.

2.4. Overview of the Cheese Industry

The cheese-making industry in the U.S. and Canada is highly diverse, both in terms of the number and types of products produced and in the diversity of the producers. For example, the September 2007 A.C. Nielsen database of total cheese sales lists over 16,500 Universal Product Codes (UPC) and several hundred brand names used by large retailers in the U.S. The Nielsen

database contains scanner data from a set of large retailers and national merchandisers. The data do not include products marketed directly to consumers or through small or specialty retailers. The USDA National Agricultural Statistics Service (NASS) estimates that over 400 million kilograms (900 million pounds) of cheese were manufactured in 2007.

There are few data available on the amount of Brie and Camembert produced yearly in the U.S. and Canada. The USDA NASS does not gather data on domestic production of these cheeses, although USDA import reports show that approximately 12 million kg (26 million pounds) of soft-ripened cheeses of all sorts are imported into the U.S. yearly. The Nielsen database contains over 100 UPCs for Brie and over 50 UPCs for Camembert products (imported and domestically produced). The sales associated with these UPCs total approximately 2 million kg (4.4 million pounds) of Brie and 0.4 million kg (0.9 million pounds) of Camembert yearly through the retail outlets reported in the Nielsen data base.

Brie and Camembert production in the U.S. and Canada generally occurs in facilities that can be characterized as either large producers or as small (artisanal or farmstead) producers. Although the outline of the cheese production process is the same regardless of scale, there are a number of factors that differ between the large and small producers that may have a significant impact on the microbiological safety of the final product. These factors include, for example, the need to pool milk from multiple herds or farms and the time for and conditions experienced during transport and storage of the milk prior to cheese-making.

Unfortunately, there are few data available in either the U.S. or Canada that characterize production volumes and distribution patterns for soft-ripened cheeses, or the specific types Brie and Camembert, and no data on the practices used by artisanal and farmstead producers, the amount of cheese produced in this sector, conditions experienced during distribution and handling, or the consumption habits of consumers who purchase these products. These data gaps made it impossible to accurately model the integrated public health impacts (such as total number of illnesses per year or population illness rates) from *L. monocytogenes* in these cheeses. As an alternative, this risk assessment used baseline models and a series of scenarios to examine

the public health impact of different practices and production systems, and expressed risk on a per-serving basis for each scenario as compared to the baseline models.

2.5. Quantitative Microbial Risk Assessment

The components of a quantitative microbial risk assessment, and the role of risk assessment within food safety risk analysis, have been described in several publications (*Codex alimentarius* Commission 1999; CFSAN Risk Analysis Working Group 2002). Food safety microbial risk assessments consist of four components:

- Hazard identification – Identifies the pathogen of concern and describes the health effects associated with consumption of that pathogen.
- Hazard characterization – Characterizes the relationship between the level of exposure to a pathogen and the probability and severity of adverse responses. In a quantitative risk assessment this may include a dose-response relationship.
- Exposure assessment – Describes the frequency and level of exposure to the pathogen by consumers. This may include modeling changes in the presence and level of the hazard in a product pathway.
- Risk characterization – Integrates the hazard characterization and exposure assessment to predict the probability and severity of adverse health effects in a population of consumers.

The end result of a quantitative risk assessment is an estimate of the public health impact of exposure to a particular hazard through a particular pathway and of the uncertainties that accompany the estimate. This impact may be expressed in terms such as the probability of illness per serving of a food, or as the total number of expected cases of illness per year in either the whole population or in defined subpopulations. A quantitative risk assessment can also be used to identify the critical data gaps that are responsible for the uncertainties in the risk estimates.

Quantitative risk assessment models can also be used to compare the predicted public health impact of different conditions in the exposure pathway or the results of the use of alternate intervention strategies. This is often done using scenario (or “what-if”) analyses. For example, the potential impact of an alternate control strategy for a pathogen can be predicted by modifying a risk assessment model that describes current practices. In addition, scenarios can be used to

estimate current levels of risk in situations where the data are not adequate to allow construction of descriptive models of existing production or distribution processes or pathways. For example, as discussed below in greater detail, data describing either the practices used by artisanal cheese makers or the amount of cheese produced by this industry segment were not available for use in this risk assessment. Therefore, the public health impacts of conditions and practices associated with artisanal or farmstead cheese production were assessed using scenarios that were modifications of the basic model.

2.6. Previous Listeria Risk Assessments

Farber *et al.* (1996) evaluated the risk of listeriosis from consumption of soft cheeses in Canada. They used a Weibull-Gamma dose response model, and derived model parameters for the general and susceptible populations from surveillance data. They assessed exposure by using data on *L. monocytogenes* incidence in food from Agri-Food and Agriculture Canada together with their previous work on levels of *L. monocytogenes* in soft cheese, and used market disappearance data from Statistics Canada as a surrogate for consumption data. They used likely values for the percent of annual listeriosis cases attributable to soft cheese, the susceptible fraction of the population, and the level of illness underreporting in the epidemiological data. Using these values, the risk assessment model produced an estimate of risk that was consistent with the surveillance data and demonstrated the importance of identifying and understanding uncertainty in risk assessment.

In November of 2000, the U.S. Department of Health and Human Services issued *Healthy People 2010*, which contained a comprehensive set of disease prevention and health promotion objectives for the nation to achieve over the first decade of the century. This publication serves as a statement of national health objectives designed to identify the most significant preventable threats to health and to establish national goals to reduce these threats. One of these goals is a reduction in foodborne listeriosis.

In support of this goal, FDA in collaboration with USDA conducted a quantitative assessment of the relative risk to the public health from foodborne *L. monocytogenes* among 23 selected categories of ready-to-eat (RTE) foods (FDA/FSIS 2003). Exposure for each food category was

estimated using data on *L. monocytogenes* prevalence and levels in foods at retail (or at manufacturing in a few cases) and by modeling *L. monocytogenes* growth or decline during distribution and storage. Consumption estimates were developed for each food category for three population groups; two populations that were considered to have increased susceptibility to listeriosis: the Elderly (> 60 years of age) and the Perinatal population (fetuses from 16 weeks plus neonates through 4 weeks), and the General population with lower susceptibility. A dose-response model was developed based on animal models and surveillance data. Differential susceptibility between populations was taken into account by applying scaling factors to this dose/response model. An estimate of the annual number of listeriosis cases was derived from epidemiological studies. The risk assessment model was used to partition these illnesses among the 23 food categories. Several different metrics were used to describe risk, including the probability of illness per serving for each food category.

The results of that assessment indicated that, among dairy foods, soft unripened cheese presents a high risk of listeriosis, and that fresh soft cheese, semi-soft cheese, and soft-ripened cheese present moderate risks of listeriosis. The relative risk associated with the use of unpasteurized milk for cheese-making was examined for only one type of cheese (fresh soft cheese) but the modeling showed that this resulted in a 40-fold increase in risk over the use of pasteurized milk for cheese-making.

Two product pathway risk assessments have been published for *L. monocytogenes* in cheese. Bemrah *et al.* (1998) carried out a risk assessment for *L. monocytogenes* in soft cheese made from raw milk that modeled changes in *L. monocytogenes* levels from milk production to consumption in France in the 1990's. Their exposure assessment assumed that contaminated milk was the only source of *L. monocytogenes*, and used data from a survey of milk producers in France to estimate the distribution of *L. monocytogenes* contamination in milk prior to cheese production. Their hazard characterization used a previously published (Farber *et al.* 1996) Weibull-Gamma dose response model for two populations, one high-risk and one low-risk. Although Bemrah *et al.* did not evaluate the effect of interventions or control strategies such as pasteurization; they did examine the effect of eliminating one of the two major sources of *L. monocytogenes* in the raw milk (*L. monocytogenes* from mastitic cows). Eliminating that input

significantly reduced the frequency of milk batches with high levels of *L. monocytogenes* and resulted in a 5 fold reduction in predicted annual illnesses.

Sanaa *et al.* (2004) modeled changes in *L. monocytogenes* levels in the product production pathway for "Brie de Meaux" and "Camembert de Normandie" soft-ripened cheeses in France. In their model, all *L. monocytogenes* was assumed to originate with bulk milk at levels determined through a one year survey of farm bulk milk tanks. Changes in *L. monocytogenes* levels during cheese production, distribution, and home storage were modeled. The probability illness per serving was calculated for each cheese type for two populations (more and less susceptible) using a simple exponential dose response relationship. They did not use their model to assess the effectiveness of potential interventions or risk management strategies.

Two important assessments of the risk from *L. monocytogenes* in pasteurized-milk cheese were made available after the public comment period on the draft version of this risk assessment (Tenenhaus-Aziza *et al.* 2013; Lamboni *et al.* 2014). These interesting articles are very helpful to better explore the Critical Control Points in the cheese making process, notably regarding contamination post pasteurization. However, these articles contained no fundamentally new results or new data leading to changes in how this report represents the cheese making process.

3. Risk Assessment Modeling

To address the questions posed by the FDA and HC-SC Risk Managers, this risk assessment developed a “product pathway” model for soft-ripened cheese that included a description of known and potential sources of *L. monocytogenes* and changes in the prevalence and level of *L. monocytogenes* from the production of milk “on farm” to consumption of cheese in the home. The details of the model, model assumptions, data sources, and important data gaps are discussed in detail in the following sections.

The pasteurized-milk cheese baseline model and the raw-milk cheese baseline model were developed using parameters and input values obtained from the published literature, industry sources, public submissions, and specific expert elicitation.

The framework of this risk assessment was to gather all the available literature on the subject and to select all the literature that falls specifically into the scope of the risk assessment. The available information was then used, through statistical and probabilistic methods, to derive a distribution of the variability of the parameter and to estimate the surrounding uncertainty of this estimated distribution. Whenever possible (*i.e.* whenever more than one source was available), no specific data or dataset was used.

The baseline models were modified to estimate the relative public health impacts of alternate interventions, practices, and conditions and the results of these modifications were expressed relative to the baseline models.

Modeling was carried out using Analytica Professional 4.2 from Lumina Decision Systems (Los Gatos, CA (Lumina Decision Systems 2010)). For quality assurance, each component of the model was also programmed and tested using the R language (Version 2.8) (The R Development Core Team, Vienna, Austria (R Development Core Team 2008)). Variability and uncertainty were evaluated separately using a Second-Order Monte Carlo simulation framework (Frey 1992). Overall the model consisted of three modules: exposure assessment, hazard characterization, and risk characterization (Figure 2).

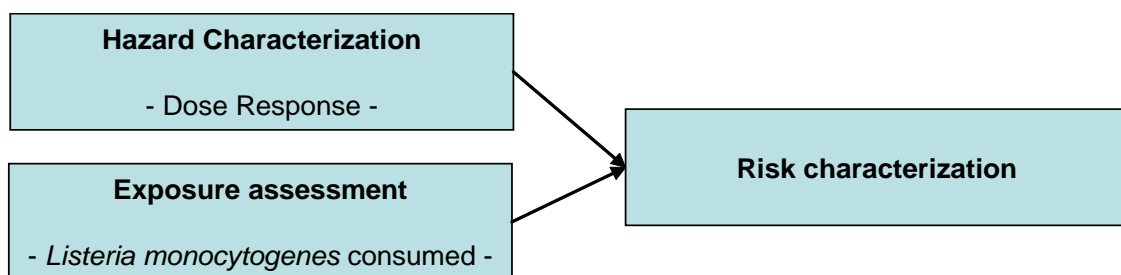


Figure 2: The 3 modules of the model.

The exposure assessment module contained a series of stages corresponding to the major stages in the process of producing, distributing, and consuming soft-ripened cheese. In particular, Camembert cheese was used as an example of a soft-ripened cheese for several stages in the exposure assessment module. These are shown in Figure 3. Each stage might consist of one or

more steps during which the prevalence and level of *L. monocytogenes* might change. These steps are described in detail in the following sections. Changes in input values or process parameters in the exposure assessment module were used to address alternate intervention or process scenarios.



Figure 3: The 5 stages of the exposure assessment.

In addition to the individual steps, sub-routines were developed to describe basic processes (Nauta 2008) that affect *L. monocytogenes* prevalence and levels in multiple steps. These included microbial growth, microbial inactivation, environmental contamination, removal, and partitioning and mixing.

The hazard characterization module contained dose-response functions for each of the populations considered in this risk assessment. The assessment considered four populations, the General (non-susceptible) population and three susceptible groups (the Elderly, the Immunocompromised, and Pregnant woman) in both the U.S. and Canada.

The risk characterization module combined the results of the exposure assessment for each population with the hazard characterization for that population to estimate risk. Risk was expressed on a “per serving” basis because the lack of data on overall levels of cheese production (particularly for small cheese makers) and on possible differences in cheese source preference among the different populations prevented developing integrated estimates of risk. The impacts of the changes evaluated in the different scenarios were expressed as relative risk compared to baseline models.

The results of the risk assessment are discussed in detail in the sections 9 and 10.

4. Hazard Identification

The biology, pathology, and ecology of *Listeria monocytogenes* and the problem of *L. monocytogenes* as a foodborne hazard have been extensively described in previous risk

assessments (FDA/FSIS 2003; FAO/WHO 2004) and in the microbiological literature (e.g. Swaminathan and Gerner-Smidt 2007). Therefore, only a summary of this information is presented here.

L. monocytogenes is a Gram-positive pathogen that is widely distributed in the environment, including agricultural and food production environments. Most human exposure to *L. monocytogenes* is through the consumption of contaminated food, although fetuses and neonates may be infected transplacentally or during birth. The symptoms of *L. monocytogenes* infection in otherwise healthy adults may be relatively mild and transient, producing typical “flu-like” symptoms or gastroenteritis. There are few data on the incidence or epidemiology of mild listeriosis.

L. monocytogenes infection can also result in invasive listeriosis, particularly in susceptible individuals. The consequences of invasive listeriosis include meningitis, encephalitis, abortion, and stillbirth. Because invasive listeriosis often results in the need for medical care, frequently including culturing of *L. monocytogenes* from internal tissues, information on disease rates as well as on the characteristics of the affected populations is available. For this risk assessment, only the public health burden of invasive listeriosis was considered.

The CDC Foodborne Diseases Active Surveillance Network (FoodNet) tracks cases of listeriosis at 10 sites in the U.S. FoodNet data for 2010 showed an incidence of approximately 3 cases per 1 million individuals (CDC 2006; CDC 2011). The overall incidence in Canada in 2004 to 2007 was 3.0, 3.3, 3.0 and 4.2, respectively, cases per million individuals (Clark et al. 2010). These incidence rates are similar to those seen in other countries (OzFoodNet 2007; Clark et al. 2010).

The consequences of invasive listeriosis are severe. FoodNet data for 2010 showed that 90% of listeriosis cases required hospitalization, more than twice the hospitalization rate for *E. coli* O157:H7. *L. monocytogenes* caused 24% of the deaths associated with foodborne infections in that year, more than twice as many deaths as were caused by *Campylobacter* (CDC 2011).

The risk of illness from exposure to *L. monocytogenes* is not uniformly distributed in the population. FoodNet data show that the very young and the Elderly are more susceptible to listeriosis than is the General population. For example, Figure 4 shows the incidence of listeriosis by age and gender in the FoodNet catchment area for 2003 and 2004. A similar pattern has been seen in Canada (Figure 5). The relatively high incidence for the lowest age group reflects increased susceptibility for pregnant woman and fetuses. Although pregnant woman with *L. monocytogenes* infections may have mild symptoms, infection of the fetus may result in stillbirth, spontaneous abortion, or birth of a critically ill newborn.

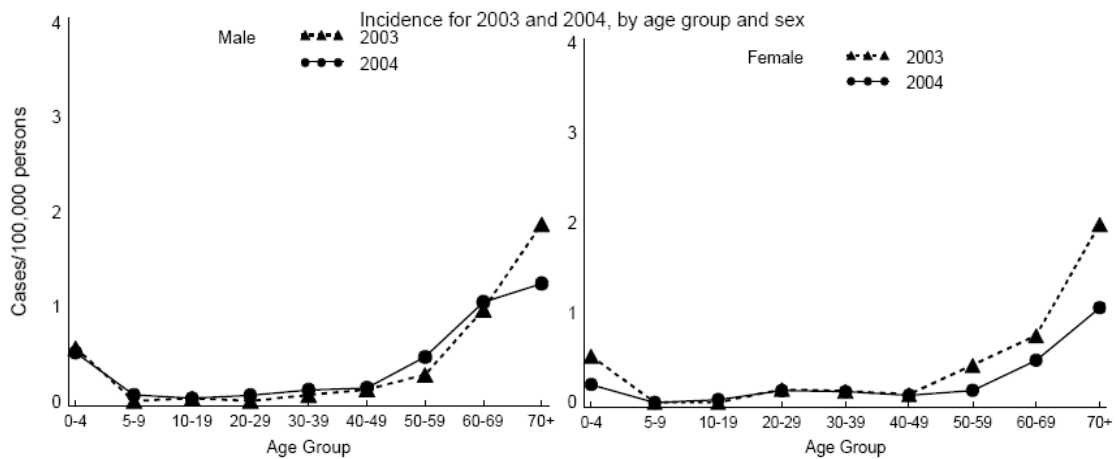


Figure 4: CDC FoodNet data on the incidence of listeriosis by age and gender in the U.S. (CDC 2006).

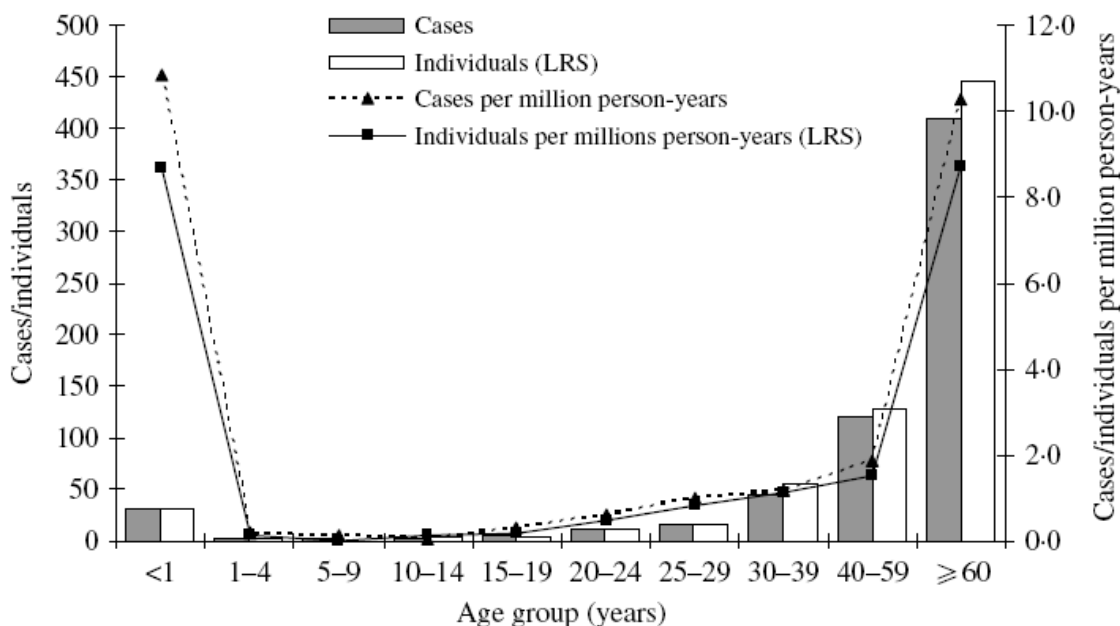


Figure 5: Canadian data on the incidence of listeriosis from the National Listeriosis Reference Service and the National Notifiable Diseases system (Clark et al. 2010).

A variety of medical conditions that affect the immune system can also result in increased susceptibility to listeriosis. These include myeloproliferative disorder; multiple myeloma; acute leukemia; giant cell arteritis; dialysis; liver, esophageal, stomach, pancreas, lung, and brain cancer; cirrhosis; and organ transplantation. Unfortunately, the listeriosis surveillance systems in the U.S. and Canada do not routinely collect data on the presence of underlying medical conditions, so it is not possible to accurately estimate relative susceptibility in different patient populations. Marchetti (cited by FAO/WHO 2004) and Goulet *et al.* (2012) were able to review medical records for listeriosis cases in France in 1992 and from 2001 to 2008, respectively. Their analyses suggested that some patient populations are more than 1,000 times more susceptible than the population of individuals <65 years old without underlying conditions. Because similar data on underlying conditions are not available for either the U.S. or Canada and, following FAO/WHO (2004), this risk assessment considered the General population and three representative high susceptibility groups: Pregnant women, the Elderly, and the Immunocompromised.

Strains of *L. monocytogenes* can be differentiated by serotyping, molecular fingerprinting, ribotyping, or DNA sequencing. There is evidence that virulence differs among strains. For

example, Clark *et al.* (2010) showed that 77% of human *L. monocytogenes* isolates in Canada from 1995 to 2004 were of serotype 1/2a or 4b. However, they also showed that all of the other *L. monocytogenes* serotypes were also found among human isolates, indicating that all serotypes are capable of causing listeriosis. The major knowledge about strain virulence is the variation in relation to subtypes encoding a full-length or truncated Internalin A (Lecuit *et al.* 1999; Lecuit *et al.* 2001; Chen *et al.* 2011). Studies of the microbial ecology of food production environments also show that some strains are better able to survive in particular locations over long periods of time (Sauders *et al.* 2004; Sauders *et al.* 2006). Strain-specific differences exist in susceptibility to control measures such as heat (pasteurization) (Doyle *et al.* 2001). Further, properties such as virulence and resistance to environmental stress are also affected by growth history (Skandamis *et al.* 2009). Because there are no data available on whether particular strains of *L. monocytogenes* are preferentially associated with milk used for cheese-making or with cheese-making environments, it was not possible to model specific serotype differences in this risk assessment. However, where data permit, we do account for among-strains characteristics' variability, *e.g.* for growth characteristics like growth rates and minimum growth temperatures.

There are several routes by which dairy products in general, and cheese in particular, may become contaminated with *L. monocytogenes*. First, cows infected with *L. monocytogenes* may shed cells directly into their milk. It has been shown that *L. monocytogenes* shedding can occur in cows with subclinical mastitis (Winter *et al.* 2004), making it difficult to control this source of *L. monocytogenes* without microbial testing. Second, *L. monocytogenes* has been shown to occur in the natural conditions in feed, water and soil on dairy farms and on farm equipment (Latorre *et al.* 2009). These environmental reservoirs are difficult to control, potentially leading to on-going or sporadic contamination of raw milk. Third, *L. monocytogenes* may occur in the cheese processing environment (Pritchard *et al.* 1994; D'Amico and Donnelly 2009), potentially leading to contamination during cheese-making. *L. monocytogenes* presence in cheese processing facilities can lead to contamination after the major microbial control points (*i.e.*, after pasteurization) and because of the need for extensive manipulation during cheese-making that occurs in cheese-making facilities.

There are limited data that can be used to model the level and frequency of *L. monocytogenes* contamination in cheese from these sources. Published surveys of *L. monocytogenes* in bulk milk can be used to anchor estimates of *L. monocytogenes* levels in the raw milk used for cheese-making (discussed in detail in the Exposure Assessment section 7). However, significant uncertainty exists as to how differences in milk sourcing practices between small-scale and large-scale producers affects the probability of *L. monocytogenes* presence in the raw milk used. For example, pooling milk from many individual cows in multiple herds for the large volumes of milk that a large volume cheese producer needs, might increase the probability of having *L. monocytogenes* in any batch of milk, but the organism would be diluted. On the other hand, the lack of dilution might lead to intermittent high levels of contamination in the smaller volume batches used by a small volume cheese producer.

The cheese-making process involves a number of steps that may present an opportunity for environmental contamination to spread to the cheese. Large scale commercial cheese operations are highly automated with little direct hands-on manipulation of the cheese; smaller scale artisanal and farmstead manufactures typically employ more extensive hands-on manipulation of cheese (Hassan et al. 2000; Hassan et al. 2001; Meyer-Broseta et al. 2003; Nightingale et al. 2004; Nightingale et al. 2005; D'Amico et al. 2008b). Due to the number of steps that involve manipulation, the cheese-making process presents multiple opportunities for environmental contamination and spread from the equipment and facilities. Because data do not exist to characterize contamination associated with individual steps in the cheese-making process, these sources of potential contamination were modeled as if all contamination occurs at a single point (see the Contamination section 6.4). The probability and level of contamination at this point was modeled by using data from Gombas *et al.* (2003) on the frequency and levels of *L. monocytogenes* in soft-ripened cheese at retail. The process for using these data is described in detail in the Exposure Assessment section.

5. Hazard Characterization

Hazard characterization describes the health effects that result from exposure to a pathogen. In a quantitative microbiological risk assessment, this is done through a dose response function that is used to link the ingested dose of a pathogen to the probability of a given specified endpoint. For

a given illness endpoint, the dose response function calculates the probability that illness occurs given ingestion of a quantity of pathogen.

The FAO/WHO and FDA/FSIS risk assessments of *L. monocytogenes* in ready-to-eat foods (FDA/FSIS 2003; FAO/WHO 2004) both included detailed hazard characterizations for *L. monocytogenes* (including characterization of severity and the selection of appropriate human health endpoints to be considered, factors that affect dose-response relations, and approaches to mathematical modeling of dose-response). Both documents described and contrasted the various dose-response models in the microbiological literature. Their discussions included detailed analyses of the assumptions underlying each mathematical model, the implications of using each at high, medium and low doses, various model forms, and various parameterizations that might be used. This information is not repeated here. The exact form of a dose response model for *L. monocytogenes* remains a topic of considerable research. To date, risk assessments have considered several different forms. Presently, the most common class of dose response models is the “linear at low-dose” model. The choice of model and data sources along with modeling assumptions can have a substantial effect on absolute measures of risk.

Both the FDA/FSIS (2003) and FAO/WHO (2004) risk assessments developed human dose-response models that were scaled using U.S. exposure (FDA/FSIS 2001) and U.S. epidemiological (Mead et al. 1999) data for susceptible and general populations. The FAO/WHO model was used here because, while the two models are functionally equivalent and linear within the dose range of interest, the FAO/WHO model requires fewer parameters and is thus more straightforward to implement. The details of this model are described in FAO/WHO (2004).

The FAO/WHO used an exponential dose-response model with invasive listeriosis as the human health endpoint of concern. Model parameters were developed for two subpopulations: one with higher susceptibility (including neonates, the elderly and the immunocompromised, these subpopulations being referred as “susceptibles” in FAO/WHO (2004)), and another with a lower level of susceptibility (the general population, referred as “non-susceptible” in FAO/WHO (2004)). This dose-response model can be written as

$$\Pr\{\text{endpoint} \mid D\} = 1 - \exp(-rD)$$

where $\Pr(\text{endpoint}|D)$ is the fraction of a population that develops invasive listeriosis when individuals within that population ingest doses of *L. monocytogenes* that follow a Poisson distribution with mean D . The exponential dose response model is a single-hit model; that is, it assumes that each ingested cell of the pathogen acts independently and that each cell has a finite, non zero probability of causing illness (Haas et al. 1999). The parameter of this model, r , is often interpreted as the probability that 1 cell will cause illness in a random consumer in the considered population. In the exponential model, this parameter is considered to be constant for a specific population. More explicitly, we assume the single-hit, independent action model:

$$\pi(d; p) = \Pr(\text{ill} | d) = 1 - (1 - p)^d, \quad d \geq 0; \quad 0 < p < 1$$

where d is ingested dose and p is the probability of illness from ingesting a single *L. monocytogenes* cell. The model can be reparameterized using the simple relationship

$$e^{-r} = 1 - p$$

yielding the exponential dose-response function form

$$\pi(d; r) = \Pr(\text{ill} | d, r) = 1 - \exp(-rd), \quad r > 0, \quad d > 0$$

where r is the single adjustable parameter of the dose response model.

When no uncertainty is considered, point estimate 1.06×10^{-12} , is used for the r parameter for the Elderly, Immunocompromised and Pregnant women populations' dose response function (*i.e.* the “susceptible population” (FAO/WHO 2004)), and another value of the parameter r , point estimate 2.37×10^{-14} , is used for the r parameter for the remaining “General” population's dose-response function (“non-susceptible” (FAO/WHO 2004)) (see Table 2.17, p. 56 and Table 2.20, p.58, FAO/WHO 2004).

The r parameter for a population may also be treated as a fixed, but unknown value when uncertainty is considered. The FAO/WHO (2004) risk assessment inferred the susceptible and non-susceptible population unknown r parameter values by representing attack rates –the annual number of listeriosis cases and the annual exposure— constructed using exposure data from a draft FDA/FSIS report (FDA/FSIS 2001) and from the estimated annual number of cases of listeriosis in the U.S. (Mead et al. 1999), subject to uncertainty about

- the fraction of the population that is susceptible rather than non-susceptible (15% to 20%);
- the fraction of cases in the epidemiological record that were attributed to susceptible consumers (80% to 98%);
- the annual number of listeriosis cases (1,888 to 3,148); and
- the fraction of servings with <1 , $1-10^3$, 10^3-10^6 , 10^6-10^9 $>10^9$ *L. monocytogenes* (FDA/FSIS 2001).

Uncertainty in the r parameters follows. The FAO/WHO (2004) risk assessment, for example, used a Monte-Carlo simulation to derive an empirical distribution of uncertainty for each of the r parameters (Analytica[®], 10,000 iterations, Median Latin Hypercube Sample, minimal standard randomization method, Table 6 (see Table 2.17, p. 56 and Table 2.20, p.58, FAO/WHO 2004)).

The distribution of uncertainty for the fixed, unknown r parameter for the susceptible population has a mean of 2.47×10^{-12} , a median of 1.06×10^{-12} and 0.025th and 0.975th quantiles of 3.87×10^{-14} and 1.03×10^{-11} , respectively. For the non-susceptible population the mean is 6.46×10^{-14} , the median is 2.72×10^{-14} and the 0.025th and 0.975th quantiles are 9.83×10^{-16} and 3.42×10^{-13} , respectively (1,000,000 Random Monte Carlo iterations).

Table 6: Uncertainty distributions for r parameter values.

Non-susceptible population	Pr(r)	Susceptible population	Pr(r)
[0, 1.54×10^{-14}]	0.395	[0, 5.57×10^{-13}]	0.3576
[1.54×10^{-14} , 3.70×10^{-14}]	0.1922	[5.57×10^{-13} , 1.13×10^{-12}]	0.1596
[3.70×10^{-14} , 5.85×10^{-14}]	0.0982	[1.13×10^{-12} , 1.70×10^{-12}]	0.076
[5.85×10^{-14} , 8.00×10^{-14}]	0.0652	[1.70×10^{-12} , 2.27×10^{-12}]	0.0684
[8.00×10^{-14} , 1.02×10^{-13}]	0.0464	[2.27×10^{-12} , 2.83×10^{-12}]	0.0498
[1.02×10^{-13} , 1.23×10^{-13}]	0.0342	[2.83×10^{-12} , 3.40×10^{-12}]	0.0192
[1.23×10^{-13} , 1.45×10^{-13}]	0.0256	[3.40×10^{-12} , 3.97×10^{-12}]	0.033
[1.45×10^{-13} , 1.66×10^{-13}]	0.0256	[3.97×10^{-12} , 4.54×10^{-12}]	0.0384
[1.66×10^{-13} , 1.88×10^{-13}]	0.0196	[4.54×10^{-12} , 5.11×10^{-12}]	0.0298
[1.88×10^{-13} , 2.09×10^{-13}]	0.0186	[5.11×10^{-12} , 5.68×10^{-12}]	0.0204
[2.09×10^{-13} , 2.31×10^{-13}]	0.0128	[5.68×10^{-12} , 6.25×10^{-12}]	8.40×10^{-3}
[2.31×10^{-13} , 2.52×10^{-13}]	0.0104	[6.25×10^{-12} , 6.82×10^{-12}]	0.013
[2.52×10^{-13} , 2.74×10^{-13}]	7.60×10^{-3}	[6.82×10^{-12} , 7.39×10^{-12}]	0.0164
[2.74×10^{-13} , 2.95×10^{-13}]	8.20×10^{-3}	[7.39×10^{-12} , 7.96×10^{-12}]	0.0206
[2.95×10^{-13} , 3.17×10^{-13}]	7.80×10^{-3}	[7.96×10^{-12} , 8.53×10^{-12}]	0.0194
[3.17×10^{-13} , 3.38×10^{-13}]	6.60×10^{-3}	[8.53×10^{-12} , 9.10×10^{-12}]	0.0142
[3.38×10^{-13} , 3.60×10^{-13}]	6.40×10^{-3}	[9.10×10^{-12} , 9.67×10^{-12}]	0.0166
[3.60×10^{-13} , 3.81×10^{-13}]	4.80×10^{-3}	[9.67×10^{-12} , 1.02×10^{-11}]	0.0128
[3.81×10^{-13} , 4.03×10^{-13}]	3.80×10^{-3}	[1.02×10^{-11} , 1.08×10^{-11}]	0.0102
[4.03×10^{-13} , 4.25×10^{-13}]	2.80×10^{-3}	[1.08×10^{-11} , 1.14×10^{-11}]	8.00×10^{-3}
[4.25×10^{-13} , 4.46×10^{-13}]	2.60×10^{-3}	[1.14×10^{-11} , 1.19×10^{-11}]	6.20×10^{-3}
[4.46×10^{-13} , 4.68×10^{-13}]	2.80×10^{-3}	[1.19×10^{-11} , 1.25×10^{-11}]	1.40×10^{-3}
[4.68×10^{-13} , 5.04×10^{-13}]	2.80×10^{-3}	[1.25×10^{-11} , 1.36×10^{-11}]	6.00×10^{-4}

(unpublished, from FAO/WHO 2004).

As discussed in the Hazard identification section, this risk assessment considers four populations: the General population and three susceptible populations: Pregnant women, the Immunocompromised, and the Elderly. The dose-response r parameter for the non-susceptible population in the FAO/WHO assessment is used for the “General” population and the dose-response r parameter for the FAO/WHO susceptible population is used for “Pregnant”, the “Immunocompromised” and the “Elderly” populations.

The exponential FAO/WHO (2004) dose-response that is used in this risk assessment is an averaged dose response regarding variability in strain virulence, as its value is inferred from epidemiological data that implies multiple strains. Since 2004, the major knowledge about strain virulence is the variation in relation to subtypes encoding a full-length or truncated Internalin A (Lecuit et al. 1999; Lecuit et al. 2001; Chen et al. 2011). The “averaged” dose-response could be biased when used for soft-ripened cheese if the distribution of subtypes of *L. monocytogenes* in soft cheese differs from the one in other products. Using data from Chen *et al.* (2011) issued from an analysis of the strains isolated in the Gombas *et al.* (2003) study, the repartition of *inlA* subtypes is not significantly different in soft cheese compared to other food items (8 vs. 4 strains

with/without premature stop codon (PMSC) for soft-ripened cheese, 219 vs. 271 for other food, $p = 0.15$). Without further data, the FAO/WHO (2004) dose response was used in this risk assessment without further considering a specific distribution of serotypes for cheese as compared to other commodities.

6. Basic Processes

The exposure assessment model consists of product pathway-specific elements in a set of “basic processes” (Nauta 2008). Six basic processes that may affect the prevalence and/or level of any microbial hazard in a food at multiple steps in the product pathway have been described. These basic processes are:

- **Growth:** the multiplication of bacteria or an increase in the size of the population;
- **Inactivation:** the decrease in the number of bacteria or in the size of the population that results from the application of a food safety or preservation strategy. Inactivation may also be the consequence of the natural environment in the food, *e.g.* low pH or low water activity;
- **Partitioning:** redistribution of bacteria that occurs when a large unit of food is split into two or more smaller units;
- **Mixing:** redistribution that is the opposite of partitioning, and occurs when smaller units of food are combined to form a new, larger unit;
- **Contamination:** (in this report) occurs when bacteria are transferred to milk or from the environment to food;
- **Removal:** this occurs when some units of food are removed from the product pathway. Non-selective removal might occur when some units of food are diverted to an alternate product pathway and selective removal might occur when some units are removed as a result of testing (Nauta 2008).

The impact of each of the basic processes on bacterial prevalence, the total number of bacterial cells and on the unit size of the food, is shown in Table 7.

Table 7: Basic processes and their qualitative effects.

Basic processes	Effect on prevalence	Effect on the total number of bacteria	Effect on the food unit size
Growth	=	+	=
Inactivation	-	-	=
Mixing	+	=	+
Partitioning	-	=	-
Removal	-	-	=
Contamination	+	+	=

(adapted from Nauta 2008). Notes: = same, + increase, - decrease.

In the product pathway for soft-ripened cheeses, these 6 basic processes are encountered in several steps (Figure 6). Bacterial growth is observed throughout the product pathway, both in milk and then in cheese. Bacterial inactivation occurs during cheese-making through the application of food safety strategies (*e.g.* pasteurization) and naturally as a result of acidification during initial ripening. Mixing and partitioning are encountered on farm (*e.g.* mixing of milk from different cows), during cheese processing (*e.g.* mixing of milk from different farms, separation of milk into curds and whey, partitioning of curd into individual cheeses) and at home (partitioning of a cheese into servings). Removal of pathogen containing lots of milk or cheese is a risk mitigation strategy that may result from microbiological testing. Contamination with bacteria from the environment may occur on the farm, in the plant, at retail and at the consumer depending on circumstances.

This section describes the general rules and data used to model these basic processes. The specific uses of these basic processes within each stage of the product pathway are described in detail in the Exposure Assessment section 7.



Figure 6: The product pathway and the corresponding basic processes.

6.1. Growth

Bacterial growth is one of the most important basic processes that must be considered in a quantitative microbiological risk assessment for *Listeria* (FDA/FSIS 2003; FAO/WHO 2004).

Assuming that bacterial populations in a defined environment behave in a reproducible manner, predictive microbiology models can be used to model changes in bacterial populations based on the level of initial contamination and the properties of the food environment (Ross and McMeekin 2003).

This section describes the models used to predict growth of *L. monocytogenes* and the procedures used to derive point or distribution estimates for the parameters used in these models (Table 8). A single *L. monocytogenes* growth model, with different parameters, was used to predict growth in milk (all stages before the cheese processing) and in the cheeses after ripening (aging and all stages after cheese-making). A different model was used for growth during ripening.

Table 8: Growth models used in this risk assessment.

Unpasteurized-milk cheese	Description
On farm (milk)	Lag phase: “relative lag time” concept (Ross and McMeekin 2003) Secondary model: square root model for temperature (Ratkowsky et al. 1982) Primary model: three phase linear model (Buchanan et al. 1997)
Initial ripening	Inactivation (Ryser and Marth 1987)
Secondary ripening	Lag phase (for newly inoculated bacteria issued from the environment): “relative lag time” concept (Ross and McMeekin 2003) Secondary model: Augustin <i>et al.</i> (2005) model for temperature, pH, a_w and interactions Primary model: three phase linear model (Buchanan et al. 1997)
Aging (from packaging to consumption)	Remaining lag phase: “relative lag time” concept (Ross and McMeekin 2003) Secondary model: square root model for temperature (Ratkowsky et al. 1982) Primary model: three phase linear model (Buchanan et al. 1997)

Table : Growth models used in this risk assessment for pasteurized milk cheese.

Pasteurized-milk cheese	Description
Before secondary ripening	No bacteria
Secondary ripening	Lag phase (for newly inoculated bacteria issued from the environment): “relative lag time” concept (Ross and McMeekin 2003) Secondary model for temperature, pH, a_w and interactions: Augustin <i>et al.</i> (2005) model Primary model: three phase linear model (Buchanan et al. 1997)
Aging (from packaging to consumption)	Remaining lag phase: “relative lag time” concept (Ross and McMeekin 2003) Secondary model: square root model for temperature (Ratkowsky et al. 1982) Primary model: three phase linear model (Buchanan et al. 1997)

6.1.1. Growth in Milk and Growth in Cheese after Ripening

Description of the Model

Primary Model

In predictive microbiology, a primary growth model predicts changes in a bacterial population over time in a given environment. The three-phase linear model is a commonly used primary model for growth in a constant environment. (Buchanan et al. 1997; van Gerwen and Zwietering 1998). This model assumes that there is an exponential increase in the bacterial population with time, until a maximum population density is reached. A lag phase may be included by delaying the start of exponential growth. The model is written as:

$$\begin{cases} y(t) = y(0) & t < \lambda \geq 0 \\ y(t) = \min(y(0) + EGR \times (t - \lambda), y_{\max}) & t \geq \lambda \geq 0 \end{cases}$$

where⁸ $y(t)$ (log(cfu)/g) is the bacterial concentration at time t (d), λ (d) is the lag time observed in a particular environment T , EGR (log(cfu)/g/d) is the exponential growth rate observed in environment T and y_{\max} (log(cfu)/g) is the maximum population density in environment T . Figure 7 illustrates this model.

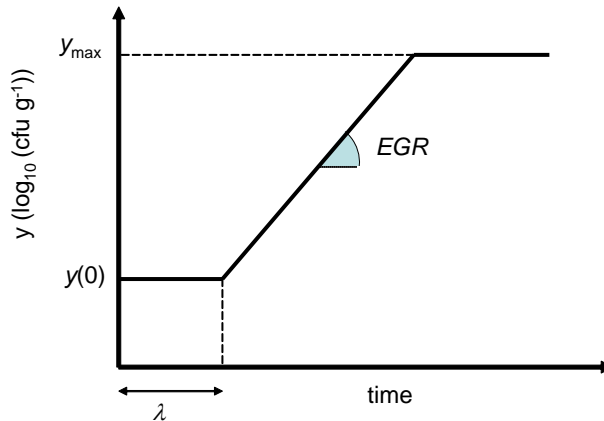


Figure 7: The three-phase linear primary growth model.

⁸ Recall: in this report, $\log(x)$ or $\log_{10}(x)$ stands for the $\log_{10}(x)$ (logarithm base 10) and $\ln(x)$ stands for $\log_e(x)$ (natural logarithm or logarithm base e).

Secondary model

Secondary growth models are enhancements of a primary model that alter parameters (namely EGR , λ and y_{\max}) in the primary model to reflect changes in the environment.

Characterization of the Environment

With the exception of the ripening phase, the model used in this assessment treated temperature as varying from step to step in the product pathway (farm storage for milk; aging, transport and marketing, storage at retail and storage at home for ripened cheeses) but that it is constant within each step. Other environmental factors that impact bacterial growth were treated as varying from batch to batch depending on context within the product pathway, and variation in the EGR_T caused by varying environmental factors were modeled by specifying a probability distribution for EGR_T that implicitly accounts for these factors (Ross and McMeekin 2003).

Secondary Model – Growth Rate

The popular square root approach (Ratkowsky et al. 1982) was used to model the effect of temperature T ($^{\circ}\text{C}$) on growth rate. This model assumes that there is a linear relationship between the temperature and the square root of EGR_T , with $EGR_T = 0$ when $T \leq T_{\min}$, where T_{\min} ($^{\circ}\text{C}$) is the minimum growth temperature. This can be written as:

$$\begin{cases} EGR_T = EGR_{T_{ref}} \left(\frac{T - T_{\min}}{T_{ref} - T_{\min}} \right)^2 & T > T_{\min} \\ EGR_T = 0 & T \leq T_{\min} \end{cases}$$

where $EGR_{T_{ref}}$ is the exponential growth rate in a specific food at a chosen T_{ref} ($^{\circ}\text{C}$) temperature. This model is valid for $T \ll T_{opt}$. The optimal growth temperature, T_{opt} , for *L. monocytogenes* is $\approx 37^{\circ}\text{C}$ (Augustin and Carlier 2000). T_{ref} was arbitrarily set to 20°C . $EGR_{T_{ref}}$ is a function of the growth medium and varies among strains. A similar square root model was used in the FDA/FSIS risk assessment for *L. monocytogenes* in ready to eat foods with $T_{ref} = 5^{\circ}\text{C}$, a constant $T_{\min} = -1.18^{\circ}\text{C}$ and a distribution of EGR_5 developed through a literature review (FDA/FSIS 2003).

Secondary Model - Lag

A growth lag (λ_T) may be observed when bacteria are transferred to a new environment. This delay in the growth is assumed to be the result of the time needed for the cells to readjust their physiology to the new environment. For bacteria in a given physiological state, the lag time is approximately proportional to the generation time (time for the population to double) in the new environment (Delignette-Muller 1998; Ross and McMeekin 2003). That is:

$$\lambda_T = K_\xi GT_T$$

in an environment T , where K_ξ (called “Relative Lag Time”, RLT (Ross and McMeekin 2003)), is a function of the physiological state ξ of the cells before transfer and

$$GT_T = \frac{\log_{10}(2)}{EGR_T}$$

is the generation time (d) in environment T . Note that K_ξ is linked to the “work to be done” during the lag phase h_0 (Baranyi and Roberts 1994), RLT and h_0 being proportional to each other.

Some studies have attempted to describe how K_ξ depends on the cells’ history (Breand et al. 1997; Delignette-Muller 1998; Breand et al. 1999; Mellefont et al. 2003; Mellefont et al. 2004) but no universally accepted model is currently available. Nevertheless, these studies have shown that the larger the shift in environmental conditions, the higher is the K_ξ . In the absence of a generally accepted model, Ross and McMeekin (2003) suggested the use of a value or a distribution of K_ξ taken from the relevant literature. We used a distribution from Ross *et al.* (2009) to describe how K_ξ varies.

Here, we modeled a lag of $\lambda > 0$ in the growth of bacteria introduced to milk from either the farm environment or mastitis and in the growth of bacteria introduced to cheese from the environment. No extra lag in growth was included to account for moving from step to step in the product pathway to account for steps’ temperature shifts. Step to step temperature shifts might occur when milk is transferred from the farm bulk tank to a dairy silo, for example, or when a cheese is transferred from retail display to a consumer’s refrigerator.

Secondary Model - y_{maxT}

The maximum population density y_{maxT} is usually considered to be a function of only the growth medium. Few studies have specifically evaluated the impact of temperature on the maximum population density y_{maxT} . The FDA/FSIS (2003) risk assessment assumed that y_{maxT} increased with increasing temperature. The same temperature dependence for y_{maxT} was used here.

Growth in Temperature Varying Processes

As described above, temperature was considered to be constant within the storage and handling steps, changing only as part of the transition from one step to the next. The bacterial population was assumed to react immediately in a manner described by the growth curve for the new conditions and without an additional lag phase. We permit the lag phase from the previous stage to persist into the next stage(s) until it is completed. If the cells entered a new step while still in lag phase, the lag time was transferred from step to step until completion of the total lag. This was modeled as if the whole lag time corresponded to a certain amount of work w to be done by the cells. During a given step j , a part of this work equal to

$$\left(w \times \frac{t_j}{\lambda_{Tj}} \right)$$

was done, where t_j is the duration of the step and λ_{Tj} is the lag time needed in the environment of the step j . When all of the work w was done, the lag was finished. Thus for a given step m a lag period equal to:

$$\lambda_m^* = \max \left(0, \lambda_{T_m} \times \left[1 - \sum_{j=1}^{m-1} \frac{t_j}{\lambda_{T_j}} \right] \right)$$

was used, as in Albert *et al* (2005).

The maximum population density appropriate for a new environment was applied immediately after moving from one step to the next. For example, if $y = 7.5 \log(\text{cfu})/\text{g}$ in a step where $y_{\max,n} = 8 \log(\text{cfu})/\text{g}$ and the bacteria entered another step where $y_{\max,n+1} = 7 \log(\text{cfu})/\text{g}$, the bacterial population was assumed to decrease to $y_{\max,n+1} = 7 \log(\text{cfu})/\text{g}$ by the end of step $n+1$. If the bacteria then entered a step where $y_{\max,n+2} = 8 \log(\text{cfu})/\text{g}$, the population grew again without delay.

Growth in a Solid Medium

Cheese is a solid medium where growth could be different from that normally observed in a liquid medium. For example, Sanaa *et al.* (2004) assumed that each cell was immobilized by the cheese matrix and gave rise to one colony with a maximum density population of 10^9 cfu per initial bacterial cell.

In this assessment model, bacterial growth in cheese was limited to $y_{\max T} \log(\text{cfu})$ at the level of individual units of 1 gram of cheese. The n bacteria that contaminated an individual K g cheese were assumed to be deposited among a number p of virtual cubes of 1 gram each, with $\min(K, n) \geq p \geq 1$. Growth in each of these contaminated grams was limited to $10^{y_{\max T}}$. The maximum bacterial population in the whole cheese was then $p \times 10^{y_{\max T}}$.

Specification of Distributions for Growth Model Parameters

L. monocytogenes growth varies among milk collections or cheeses sampled at random due to both strain and medium variability (pH, a_w , for example). In general, this variability is summarized using probability distributions given other known environmental parameters (temperature, time). Parameter uncertainty may be derived in some cases using classical statistical inference. In this assessment unknown parameters for these distributions were estimated using literature data, preferably from published meta-analyses. This procedure assumes that the data sets used to describe the parameters occur such as would be the case if the data set conditions were a random sample of the conditions in the population of cheeses of interest.

Minimum Growth Temperature T_{\min}

The minimum growth temperature was described using data from the meta-analysis of Augustin *et al.* (2005) (Table 3, pg. 1025-1026). Augustin *et al.* (2005) estimated minimum growth temperatures ($^{\circ}\text{C}$) for 25 studies in liquid microbiological media. Variability in T_{\min} among bacterial strains was described by the equation

$$T_{\min} \sim N(\mu_{T_{\min}}, \sigma_{T_{\min}}^2)$$

Uncertainty for the unknown parameters $\mu_{T_{min}}$ and $\sigma_{T_{min}}^2$ was described using classical statistical theory. The parameters and their uncertainty distributions that were derived are shown in Table 9.

Table 9: Maximum likelihood estimates for minimum growth temperature.

Parameter	Point estimate (m.l.e.)	Uncertainty distribution
$\mu_{T_{min}}$	-1.72	Normal(-1.72, 0.51 ²)
$\sigma_{T_{min}}^2$	2.55 ²	Gamma ⁻¹ (12.5, 81.28 ⁻¹)

Normal(μ_{min} , $\sigma_{T_{min}}^2$) distribution.

EGR₂₀ in Milk

The optimal growth rate (h⁻¹) for *L. monocytogenes* in milk at the optimal temperature for growth was modeled as

$$\mu_{opt} \sim \text{Normal}(\theta_{\mu_{opt}}, \sigma_{\mu_{opt}}^2)$$

truncated on [0; ∞), where

$$\mu_{opt} = \frac{EGR_{opt}}{\ln(10)}$$

is the specific growth rate at the optimal temperature. Pouillot *et al.* (2003) used a Bayesian meta-analysis of 124 growth curves for *L. monocytogenes* in milk from 12 publications to obtain the point estimates and the uncertainty distribution for $\theta_{\mu_{opt}}$ and $\sigma_{\mu_{opt}}^2$ shown in Table 10.

Table 10: Estimates for optimal growth rate in milk distribution.

Parameter	Point estimate (Bayesian inferences)	Uncertainty distribution
$\theta_{\mu_{opt}}$	0.69	Gamma(601, 502.5)-0.508
$\sigma_{\mu_{opt}}^2$	0.18 ²	(LN(-1.73, 0.160 ²) - 9.06×10 ⁻⁴) ²

(Pouillot *et al.* 2003). Normal truncated on [0, ∞).

The specific growth rate (ln(cfu)/h) was transformed to an exponential growth rate (log(cfu)/d) at a reference temperature of 20°C using a multiplying factor of 4.64:

$$EGR_{20} = 4.64 \times \mu_{opt}$$

This value was obtained using the secondary cardinal model of Rosso *et al.* (1993) with temperature parameters $T_{min} = -1.72^\circ\text{C}$, $T_{opt} = 37^\circ\text{C}$, $T_{max} = 45.5^\circ\text{C}$ (Augustin *et al.* 2005).

EGR₂₀ in Camembert

As described above, the product pathway model was developed using parameters appropriate for the manufacture of soft-ripened cheese, and where necessary, Camembert was used as an example of a soft-ripened cheese. Because no published meta-analysis for *EGR* of *L. monocytogenes* in soft-ripened cheese was available, a literature search was carried out to identify papers with data that could be used to develop an EGR model for Camembert appropriate to the intended application in this risk assessment, that is, to describe the EGR variability for a *L. monocytogenes* strain at random in the rind and in the core of a cheese at random (Table 11). Authors' design characteristics are in the rightmost 6 columns in Table 11. Table 11's EGR_T is a reported *EGR* at study temperature $T^\circ\text{C}$ directly from the article's text or tables or it was read or measured from the article's graphs. In studies that used inoculated cheeses, the EGR_T sometimes refers to the average among several *L. monocytogenes* strains pooled into the same inoculant. Some articles' EGR_T values were averages over several independent replicates of the articles' experiments (*Trials* avgd), usually replicating cheese-making and possibly the preparation of the *L. monocytogenes* used in the experiment. Some articles' EGR_T values were averages of growth in cheeses over several individual cheeses within the same cheese-making (*Cheeses* avgd). Some articles' EGR_T values were averages of growth over several *L. monocytogenes* strains (*Lm strains* pooled). We encode the milk characteristics that the study used as *Pasteurized milk* (PM), *Raw milk* (RM) and *Unknown* (UNK). Our references to *Table* or *Figure* in the *Notes* column in Table 11 indicate the source of the information within the reference article, not to a *Table* or *Figure* in this report.

Table 11: Data for Camembert aging and holding growth rates.

Ident numb.	Source	Cheese part	egr_T	Temp ($^\circ\text{C}$)	<i>Lm strains</i> pooled	<i>Trials</i> avgd	<i>Cheese</i> avgd	Milk	Notes
1	(Ryser and Marth 1987)	Rind	.0298	6	1	1	3	PM	Scott A, Figure 5
2	(Ryser and Marth 1987)	Rind	.0000	6	1	1	3	PM	V7, Figure 5
3	(Ryser and Marth 1987)	Rind	.0207	6	1	1	3	PM	CA, Figure 5
4	(Ryser and Marth 1987)	Rind	.0658	6	1	1	3	PM	OH, Figure 5
5	(Ryser and Marth 1987)	Rind	.0970	6	1	1	3	PM	Scott A, Figure 1
6	(Ryser and Marth 1987)	Core	.0450	6	1	1	3	PM	Scott A, Figure 1
7	(Ryser and Marth 1987)	Rind	.1050	6	1	1	3	PM	CA, Figure 3
8	(Ryser and Marth 1987)	Core	.0780	6	1	1	3	PM	CA, Figure 3
9	(Ryser and Marth 1987)	Rind	.1000	6	1	1	3	PM	V7, Figure 2
10	(Ryser and Marth 1987)	Core	.0538	6	1	1	3	PM	V7, Figure 2
11	(Ryser and Marth 1987)	Rind	.0750	6	1	1	3	PM	OH, Figure 4
12	(Ryser and Marth 1987)	Core	.0730	6	1	1	3	PM	OH, Figure 4

Ident numb.	Source	Cheese part	egr_T	Temp (°C)	<i>Lm</i> strains pooled	Trials avgd	Cheese avgd	Milk	Notes
13	(Genigeorgis et al. 1991)	Core	.8655	30	5	1	1	UNK	Table 1, pg. 664
14	(Genigeorgis et al. 1991)	Core	.1456	8	5	1	1	UNK	Table 1, pg. 664
15	(Genigeorgis et al. 1991)	Core	.0197	4	5	1	1	UNK	Table 1, pg. 664
16	(Genigeorgis et al. 1991)	Rind	.8655	30	5	1	1	UNK	Table 1, pg. 664
17	(Genigeorgis et al. 1991)	Rind	.0927	8	5	1	1	UNK	Table 1, pg. 664
18	(Genigeorgis et al. 1991)	Rind	.0183	4	5	1	1	UNK	Table 1, pg. 664
19	(Sulzer and Busse 1991)	Rind	.0608	6	1	1	1	PM	<i>Li</i> , Figure 1
20	(Sulzer and Busse 1991)	Rind	.0473	6	1	1	1	PM	<i>Li</i> , Figure 1
21	(Sulzer and Busse 1991)	Rind	.0583	6	1	1	1	PM	<i>Lm</i> , Figure 3
22	(Sulzer and Busse 1991)	Rind	.0288	6	1	1	1	PM	<i>Lm</i> , Figure 3
23	(Sulzer and Busse 1993)	Rind	.0909	6	1	1	1	PM	<i>Li</i> , Figure 1
24	(Sulzer and Busse 1993)	Core	.0606	6	1	1	1	PM	<i>Li</i> , Figure 1
25	(Sulzer and Busse 1993)	Rind	.0500	4	1	1	1	PM	<i>Li</i> , Figure 6
26	(Sulzer and Busse 1993)	Rind	.1500	7	1	1	1	PM	<i>Li</i> , Figure 6
27	(Sulzer and Busse 1993)	Rind	.475	15	1	1	1	PM	<i>Li</i> , Figure 6
28	(Maisnier Patin et al. 1992)	Core	.1464	11	1	1	3	PM	Nis-
29	(Maisnier Patin et al. 1992)	Rind	.2107	11	1	1	3	PM	Nis-
30	(Back et al. 1993)	Rind	.0600	3	1	2	6	PM	Figure 1a
31	(Back et al. 1993)	Rind	.0740	6	1	2	6	PM	Figure 1a
32	(Back et al. 1993)	Rind	.1200	10	1	2	6	PM	Figure 1a
33	(Back et al. 1993)	Rind	.0467	3	1	1	1	UNK	Table 1
34	(Back et al. 1993)	Rind	.1467	6	1	1	1	UNK	Table 1
35	(Back et al. 1993)	Rind	.0867	3	1	1	1	UNK	Table 1
36	(Back et al. 1993)	Core	-0.028	3	1	2	6	PM	Figure 1b
37	(Back et al. 1993)	Core	-0.028	6	1	2	6	PM	Figure 1b
38	(Back et al. 1993)	Core	0	10	1	2	6	PM	Figure 1b
39	(Back et al. 1993)	Core	.092	15	1	2	6	PM	Figure 1b
40	(Murphy et al. 1996)	Core	.0070	4	1	1	1	UNK	Table 4, Gompertz
41	(Murphy et al. 1996)	Core	.0836	10	1	1	1	UNK	Table 4, Gompertz
42	(Wan et al. 1997)	Rind	.2493	15	2	1	3	PM	control
43	(Wang and Johnson 1997)	Rind	.0943	4	1	1	2	UNK	Figure 6A control
44	(Liu et al. 2004)	Rind	.0700	7	1	3	8	PM	TS
45	(Liu et al. 2004)	Core	.1100	7	1	3	8	PM	C
46	(Liu et al. 2007)	Rind	.0600	7	1	3	8	PM	TS
47	(Liu et al. 2007)	Core	.0467	7	1	3	8	PM	C
48	(Liu et al. 2009)	Core	.0333	7	1	3	8	PM	<i>Lm</i> , Figure 6c, C
49	(Liu et al. 2009)	Core	.0417	7	1	3	8	PM	<i>Li</i> , Figure 6c, C
50	(Liu et al. 2009)	Rind	.0600	7	1	3	8	PM	<i>Lm</i> , Figure 6b, TS
51	(Liu et al. 2009)	Rind	.0533	7	1	3	8	PM	<i>Li</i> , Figure 6b, TS
52	(D'Amico et al. 2008a)	Rind	.0429	4	5	1	3	RM	Figure 4
53	(D'Amico et al. 2008a)	Rind	.0393	4	5	1	3	PM	Figure 4
54	(D'Amico et al. 2008a)	Rind	.0881	4	5	1	3	RM	Figure 4
55	(D'Amico et al. 2008a)	Rind	.0536	4	5	1	3	PM	Figure 4

PM: pasteurized milk; RM: raw milk; UNK: unknown.

Some studies, or some data from some studies listed in Table 11, were excluded from this analysis for several reasons:

- Growth was measured in soft-ripened cheeses other than Camembert (Genigeorgis et al. 1991; Back et al. 1993; Guerzoni et al. 1994; Whitley et al. 2000; Faleiro et al. 2003; Arqués et al. 2005; Modzelewska-Kapitula and Marin-Iniesta 2005; Kongo et al. 2006;

Tan et al. 2008) or in processed, fresh, unripened, surface smear, soft, semi-soft, semi-hard and hard cheeses based on article title or keywords;

- Growth was measured for pathogens other than *L. monocytogenes* or *L. innocua* such as *E. coli*, *Salmonella* spp. or *Yersinia enterocolitica* (Ramsaran et al. 1998; Leuschner and Boughtflower 2002; Modzelewska-Kapitula and Marin-Iniesta 2005);
- Growth was clearly affected by reaching maximum population densities (Back et al. 1993);
- Inhibitor treatments other than milk pasteurization or additives applied to milk or cheese were used (Sulzer and Busse 1991; Maisnier Patin et al. 1992; Bougle and Stahl 1994; Wan et al. 1997; Wang and Johnson 1997; Ramsaran et al. 1998; Garcia-Graells et al. 2000; Loessner et al. 2003; Modzelewska-Kapitula and Marin-Iniesta 2005);
- Growth was measured using blended core and rind samples (Ryser and Marth 1987; Maisnier Patin et al. 1992; Wang and Johnson 1997; Ramsaran et al. 1998; Leuschner and Boughtflower 2002; Helloin et al. 2003; Liu et al. 2004; Gay and Amgar 2005; Liu et al. 2007; Liu et al. 2009); and,
- Growth was measured only during ripening before aging and holding (Helloin et al. 2003; Linton et al. 2008).

Separate growth rate distributions were developed for the cheese core and rind because it has been consistently observed that the growth rate is higher in the rind than in the core. The common physical reason is that pH is higher in the rind than in the core, and increases more rapidly during ripening (Ryser and Marth 1987; Sanaa et al. 2004; Liu and Puri 2008). Additionally, differences in oxygen tension as well as in water activity between the interior and rind of the cheese could explain this observation. Growth profiles from 55 data sets from 13 references (Table 11) that address growth in the core (19 data sets) and rind (36 data sets) during Camembert aging and holding at study-varying temperatures were used to derive EGR_T ($\log(\text{cfu})/\text{g/d}$) values, where EGR_T is the mean exponential growth rate observed during a specific study at temperature T . The corresponding EGR_{20} values were calculated using the Ratkowsky's square root model (Ratkowsky et al. 1982). Figure 8 shows the EGR_{20} s obtained using fixed $T_{min}=-1.72^\circ\text{C}$, for illustration.

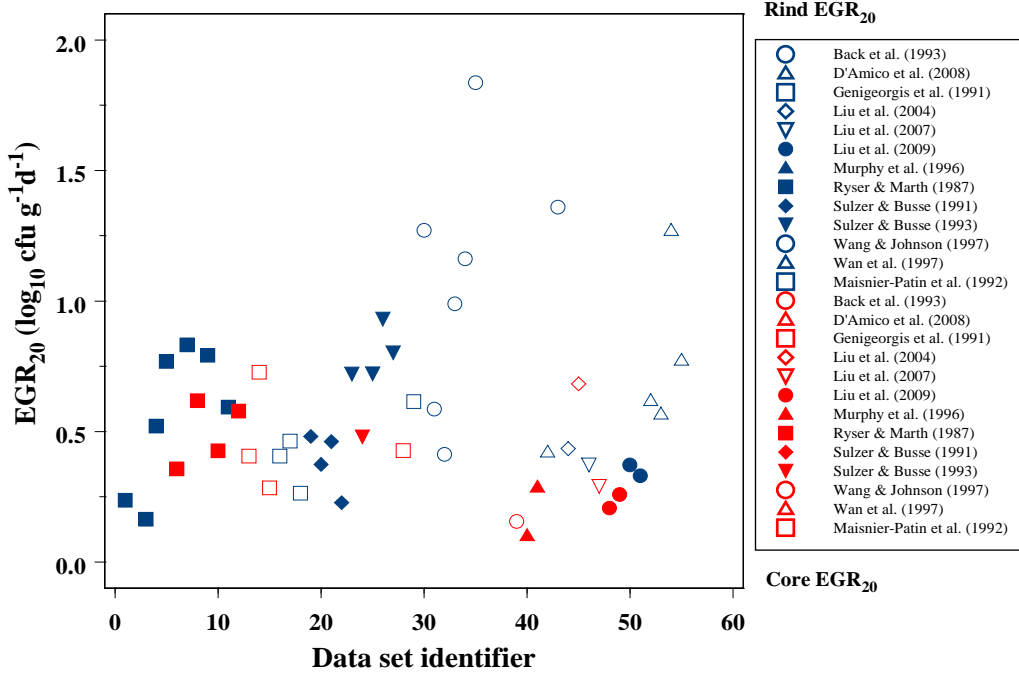


Figure 8: Study EGR_{20} (study-temperature EGR_7 -transformed) in Camembert rind (blue symbols) and core (red symbols).

These data were used to estimate parameters for a hierarchical model of EGR_{20} that accounted for variability among ($L. monocytogenes$ strains \times cheese-making) and among cheeses within ($L. monocytogenes$ strains \times cheese-making). The zero-inflated Gamma distribution is used to describe EGR_{20} variability among $L. monocytogenes$ strains and cheese-making and the Normal distribution is used to describe EGR_{20} variability among individual cheeses within the same cheese-making ($L. monocytogenes$ strain). For example, for the core

$$\begin{cases} EGR_{20_c} = 0 & \text{with probability } \theta_c \\ EGR_{20_c} = \max(X_c + Y_c, 0) & \text{with probability } (1 - \theta_c) \end{cases}$$

with

$$\begin{cases} X_c \sim \text{Normal}(0, \sigma_c^2) \\ Y_c \sim \text{Gamma}(\alpha_c, \lambda_c) \end{cases}$$

Among studies, only Back *et al.* (1993) included a datum point that shows decline in the $L. monocytogenes$ concentration, but there is not enough information in the article to distinguish that decline from merely measurement error. So, the collection of data sets, itself, points to either growth or to no growth. This mixture of distribution models satisfies that indication.

Values for α_C , λ_C , θ_C , σ_C (core) and α_R , λ_R , θ_R , σ_R (rind) were estimated using maximum likelihood methods (Table 12, Table 13). Figure 9 shows the marginal rind and core EGR_{20} density functions for $EGR_{20} > 0$.

Table 12: Maximum likelihood estimates, for Camembert rind and core EGR_{20} .

Rind	m.l.e. \pm se [Wald-type CI95%]	Core	m.l.e. \pm se [Wald-type CI95%]
α_R^*	2.25 \pm 1.50 [-0.791, 5.29]	α_C^*	1.15 \pm 0.629 [-0.179, 2.48]
λ_R^*	-2.83 \pm 1.51 [-5.90, 0.235]	λ_C^*	-2.08 \pm 0.692 [-3.54, -0.618]
θ_R^*	-3.61 \pm 1.07 [-5.78, -1.44]	θ_C^*	-1.74 \pm 0.663 [-3.14, -0.341]
σ_R^*	-1.91 \pm 0.950 [-3.85, 0.018]	σ_C^*	-2.27 \pm 0.955 [-4.28, -0.251]

Normal-zero-inflated Gamma distribution. (α^* , λ^* , θ^* , σ^*)=($\ln[\alpha]$, $\ln[\lambda]$, $\text{logit}(\theta)$, $\ln(\sigma)$).

Table 13: Correlations among parameters' maximum likelihood estimates.

Rind	$\hat{\lambda}_R$	$\hat{\theta}_R$	$\hat{\sigma}_R$	Core	$\hat{\lambda}_C$	$\hat{\theta}_C$	$\hat{\sigma}_C$
$\hat{\alpha}_R$	-0.9989	0.0033	0.8544	$\hat{\alpha}_C$	-0.9815	-10^{-5}	0.5758
$\hat{\lambda}_R$	1	-0.0033	-0.8505	$\hat{\lambda}_C$	1	0.0005	-0.6097
$\hat{\theta}_R$	-0.0033	1	-0.0083	$\hat{\theta}_C$	0.0005	1	-0.0044

The EGR_{20s} in the rind and the core of the same cheese are linked, due to common physical and chemical properties (the food matrix) and the assumed presence of a single bacterial strain. The joint EGR_{20r} and EGR_{20c} distribution was modeled to have these characteristics:

- $EGR_{20C} = EGR_{20C} = 0$ with a probability θ_R . This corresponds to situations where growth does not occur in the rind or in the core;
- $EGR_{20C} = 0$ with an additional probability ($\theta_C - \theta_R$). This corresponds to situations where growth occurs in the rind only;
- rank correlation $\rho(EGR_{20R}, EGR_{20C}) = 0.72$ for a single cheese in the ($EGR_{20R} > 0$, $EGR_{20C} > 0$) region. This rank correlation was estimated using 11 paired data sets (identification numbers: (5, 6), (7, 8), (9, 10), (11, 12), (23, 24), (29, 28), (32, 38), (44, 45), (46, 47), (48, 50), (49, 51) in Table 11). The sampling distribution for this rank correlation was estimated by non-parametric bootstrap from the 11 paired data sets and that sampling distribution was used as an expression of uncertainty.

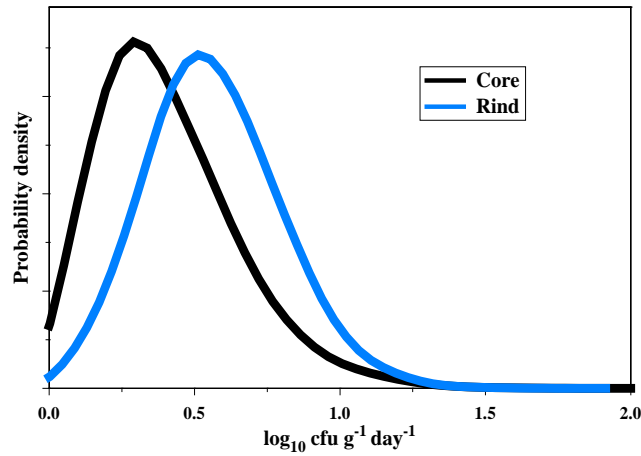


Figure 9: Marginal density functions for single Camembert cheese rind EGR_{20} (blue) and single Camembert cheese core EGR_{20} (black), when EGR_{20} greater than 0.
(Normal Gamma distribution, at Table 12 m.l.e.)

Maximum Population Density

The maximum population density y_{\max} (log(cfu)/g) in milk and cheese was set using values from FDA/FSIS (2003). These values are a function of the temperature and the media as shown in Table 14. It was assumed that there are no among *L. monocytogenes* strain and among cheese effects and that temperature alone accounts for all variability in the maximum population density.

Table 14: Maximum population density (log(cfu)/g) as a function of temperature and medium.

Medium	<5°C	5-7°C	>7°C
Milk	7	7.5	8
Soft-ripened cheese	5	6.5	8

(FDA/FSIS 2003).

Following the procedure used in FDA/FSIS (2003), a range of one log(cfu)/g was used to represent the uncertainty around these point estimates, specifically $y_{\max_T} = f(T) + X$, $X \sim \text{uniform}(-0.5, 0.5)$ and $f(T)$ from Table 14.

Lag

There were no data available to derive the lag time or the number of relative generations that occur following the incorporation of bacteria into milk or cheese from the environment. In a

meta-analysis of 74 publications, Augustin and Carlier (2000) derived a median value of $K_\xi = 3.09$ for this lag. Most of their data came from studies using bacteria in good physiological condition, *i.e.* this value for K is probably lower than would be expected for bacterial transfer in milk or during cheese-making. Ross and McMeekin (2003) showed that K for many bacterial pathogens appears to have a pronounced peak in the range 3–6 under a very wide range of experimental conditions. Mellefont *et al.* (2003) found that most relative lag times were in the range of 4–6 and that relative lag times greater than 8 could not be found with the experimental system used. Ross *et al.* (2009) used a logNormal distribution with a mean of 5.29 and a standard deviation of 5.72 (equivalent to a distribution such as $\ln(x) \sim Normal(1.28, 0.88^2)$) for K in a model of *L monocytogenes* in ready-to-eat meat. This distribution, $\ln(K_\xi) \sim Normal(1.28, 0.88^2)$, was used here to model the lag time. The uncertainty for the μ_ξ and σ_ξ^2 parameter estimates was specified as

$$\hat{\mu}_\xi \sim Normal\left(1.28, \frac{.88^2}{284}\right)$$

and

$$\hat{\sigma}_\xi^{-2} \sim Gamma\left(283/2, [.5 \times 283 \times .88 \times .88]^{-1}\right).$$

Note that Sanaa *et al.* (2004) modeled the growth lag in cheese using $\text{lag} \sim \text{Triangular}(14, 32, 54)$ (in days) based on unpublished data. This lag period leads to an absence of growth during a large part (or all) of the process. There is no published literature that supports using such a long lag period.

6.1.2. Growth in Cheese During Processing

During cheese processing, the bacterial environment is characterized by complex changes of temperature, pH and a_w (Liu and Puri 2004). Measurements of *L. monocytogenes* levels during Camembert cheese-making have shown that bacterial populations decrease due to low pH values during the first 12 days. After these 12 days, these populations increase for the remaining ripening period (Ryser and Marth 1987; Ryser 2007; Liu et al. 2009). Some complex models have been written that model growth in this environment (Sanaa et al. 2004; Liu and Puri 2008; Schwartzman et al. 2011). We model the influence of the temperature, the pH, the water activity

(a_w) and their interactions on the specific growth rate μ (h^{-1}) using Sanaa *et al.* (2004) and Augustin *et al.* (2005).

Initial Ripening

Ryser and Marth (1987) and Ryser (2007) indicate that “during the 17 days of cheese ripening, populations of three out of four *L. monocytogenes* strains decreased 10- to >1000-fold.” Liu and Puri (2008) observed a 1 log decrease during this period. Sanaa *et al.* (2004) used a *Triangular*(0.5, 1, 2) log reduction to model this decrease. The model used here assumes a 1 log(cfu)/g population decline during the initial ripening process. Cheese-to-cheese variability was modeled by assuming independent inactivation of each bacterial cell with an equal probability ($1 \cdot 10^{-1}$). The number of bacteria in an individual cheese at the end of the initial ripening period X_1 follows:

$$X_1 \sim \text{Binomial}(X_0, 10^{-1})$$

where X_0 is the number of bacteria in that individual cheese at the beginning of ripening.

Secondary Ripening

Cheese ripening is characterized by a complex and rapid evolution of the pH and the a_w , associated with changes in temperature. These complex changes justify the use of a more complex model to evaluate *L. monocytogenes* growth. We model the influence of the temperature, pH, a_w and their interactions on the specific growth rate μ (h^{-1}) using the model #5 of Augustin *et al.* (2005), that is,

$$\mu = \mu_{opt} CM_2(T) CM_1(pH) SR_1(a_w) \xi(T, pH, a_w)$$

with $T_{min} = -1.72^\circ\text{C}$, $T_{opt} = 37^\circ\text{C}$, $T_{max} = 45.5^\circ\text{C}$, $a_{wmin} = 0.913$, $a_{wopt} = 0.997$, $pH_{min} = 4.26$, $pH_{opt} = 7.1$, $pH_{max} = 9.61$ in

$$CM_n(X) = \begin{cases} 0, & X \leq X_{min} \\ \frac{(X - X_{max})(X - X_{min})^n}{(X_{opt} - X_{min})^{n-1} \left\{ (X_{opt} - X_{min})(X - X_{opt}) - (X_{opt} - X_{max}) \left[(n-1)X_{opt} + X_{min} - nX \right] \right\}}, & X_{min} \leq X \leq X_{max} \end{cases}$$

$$SR_n(X) = \begin{cases} 0, & X \leq X_{min} \\ \left(\frac{X - X_{min}}{X_{opt} - X_{min}} \right), & X_{min} \leq X \leq X_{max} \end{cases}$$

$$\xi = \begin{cases} 1, & \psi \leq .5 \\ 2(1-\psi), & .5 < \psi < 1, \text{ with } \psi = \sum_{i=\{T, pH, a_w\}} \left(\frac{\varphi(i)}{2 \prod_{j=\{T, pH, a_w\} \neq i} (1-\varphi(j))} \right), \text{ with } \varphi(X) = \left(\frac{X_{opt} - X}{X_{opt} - X_{min}} \right)^3 \\ 0, & \psi \geq 1 \end{cases}$$

Sanaa *et al.* (2004) used $\mu_{opt} = 0.1060 \text{ h}^{-1}$ for Camembert, a value that takes into account the a_w impact. Assuming $a_w = 0.98$ in their process, we used $\mu_{opt} = 0.133 \text{ h}^{-1}$ for a Camembert at T_{opt} , pH_{opt} and a_{wopt} .

Evolution of the pH in Stabilized and non-Stabilized Cheeses

Sanaa *et al.* (2004) described the evolution of pH during the manufacture of “Camembert de Normandie”, Camembert cheeses that are not stabilized, as controlled French designation of origination. They developed a polynomial describing the pH evolution fitted from the data from Ryser and Marth (1987), with very good correspondence with Lawrence *et al.* (1987)’s description for non-stabilized Camembert. After molding, the pH is as low as 4.58 in the core and 4.25 in the rind, and increases during the ripening step to reach 6.55 in the core and 7.09 in the rind at day 55. From Kosikowski (1987) and Lawrence (1987)’s description, we propose that the pH in stabilized cheese increases from a starting value of 5.5 and ends at the same value as classical cheese after 55 days, with a similar shape (see Appendix, section “*L. monocytogenes* growth in Camembert cheese”).

Evolution of the Temperature and a_w

The temperature profile during Camembert ripening was the one used by Sanaa *et al.* (2004): 12 days at 14°C and 38 days at 9°C (ripening). The a_w profile was obtained from Schlessner *et al.* (1992, Table 5).

Results

Figure 10 shows the number of generations⁹, as a function of time, in the rind and in the core, for classical and stabilized cheeses. For cheeses made with the classical process, we obtain a profile comparable to the one obtained by Ryser and Marth (1987) or Back *et al.* (1993), with, perhaps,

⁹ The number of generations is linked to the growth rate following $nbG = \int_t \frac{\mu(t)}{\log(2)} dt$.

a lower predicted increase in the core of the cheese. The growth is higher in stabilized cheeses. These curves do *not* include the 1 log₁₀ reduction observed during the first days of ripening in Camembert cheeses made with the classical process. More details and graphical illustrations are available in the appendix (section “*L. monocytogenes* growth in Camembert cheese”).

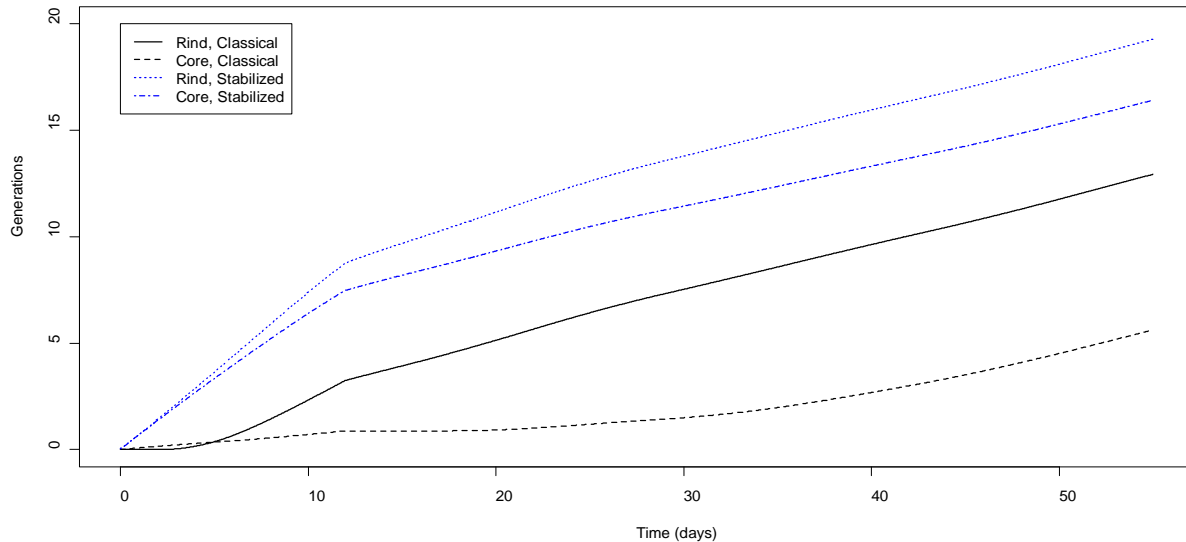


Figure 10: Modeled number of generations during Camembert cheese ripening and manufacturing.

6.2. Inactivation

Inactivation is the second of the six basic processes that govern the size of bacterial populations (Nauta 2008). This basic process is characterized by a decrease in the number of organisms per unit of food. Two steps in the product pathway model include inactivation processes:

- The initial ripening step, because the bacterial population decreases during the first day of the ripening due to low pH (Ryser and Marth 1987; Ryser 2007; Liu et al. 2009);
- The “mitigation” step that includes several options for treating milk at the beginning of cheese-making. The options available at this step, treated as alternative scenarios, include no inactivation (*i.e.*, use of raw milk), heat treatments such as pasteurization, or any other treatment characterized by a time-temperature couple, complete inactivation of all the *L. monocytogenes* present in the milk at that time (designated as “full pasteurization”), and partial reductions of the number of bacteria by defined amounts.

The general inactivation model is described below. The application of this model for the initial ripening and mitigation steps is straightforward because the log reduction of bacteria is fixed.

6.2.1. General Inactivation

This model assumes that the effect of an inactivation process is applied independently to each cell of *L. monocytogenes* present in the food. Moreover, the probability of survival for each cell (s) is equal for all cells¹⁰. If the number of bacteria in a unit of food before the inactivation step is X_0 cfu/unit, the number of bacteria at the end of the inactivation process X_1 will thus be (Nauta 2008):

$$X_1 \sim \text{Binomial}(X_0, s).$$

The expected value of X_1 is $s \times X_0$. s may be expressed as $S = \log(1/s)$, the expected log reduction during the inactivation process. As an example, $s = 0.01$ is equivalent to an expected log reduction of $S = \log(1/0.01) = 2$. This inactivation model can lead to the inactivation of all the bacteria in a particular unit of food, and thus in a decrease in the prevalence of contaminated food. The probability that all the X_0 bacteria in the unit are inactivated is

$$\Pr(X_1 = 0 | X_0, s) = (1-s)^{X_0}.$$

As an example, if $s = 0.01$ (*i.e.* $S = 2$ log reduction) and $X_0 = 100$ cfu/unit, the probability that all the bacteria are inactivated in a unit is

$$\Pr(X_1 = 0) = (1-0.01)^{100} = 0.37.$$

Given that the prevalence of contaminated products at the beginning of the inactivation step is p_0 , the prevalence of contaminated products at the end of the inactivation step will be

$$p_1 = p_0 \times (1 - (1-s)^{X_0}).$$

6.2.2. Inactivation During Initial Ripening

As discussed above, based on literature data the model assumed a constant $S = 1$ log reduction occurs during the initial ripening step, that is $s = 0.1$ (see Section 6.1).

6.2.3. Inactivation During Mitigation using a Defined Log Reduction

Three specific mitigation situations were modeled.

- No treatment, and thus no inactivation. In this case, obviously $s = 1$ *i.e.* $S = 0$. Then, $X_1 = X_0$;

¹⁰ s can, nevertheless, vary from product to product.

- “Full pasteurization”, which is assumed to completely eliminate all *L. monocytogenes* in the milk used for cheese-making. In this case, $s = 0$, *i.e.* $S = -\infty$, corresponding to a perfect pasteurization and $X_1 = 0$ with probability 1 ($X_1 \equiv 0$). In this situation, any *L. monocytogenes* present in the cheese did not originate in milk from the farm;
- A specified log reduction, which assumes that the level of *L. monocytogenes* present in milk is reduced by a defined amount without specifying a mechanism of reduction. Using this option allows the model to test the impact of defined levels of mitigation without restricting the types of technology considered.

6.3. Partitioning and Mixing

Partitioning and Mixing are the third and fourth basic processes that need to be considered in an exposure assessment model (Nauta 2008). Partitioning occurs when a large unit of food is split into several small units. Mixing is the opposite of partitioning, when two or more units are joined to form a new larger unit. The total number of cells does not change but the bacteria are redistributed among the basic units. In this model, partitioning occurs:

- During curd formation, when some of *L. monocytogenes* in the milk are trapped in the curd while the remaining cells are lost in the whey¹¹;
- During cheese formation, when the bacteria trapped in the bulk curds are distributed among the different individual cheeses;
- Between the “exterior” (“rind”) and “interior” (“core”) of an individual cheese where the cells experience different environments;
- In the spatial partition of each cheese into 1 gram units to mimic growth of the bacteria in a solid medium (see section 6.1);
- In the home, when cheeses (and the cheeses’ contaminating bacteria) are partitioned into individual servings.

Mixing occurs on farm, when milk from each quarter of an individual cow, milk from several cows and, possibly, milk from several farms are gathered in the dairy silo. Figure 11 illustrates these mixing and partitioning processes.

¹¹ Since the process no longer uses whey, then one could view this as an inactivation process.

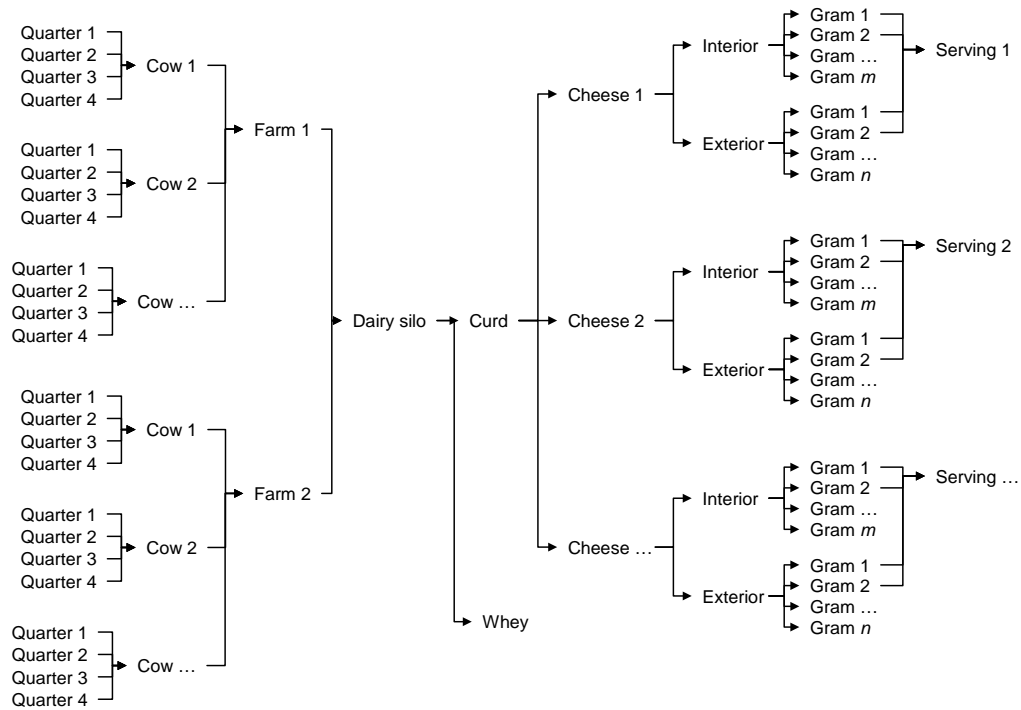


Figure 11: Mixing and partitioning process in the exposure assessment model.

6.3.1. Partition Model

This model assumes that bacteria are distributed evenly in the milk and the curd and that the bacteria are independently partitioned into the sub-units (from milk to curd, from curd to cheeses, from cheeses to interior and exterior, etc.). Partitioning is thus a multinomial process. When we focus on one sub-unit at random, among all the sub-units, that multinomial process simplifies to a binomial process. If the number of bacteria in a unit before the partition step is X_0 cfu/unit with s_0 the size of the unit, the number of bacteria in one random sub-unit of size s_1 at the end of the partition process X_1 will thus follow:

$$X_1 \sim \text{Binomial}\left(X_0, \frac{s_1}{s_0}\right)$$

The expected value of X_1 is $s_1 / s_0 \times X_0$. This partition model leads to a lower prevalence of contaminated sub-units compared to the prevalence of contaminated units. Indeed, the probability that a sub-unit includes 0 bacteria is

$$\Pr\left(X_1 = 0 \mid X_0, \frac{s_1}{s_0}\right) = \left(1 - \frac{s_1}{s_0}\right)^{X_0}.$$

Given that the prevalence of contaminated units is p_0 , the prevalence of contaminated sub-units will be

$$p_1 = p_0 \times \left(1 - \left(1 - \frac{s_1}{s_0}\right)^{X_0}\right).$$

6.3.2. Mixing Model

The mixing model may be described as a simple gathering of objects: if k sub-units of size b_i ($i = (1, \dots, k)$) are gathered into a single (larger) unit and if each of these sub-units contains X_i bacteria, the number of bacteria in the final unit will be

$$X = \sum_i X_i.$$

The final concentration will be

$$C = \frac{\sum_i X_i}{\sum_i s_i}$$

in the larger unit, *i.e.* $\sum_i X_i$ bacteria in the $\sum_i s_i$ volume. Mixing will generally lead to a decrease in the concentration of bacteria (dilution), for example, when milk from one mastitic cow is mixed with the milk from other non-mastitic cows in the bulk tank. The prevalence of contaminated units will be higher than the prevalence of contaminated sub-units. Assuming random homogeneous mixing and a prevalence of contaminated sub-units p_0 , the prevalence of contaminated units will be

$$p_1 = 1 - (1 - p_0)^k$$

after mixing $k > 1$ sub-units. As an example, if the prevalence of infected cows is $p_0 = 1\%$, the prevalence of contaminated bulk tanks from the mixing of the milk of 50 cows at random is 39%.

6.4. Contamination

Three sources of contamination were considered in the exposure assessment:

- contamination of milk from a mastitic cow;
- contamination of milk from the farm environment;

- contamination of cheese from the plant environment.

Each contamination process required an estimate of *i*) the frequency of contamination and; *ii*) the number of *L. monocytogenes* cfu per contaminated unit when contamination occurs. The models and data used for the two contamination processes that occur on the farm are described in the section 7.1 below and in the Appendix (section “On Farm”). The following deals with contamination of cheese in the plant during cheese-making.

While it has been shown that cheese processing facilities can become contaminated with *L. monocytogenes* (Pritchard et al. 1994; Pritchard et al. 1995), there are no data describing the process or rate of transfer of bacteria from the environment to the product in the plant. The most relevant data that include values for both the prevalence and the level of contamination of soft-ripened cheeses in the United States and Canada were from a random sample of cheeses obtained at retail in Maryland and California (U.S.) (Gombas et al. 2003) as part of a larger survey of ready to eat foods. The relevant results from this survey are shown in Table 15. These data on prevalence and levels in cheeses at retail were used to infer the frequency and level of contamination from the plant environment at an earlier step in the process model. The process of reconstructing model inputs using data obtained at another point downstream in the same process has been used in fields ranging from infectious diseases (Ghani et al. 1998; Deuffic et al. 1999) to food safety risk assessment (Albert et al. 2008).

The relevant results from Gombas *et al.* (2003) for soft-ripened cheeses are shown in Table 15. Raw results from this study are available on the FoodRisk.org website¹² (Table 16). From this dataset, the distribution of the level of contamination occurring in the plant was estimated by “subtracting” the distribution of the growth that occurs during aging, marketing and retail from the distribution of the level of contamination at retail for soft-ripened cheese in the United States and Canada (Figure 12). The published work of Gombas *et al.* (2003) and the Gombas *et al.* (2003) dataset available on the FoodRisk.org website, let us easily distinguish the soft-ripened

¹² <http://foodrisk.org/exclusives/SLMREF/>, accessed 12/19/2011.

cheese results of interest to this risk assessment from the many other RTE foods that Gombas *et al.* (2003) reported on.

Table 15: Results reported in Gombas *et al.* (2003) for soft-ripened cheeses.

Site	no. of cheeses sampled	no. with no <i>Lm</i> detected in a 25g sample	0.04-0.1 MPN/g	>0.1-1 MPN/g	>1-10 MPN/g	>10-100 MPN/g
Maryland	517	516	1	0	0	0
California	830	817	11	0	2	0

Table 16: Raw results, as available on the FoodRisk.org website.

FoodNet site	Freq.	Screening Vol.	Screening Result	Vol.	Result	Vol.	Result	Vol.	Result	Vol.	Result	Vol.	Result
California	817	25 or 2x25g	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
California	11	25 or 2x25g	+	3x1	---	3x0.1	---	3x0.01	---	0.1	0	0.002	0
California	1	25 or 2x25g	+	3x1	+++	3x0.1	+++	3x0.01	---	0.1	0	0.002	0
California	1	25 or 2x25g	+	1	+++	0.1	---	0.01	---	0.1	-	0.002	0
Maryland	516	25 or 2x25g	-	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Maryland	1	25 or 2x25g	+	3x1	---	3x0.1	---	3x0.01	---	0.1	0	0.002	0

Notes: Result: + tube positive for *L. monocytogenes*. -, tube negative for *L. monocytogenes*. NT, not tested. vol.: sample volume (g), original sample.

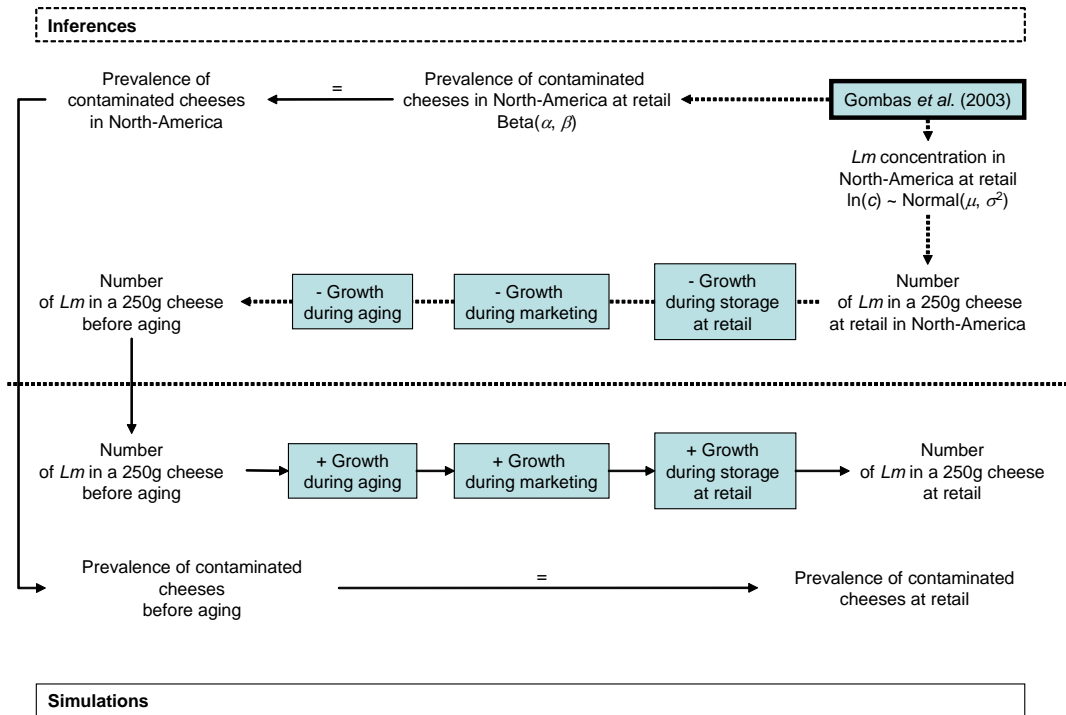


Figure 12: Schematic view of the inference process used to estimate the contamination of cheeses in plant.

Top panel: the inference part estimates distributions for the prevalence of contamination and for level of contamination of cheeses in plant from the Gombas *et al.* (2003) data set. Bottom panel: the simulation part synthesizes the prevalence and level of contamination in contaminated cheeses from plant to retail.

6.4.1. Method

The estimate of the frequency and level of in plant contamination was obtained in three steps:

i) A description of the prevalence and level of contamination in cheeses at retail was developed from the Gombas *et al.* (2003) data set. This led to estimates for:

- site-to-site variability of prevalence of cheese contamination at retail, which was assumed to follow a Beta distribution: $p \sim \text{Beta}(\alpha, \beta)$;
- the level of contamination (cfu/g) at retail for contaminated cheeses, which was assumed to follow a logNormal distribution: $\ln(c) \sim \text{Normal}(\mu, \sigma^2)$.

ii) the estimated prevalence of contaminated cheese at retail was used as an estimate of the probability that a cheese, at random, has contamination from the environment in the cheese processing plant and how that probability varies. The Gombas *et al.* (2003) data suggest that the prevalence p varies from site to site (Maryland and California). That variation was represented by a Beta distribution.

iii) the amount of in-plant environmental contamination that would grow to the estimated level of retail contamination was estimated. The process is described below, and some examples of the calculations are shown in Table 13:

- a set of integer values $\mathbf{Y} > 0$ (cfu/cheese) for the number of *L. monocytogenes* cells in a random contaminated 250g cheese at retail were sampled using the logNormal(μ, σ^2) distribution of contamination (cfu/g) at retail inferred from the Gombas *et al.* (2003) data set;
- independently, a set of values \mathbf{G} for the bacterial growth during the aging, marketing and retail steps were obtained from the growth models described previously using the time and temperature parameters for the pasteurized-milk cheese baseline model, as described in the Exposure Assessment section;
- \mathbf{Y} and \mathbf{G} were reordered to produce a rank correlation ρ . $\rho \in [-1, 1]$ is a parameter that links bacterial concentration at retail with bacterial growth during the preceding steps. A high value of ρ indicates that the highest bacterial concentrations at retail are due to the highest bacterial growth during the aging, marketing and retail steps. A value of $\rho = 0$ would indicate that those parameters are not linked, and thus that high retail

- concentrations could be due either to high bacterial growth from low initial levels of contamination or from low bacterial growth from high initial levels of contamination;
- the distribution of the number of bacteria in a 250g cheese before aging was evaluated as an empirical distribution of $\mathbf{X} = \text{round}(\mathbf{Y}/\mathbf{G})$, with $\mathbf{X} > 0$. Values of $\mathbf{X} = 0$ were discarded because at least one bacterial cell needs to be present in the cheese before aging to lead to the observation of a contaminated product at retail.

Parameter uncertainty for the α and β in the description of cheese prevalence was estimated using a parametric bootstrap. The joint uncertainty distribution for $\hat{\mu}$ and $\hat{\sigma}^2$ in the description of cheese contamination at retail was developed from the sampling distributions for the parameters' maximum likelihood estimates.

The details of this inference process are given in the Appendix (section "Environmental Contamination"). Some examples of the calculations are shown in Table 17.

Table 17: Example of the process used to derive the distribution of the number of *L. monocytogenes* in a 250g cheese before aging.

Y is the number of *L. monocytogenes* in a 250g cheese at retail, *G* is the growth (multiplying factor) that occurred during aging, marketing and retail, $X^* = \text{round}(Y/G)$ is a tentative number of *L. monocytogenes* that was present before aging and *X* is an iteration of the number of *L. monocytogenes* that were present in the cheese before aging. Note that *Y* and *G* have a rank correlation coefficient of 1 (both are in increasing order).

Iteration	<i>Y</i> : number of <i>L. monocytogenes</i> in a 250g cheese at retail	<i>G</i> : growth (multiplying factor) that occurred during aging, marketing and retail, round	X^* : round(<i>Y</i> / <i>G</i>)	<i>X</i>	Note
1	1	1.00	1	1	NA
2	2	1.00	2	2	NA
3	2	1.00	2	2	NA
4	3	1.00	3	3	NA
5	4	1.00	4	4	NA
6	5	1.00	5	5	NA
7	6	1.00	6	6	NA
8	6	1.00	6	6	NA
9	6	1.00	6	6	NA
10	12	1.35	9	9	NA
11	13	1.54	8	8	NA
12	14	1.83	8	8	NA
13	14	1.88	7	7	NA
14	14	1.96	7	7	NA
15	15	2.01	7	7	NA
16	17	2.69	6	6	NA
17	18	3.11	6	6	NA
18	18	3.12	6	6	NA
19	23	6.41	4	4	NA
20	28	10.4	3	3	NA
21	29	12.5	2	2	NA
22	38	26.2	1	1	NA
23	40	32.1	1	1	NA
24	101	730	0	NA	Discarded, because round(<i>Y</i> / <i>G</i>) = 0
25	120	1402	0	NA	Discarded, because round(<i>Y</i> / <i>G</i>) = 0
26	535	754292	0	NA	Discarded, because round(<i>Y</i> / <i>G</i>) = 0

NA Not Applicable

6.4.2. Results

Prevalence

Variability in among-site prevalence was assumed to follow a Beta(α , β) distribution. The point estimates and statistics of the joint uncertainty distributions for parameters α and β used in the simulation are given in Table 18. The overall probability of contamination for a cheese at

random was $E[p]|p \sim \text{Beta}(\alpha, \beta) = 0.0094$ when point estimates were used for α and β and 0.0103 (CI95%: [0.00270, 0.0248]) when an uncertainty distributions was used for (α, β) .

Table 18: Parameters α and β used to model the frequency of cheeses with in plant contamination.

Parameter	Point estimate (m.l.e.)	Summary statistics from uncertainty distribution ^a median (mean) [2.5%, 97.5%]
α	1.834	1.70 (2.24) [0.27, 8.8]
β	192.3	179.4 (226.2) [29.42, 711.6]; $\rho(\alpha, \beta) = 0.907$

^aThe uncertainty distribution for the ML estimates is estimated by the empirical distribution of m.l.e. from a parametric bootstrap.

Level of Contamination at Retail

Variability in the concentration at retail of *L. monocytogenes* among contaminated cheeses was assumed to follow a logNormal distribution $\ln(c) \sim \text{Normal}(\mu, \sigma^2)$. The parameters μ and σ^2 obtained are given in Table 19. Using this distribution, the estimated distribution of the number of bacteria per contaminated cheese \mathbf{Y} ($\mathbf{Y} > 0$) at retail had a mean of 51.3 (CI95%, [26.2; 104.2]) cfu, a 5th percentile of 11 [5; 24] cfu and a 95th percentile of 131 [64; 318] cfu.

Table 19: Maximum likelihood estimates, level of contamination at retail.

Parameter	Point estimate (m.l.e.)	Uncertainty distribution
μ	-1.874	Normal(-1.8737, 0.3386 ²)
σ^2	0.5265 ²	Gamma ⁻¹ (7.5, [3.949] ⁻¹) $\rho(\mu, \sigma) = -0.0127$

In concentration Normal(μ, σ^2).

Level of Contamination Before Aging

Because the actual value of ρ that links the bacterial concentration at retail with bacterial growth is unknown, the effect of using various values for ρ was tested. (see Appendix, section “Environmental Contamination”). However, these tests showed that the inferred level of contamination in retail cheeses and the inferred amount of growth from the point of contamination during cheese-making are compatible only if they are rigidly linked using $\rho = 1$ so that low retail concentrations occur when low growth occurs and high retail concentrations occur only when low level contamination is followed by high growth. In that case, the distribution of *L. monocytogenes* environmental contamination is concentrated at small cfu values (Table 20). When point estimates are used for all parameters, the estimated level of contamination for a cheese at the plant is less than 31 bacteria (cfu).

Table 20: Probability distribution of the number of *L. monocytogenes* that contaminate a 250g cheese in the plant.

Number of bacteria	Percentage
1	10.0
2	7.35
3	5.36
4	4.49
5	3.55
6	3.46
7	3.03
8	2.69
9	3.02
10	2.70
11	2.74
12	2.82
13	2.92
14	2.81
15	2.56
16	2.88
17	2.59
18	2.84
19	2.64
20	2.92
21	2.64
22	2.66
23	2.70
24	2.39
25	2.39
26	2.52
27	2.50
28	2.32
29	2.23
30	2.18
31	2.07

(25,000 Monte-Carlo iteration simulation, point estimates).

However, considering parameter uncertainty in the growth model (see section 6.1) and uncertainty around the parameters inferred from the Gombas *et al.* (2003) data set leads to uncertainty in the number of bacteria that contaminate a cheese in the plant (Table 21).

Table 21: Summary statistics for the distribution of number of *L. monocytogenes* that contaminate a 250g cheese in the plant.

Median	Mean	5 th	25 th	75 th	95 th
13 [6, 26.7]	13.9 [6.99, 28.4]	1 [1, 2]	5 [3, 11]	22 [11, 46.3]	30 [15, 63]

(25,000 × 500 two dimension Monte Carlo simulations, uncertainty and variability considered). Entries are Median [2.5, 97.5] percentiles over uncertainty distribution.

6.4.3. Assumptions and Discussion

Inferring the frequency and level of in plant contamination from data obtained at retail requires several assumptions. These assumptions include:

- That there is no decline in the bacterial population between packaging and retail;
- That the bacterial population does not reach the maximum population density in the cheese at retail. The results obtained from Gombas *et al.* (2003) data set show that this assumption is valid;
- That the prevalence and the level of contamination observed in Maryland and California are representative of the prevalence and level of contamination in the United States and Canada;
- That the cheeses that were sampled at each site were representative of the cheeses in these areas;
- That the prevalence of contaminated cheeses varies among sites but that the distribution of contamination levels does not (same distribution used for all sites);
- That the only source of contamination for the tested cheeses was environmental contamination that occurred during cheese processing during ripening and before packaging, resulting in growth of *L. monocytogenes* in the rind and not the core. This hypothesis is reasonable for pre-packed cheeses made from pasteurized milk;
- That growth rates, time and temperature between packaging and retail sale that were used in the exposure assessment model also applied to the cheeses sampled by Gombas *et al.* (2003).

Moreover, these inferences estimate the probability that a cheese has in-plant contamination, but provide no information on the process of contamination, notably the lot-to-lot and within lot structure of contamination. While this does not affect the risk characterization, it does lead to the suggestion that additional testing of each cheese lot might be a viable risk mitigation strategy (see sections 6.5 and 10.1.3.).

6.5. Removal

The final basic process that was considered was removal of a batch of bulk milk or lot of cheeses linked to a testing procedure.

6.5.1. Generality

We modeled the impact of the removal of products, *i.e.* milk or cheese, after getting a positive detection test. We assume that all bulk milk tested positive and all cheese lots tested positive are removed. We have not implemented multiple stage screening for the test procedure; decision rules that reject a tank or a lot if the enumerated cells in the test sample exceed some non-zero-criterion; or the possibility of rejection of a tank or a lot for other (quality, testing for other pathogens) reasons. We assume that test methods are fully specific, *i.e.* that the probability for a tank/lot to be rejected while non-contaminated is 0.

6.5.2. Testing Bulk Milk

Testing of bulk milk will not reduce the prevalence in the milk used for cheese-making to 0 because no testing system is 100% effective. Testing can result in a lower prevalence of contaminated bulk raw milk and can impact the concentration distribution of undetected contaminated raw milk if there is a higher probability that highly contaminated milk will test positive than less contaminated milk. The removal basic process assumes:

- that the probability that a particular batch of the bulk milk is tested is φ ;
- that the tested sample size is ν ml;
- that the *L. monocytogenes* cells are homogeneously distributed within the bulk milk such that the number of cells in the small testing volume of ν ml follows a Poisson distribution with mean $\mu\nu$ cfu when the concentration in the larger volume of bulk milk is μ cfu/ml;
- that the probability that the test detects a cfu of *L. monocytogenes* present in a sample is η ;
- that this probability is independent for each cfu of *L. monocytogenes* in the sample.

The probability of detecting a positive sample and of removing that batch of milk from production is:

$$\Pr(\text{reject} | \mu) = \varphi \times (1 - \exp(-\eta\nu\mu)),$$

i.e. the probability that the milk is tested multiplied by the probability that at least one bacterial cell is detected in the ν ml testing sample. Note that this procedure is consistent with a higher probability of rejecting a batch of contaminated milk as the concentration μ increases. Except for

specific parameterization, the method holds for testing farm bulk tank milk, tanker truck milk and dairy silo milk before mitigations.

6.5.3. Testing Cheese Lots

The model assumed that each test sample is the sum, $n \times v / n$ g, of v / n g cheese from n cheeses randomly sampled from a lot. For a batch of n cheeses produced in the same process (same batch of milk, same level of mitigation, and environmental contamination from the same distribution) until the end of the aging phase, m , the number of *L. monocytogenes* cfu present in a random sample of $n \times v / n$ g randomly sampled per cheese was evaluated assuming:

- that the probability that the lot is tested is φ . As a default, a value of 100% (all lots) is used in this report;
- that the probability that the test detects one *L. monocytogenes* cfu present in the sample is η ;
- that this probability is independent for each *L. monocytogenes* cfu in the sample.

The probability of detecting and removing a contaminated lot is:

$$\Pr(\text{reject} | \mu) = \varphi \times (1 - (1 - \eta)^m),$$

i.e. the probability that the lot is tested multiplied by the probability that at least one bacterial cfu is detected in the test sample. The number m of *L. monocytogenes* in a test sample is derived by simulation. This procedure is consistent with a higher probability to reject a contaminated lot as the fraction of *L. monocytogenes* contaminated cheeses in a lot increases, the *L. monocytogenes* in individual cheeses increases; or the *L. monocytogenes* in a test sample increases.

7. Exposure Assessment

The product pathway used in this risk assessment consisted of five stages: “On farm”, “Cheese Processing”, “Transport and Marketing”, “Retail” and “Home”. This section describes how the model estimated bacterial prevalence and level all along this pathway using the basic processes described previously and information specific to each step.

7.1. On Farm

User-specified inputs for the *L. monocytogenes* prevalence and concentration in dairy silo milk used to manufacture soft-ripened cheese begin the cheese processing portion of the exposure assessment (see section 7.2). However, the few studies that have surveyed dairy silos directly provide very limited information to describe *L. monocytogenes* prevalence and levels to inform those user inputs.

Precedents modeled the process in Figure 13 to synthesize dairy silo *L. monocytogenes* prevalence and concentration distributions. Milk collected from one or more farms' bulk raw milk tanks is transported to and mixed together in the cheese manufacturer's silo prior to cheese production. *L. monocytogenes* in farm bulk milk comes from the farm environment and, less frequently, also from *L. monocytogenes* shed in the milk from a mastitic or *L. monocytogenes* infected cow. When conditions permit, *L. monocytogenes* can grow in raw milk while held on the farm, while transported to the dairy silo and while held in the silo before the start of cheese manufacture. Dairy silo milk that has no contamination comes from farms with no contamination in their bulk tank milk. Dairy silo milk that has some *L. monocytogenes* contamination comes from one or more farms with *L. monocytogenes* contamination. At the dairy silo, the *L. monocytogenes* concentration is the result of mixing together varying volumes of milk, some of which has and some of which does not have contamination. This model simplifies for farmstead cheeses manufactured with milk from the farmer's own herd on the farm where the animals are raised. For that production, there is no mixing of milk from different farms, no storage in the farm bulk tank, no transport and no dairy silo storage. Details in Appendix (section "On Farm") supplement this section's description.

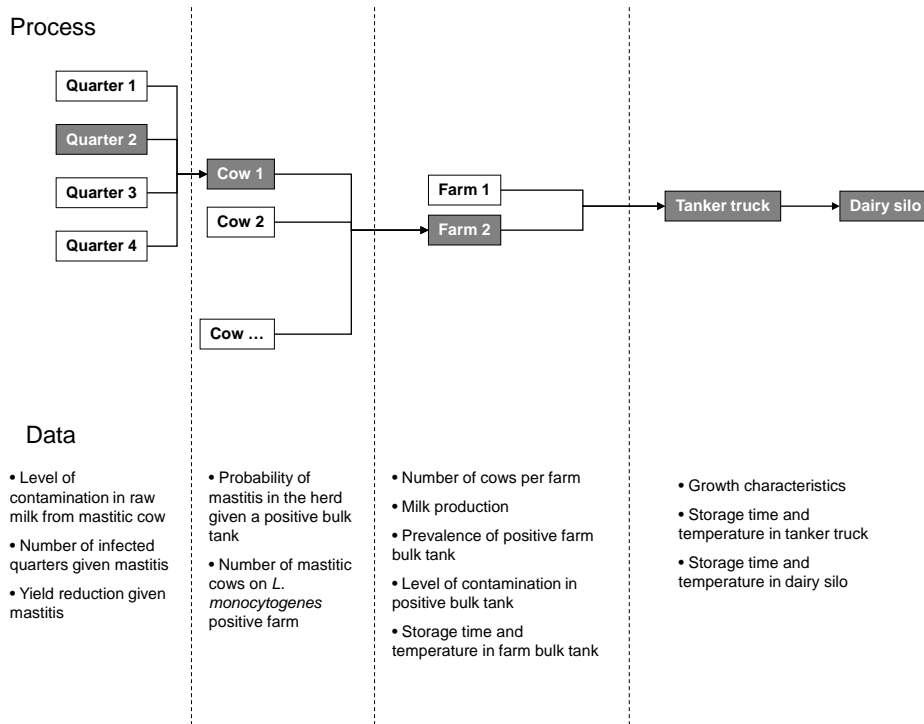


Figure 13: On farm process and data used.

Grayed boxes stand for contaminated units. In this example, the dairy silo is contaminated from the tanker truck. This tanker truck is contaminated from Farm #2. Farm #2 is contaminated from a mastitic cow (Cow #1) with one infected quarter (Quarter #2).

Large scale commercial operations, using large volumes of milk for a single cheese lot would use milk collected from more than 1 tank truck. The dairy silo *L. monocytogenes* prevalence and concentration in that commingled milk would resemble those that would result from applying mixing processes (section 6.3.2.).

7.1.1. Data and Methods

Farm Bulk Tank Prevalence and Concentration

For a particular prevalence of *L. monocytogenes* contaminated farm bulk tanks, p_{farm} , and assuming independence of contamination among farms, the dairy silo prevalence is

$$p_{dairy} = 1 - (1 - p_{farm})^n,$$

where n is the number of farms per collection (Steele et al. 1997; Bemrah et al. 1998; Sanaa et al. 2004).

Alternative p_{farm} values can come from:

- individual United States and Canadian farm bulk tank milk surveys,
 - Canada: Farber *et al.* (1988), Slade *et al.* (1988; 1989), Davidson *et al.* (1989), Fedio & Jackson (1990; 1992), Tiwari & Aldenrath (1990), Steele *et al.* (1997); and,
 - U.S.: Lovett *et al.* (1987), Patterson *et al.* (1989), Lund *et al.* (1991), Rohrbach *et al.* (1992), Jayarao & Henning (2001), Muraoka *et al.* (2003), Murinda *et al.* (2004), Van Kessel *et al.* (2004), Jayarao *et al.* (2006), D'Amico *et al.* (2008b);
- summaries of them;
- other countries' farm bulk milk surveys (FDA/FSIS (2003) references); and,
- risk factors that associate higher or lower farm bulk milk prevalence with some conditions and practices (Everson 1988; Sanaa *et al.* 1993; Sanaa and Menard 1994; Hassan *et al.* 2000; Hassan *et al.* 2001; Nightingale *et al.* 2004; Nightingale *et al.* 2005; Ho *et al.* 2007; Vilar *et al.* 2007; Antognoli *et al.* 2008; Mohammed *et al.* 2009).

The *L. monocytogenes* concentration in dairy silo milk that contains milk from *L. monocytogenes* positive farm bulk milk is a milk-volume weighted sum of the individual farm tanks' concentrations. Beckers *et al.* (1987), Lovett *et al.* (1987), Liewen & Plautz (1988), Slade & Collins-Thompson (1988a; 1988b), Fenlon & Wilson (1989), Greenwood *et al.* (1991), Harvey & Gilmour (1992), Sanaa *et al.* (1993), Fenlon *et al.* (1995), O'Donnell (1995), Desmasures & Guegen (1997), Gaya *et al.* (1998), Waak *et al.* (2002), Meyer-Broseta *et al.* (2003) and Van Kessel *et al.* (2004) contribute data points that help to describe how *L. monocytogenes* concentrations in *L. monocytogenes* positive farm raw milk vary. Albert *et al.* (2005) describe how milk production varies among dairy farms.

D'Amico & Donnelly (2010) provides data that we use to describe the number of milked cows per herd on farms producing farmstead cheese:

- between 7 and 112 milked cows per farm, with mean 45 cows (D'Amico and Donnelly 2010); and,
- concentrated between 30 and 50 milked cows per farm.

We represent this data by a rounded Beta-Pert(7, 37.75, 112) distribution.

L. monocytogenes Mastitis

L. monocytogenes is not as invasive to the udder as other pathogens that are more commonly associated with bovine mastitis. Only rarely does the literature report clinical or sub-clinical *Listeria* mastitis cases (Gitter et al. 1980; Sharp 1989; Fedio and Jackson 1990; Sanaa et al. 1993; Bourry et al. 1995; Jensen et al. 1996; Bemrah et al. 1998; Stephan et al. 2000; Wagner et al. 2000; Erdogan et al. 2001; Meyer-Broseta et al. 2003; Schoder et al. 2003; Nightingale et al. 2004; Sanaa et al. 2004; Winter et al. 2004; Rawool et al. 2007), or references in them or to them (e.g. Potel 1953; De Vries and Strikwerda 1956; Von Hartwigk 1958; Schulz 1967; Dutta and Malik 1981; Djoenne 1982; van Daelen and Jaartsveld 1988; Gilleberg and Nordhaug 1989; Wesley 2007).

An intramammary *L. monocytogenes* infection sheds *L. monocytogenes* into the affected cow's milk, often for an extended period (Doyle et al. 1987; Bourry et al. 1995; Bourry and Poutrel 1996; Schoder et al. 2003). One can synthesize the mastitis contributions to positive farm bulk milk from

- mastitis prevalence: the rate of farms with clinical *listeria* mastitis case(s); the mastitis frequency among cows on a farm with *L. monocytogenes* mastitis; and the number of quarters shedding *L. monocytogenes* (Schulz 1967; Doyle et al. 1987; Farber et al. 1988; Slade et al. 1989; Moustafa and Marth 1993; Sanaa et al. 1993; De Graaf and Dwinger 1996; Jensen et al. 1996; Sanaa et al. 1996; Bemrah et al. 1998; Yoshida et al. 1998; Erdogan et al. 2001; Nightingale et al. 2004; Nightingale et al. 2005; Rawool et al. 2007; Wesley 2007);
- concentration in milk from a mastitic cow, single occasion, single quarter concentrations in the mastitic quarter milk (Schulz 1967; Farber et al. 1988; Sharp 1989; Fedio et al. 1990; Vishinsky et al. 1993; Bourry et al. 1995; Bourry and Poutrel 1996; Wagner et al. 2000; Winter et al. 2004); and,
- mastitic quarter milk yield reduction over the yield of a healthy quarter (De Graaf and Dwinger 1996; Rajala-Schultz et al. 1999; Gröhn et al. 2004; Wilson et al. 2004).

Growth

If milk is not processed just after milking, *L. monocytogenes* in bulk milk grows while held in the bulk tank on farm, in the tank truck between farm and dairy and in the dairy silo prior to cheese-making when conditions permit (see section 6.1). Relevant conditions include:

- farm storage and tank truck transport times (Bemrah et al. 1998);
- farm storage temperatures and tank truck temperatures (Servello et al. 2004); and,
- dairy silo holding times and temperatures (IDFA 2008).

Growth is assumed to occur according to the model and the specified parameters in section 6.1.

Bulk Milk Testing

Practice, policy, and regulation set bulk milk testing frequency, place, and analytical methods. Precedents addressed how farm bulk tank and tank truck milk testing reduces *L. monocytogenes* prevalence and changes the *L. monocytogenes* concentration distribution in dairy silo milk collaterally. For this implementation, we consider only the effects of testing for *L. monocytogenes* and ignore the collateral effects of testing for milk quality and testing for other pathogens. Methods follow those in section 6.5; parameterization applies 25 ml nominal test volumes from Latorre *et al.* (2009) and Meyer-Broseta *et al.* (2003) and test sensitivities as in U.S. FSIS (2003).

Farms per Collection, Cows per Farm

We distinguish between 2 illustrative cases (scenarios, models) based on the milk collected for cheese-making operations, the number of cows from which milk is sourced, and holding and transport conditions. The two illustrative cases are:

- Farmstead-scale operations: milk is collected for cheese-making from a single herd, on the farm where the cheese-making operation resides; there is no on farm, tank truck or dairy silo holding time between milking and the start of cheese manufacture;
- Artisanal-scale operations: milk for cheese-making is collected from 2 farms and pooled; on farm and tank truck times are as described above but there is no dairy silo holding time at the cheese-making operation.

“Farmstead” and “artisanal” are terms that D'Amico & Donnelly (2010) attributed to the American Cheese Society and do not correspond to any specific regulation. We use the terms in a similar way, to name scenarios.

One can represent other particular scenarios by setting the data that describe the process to appropriate values.

7.1.2. Dairy Silo Prevalence and Concentration for the Raw-Milk Cheese Baseline Model

Prevalence of *L. monocytogenes* contaminated dairy silos and the *L. monocytogenes* concentration in contaminated dairy silo bulk milk results are reported for the farmstead-scale and artisanal-scale cases defined in the previous section. The Appendix (section “On Farm”) derives the other model inputs common to the 2 cases, which are:

- Farm bulk tank *L. monocytogenes* prevalence, one for Canada and one for the U.S.;
- *L. monocytogenes* concentration in contaminated farm bulk tank milk;
- *L. monocytogenes* mastitis prevalence (farm, cow, quarters) on *L. monocytogenes* positive farms;
- *L. monocytogenes* concentration in milk from an infected quarter;
- Milk yield (cow), one distribution for Canada and one for the U.S.;
- Milk yield reduction (*L. monocytogenes* mastitic cow);
- *L. monocytogenes* growth characteristics (EGR_{20} , T_{min} , K_{ξ} , y_{max}) in milk;
- Farm tank, tank truck and dairy silo storage time and temperature, and;
- Baselines implement no bulk milk testing.

Table 22 shows point estimates of the farm module outputs, that is, summary statistics for the distributions for the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections in Canada and in the U.S. for the baseline farmstead-scale case. Table 23 shows the same outputs for the artisanal-scale case. Data uncertainty propagated to these on farm outputs are reported separately (Table 24, Table 25).

The contaminated dairy silo milk concentration distribution is bi-modal (Figure 14). One mode corresponds to the environment source *L. monocytogenes*, which occurs at lower levels than

mastitis source *L. monocytogenes*. The location and height of the second mode are influenced by the presence of mastitis source *L. monocytogenes*: frequency of occurrence of positive farms with *L. monocytogenes* mastitis case(s); concentration in milk from a mastitic quarter; and, dilution in the total volume of milk.

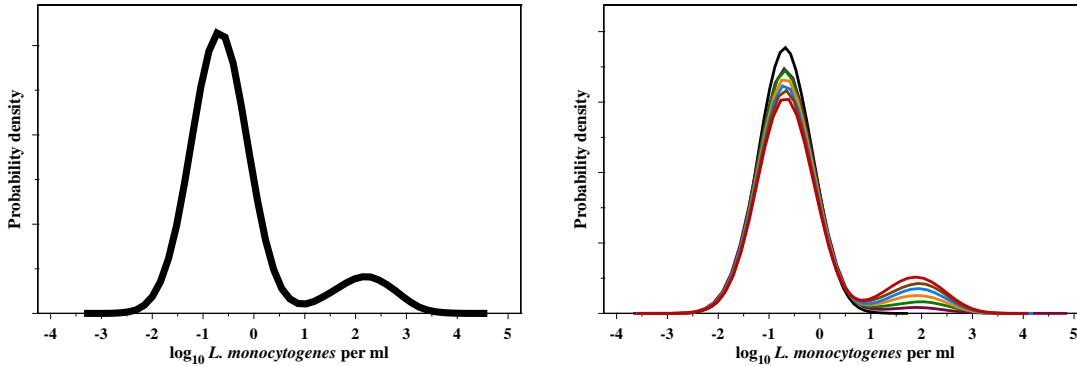


Figure 14: Distribution of the concentration (log cfu/ml) of *L. monocytogenes* in positive milk collection and change in concentration distribution as $\Pr(Lm \text{ mastitis} | Lm+ \text{ environment})$ increases over range 0 (0.025) 0.15.

Dairy silo contaminated milk prevalence is higher in the artisanal-scale case than in the farmstead-scale case, the result of gathering bulk milk from two farms in a single collection: the probability of collecting milk from at least one infected farm increases sharply as the number of farms in the collection increases. The *L. monocytogenes* concentration in a contaminated collection is slightly lower in the artisanal-scale case than in the farmstead-scale one: in the artisanal-scale case, the farms' contaminated milk is diluted in the farms' not contaminated milk, when the milk from two farms is mixed.

Table 24 and Table 25 describe the uncertainty associated with each summary statistic when we account for data uncertainty in the parameters used to synthesize the prevalence and concentration variability distributions. Note the high uncertainty in the mean prevalence.

Table 22: Point estimates of the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Baseline model, farmstead-scale operations.

Canada Summary statistics	Canada Prevalence	Canada Concentration (cfu / ml, log ₁₀ statistics)	United States Summary statistics	United States Prevalence	United States Concentration (cfu / ml, log ₁₀ statistics)
Median	0.0225	-0.289	Median	0.0371	-0.289
Mean	0.0236	1.60	Mean	0.0424	1.60
Std. Dev.	0.0878	2.29	Std. Dev.	0.264	2.29
Quantile 1%	0.0794	-1.48	Quantile 1%	0.0460	-1.48
Quantile 2.5%	0.0957	-1.30	Quantile 2.5%	0.00696	-1.30
Quantile 5%	0.0112	-1.14	Quantile 5%	0.00965	-1.14
Quantile 10%	0.0132	-0.957	Quantile 10%	0.0136	-0.957
Quantile 25%	0.0172	-0.649	Quantile 25%	0.0228	-0.649
Quantile 50%	0.0225	-0.289	Quantile 50%	0.0371	-0.289
Quantile 75%	0.0288	0.140	Quantile 75%	0.0564	0.140
Quantile 90%	0.0353	1.68	Quantile 90%	0.0780	1.68
Quantile 95%	0.0396	2.34	Quantile 95%	0.0930	2.34
Quantile 97.5%	0.0436	2.66	Quantile 97.5%	0.107	2.66
Quantile 99%	0.0485	2.93	Quantile 99%	0.125	2.93

Table 23: Point estimates of the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Raw-milk cheese, artisanal-scale operations.

Canada Summary statistics	Canada Prevalence	Canada Concentration (cfu / ml, log ₁₀ statistics)	United States Summary statistics	United States Prevalence	United States Concentration (cfu / ml, log ₁₀ statistics)
Median	0.0445	-0.584	Median	0.0729	-0.582
Mean	0.0465	1.29	Mean	0.0822	1.29
Std. Dev.	0.0171	1.98	Std. Dev.	0.0497	1.98
Quantile 1%	0.0158	-1.87	Quantile 1%	0.00918	-1.87
Quantile 2.5%	0.0191	-1.67	Quantile 2.5%	0.0139	-1.67
Quantile 5%	0.0222	-1.49	Quantile 5%	0.0192	-1.48
Quantile 10%	0.0263	-1.30	Quantile 10%	0.0271	-1.29
Quantile 25%	0.0341	-0.968	Quantile 25%	0.0451	-0.959
Quantile 50%	0.0445	-0.584	Quantile 50%	0.0729	-0.582
Quantile 75%	0.0567	-0.127	Quantile 75%	0.110	-0.131
Quantile 90%	0.0693	1.38	Quantile 90%	0.150	1.39
Quantile 95%	0.0776	2.02	Quantile 95%	0.177	2.04
Quantile 97.5%	0.0852	2.34	Quantile 97.5%	0.203	2.36
Quantile 99%	0.0946	2.62	Quantile 99%	0.234	2.62

Table 24: Prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Baseline farmstead-scale case, uncertainty considered.

Country	Summary statistics	Prevalence Median	Prevalence LB CI95%	Prevalence UB CI95%	Conc. (cfu/ml, log ₁₀ statistics) Median	Conc. (cfu/ml, log ₁₀ statistics) LB CI95%	Conc. (cfu/ml, log ₁₀ statistics) UB CI95%
Canada	Median	0.0234	6.25×10 ⁻⁴	0.208	-0.282	-0.390	-0.121
Canada	Mean	0.0252	6.77×10 ⁻⁴	0.209	1.60	1.21	1.98
Canada	Std. Dev.	0.00917	3.43×10 ⁻⁴	0.0320	2.34	2.04	2.61
Canada	Quantile 1%	0.00640	9.48×10 ⁻⁵	0.168	-1.49	-1.64	-1.33
Canada	Quantile 2.5%	0.00765	1.37×10 ⁻⁴	0.174	-1.30	-1.44	-1.16
Canada	Quantile 5%	0.00944	1.94×10 ⁻⁴	0.180	-1.15	-1.27	-1.00
Canada	Quantile 10%	0.0112	3.06×10 ⁻⁴	0.186	-0.965	-1.082	-0.826
Canada	Quantile 25%	0.0154	4.38×10 ⁻⁴	0.196	-0.652	-0.762	-0.519
Canada	Quantile 50%	0.0234	6.25×10 ⁻⁴	0.208	-0.282	-0.390	-0.121
Canada	Quantile 75%	0.0328	8.60×10 ⁻⁴	0.222	0.142	-0.0146	1.36
Canada	Quantile 90%	0.0385	0.00112	0.240	1.71	0.395	2.39
Canada	Quantile 95%	0.0438	0.00129	0.251	2.34	1.04	2.69
Canada	Quantile 97.5%	0.0487	0.00146	0.261	2.63	2.22	2.92
Canada	Quantile 99%	0.0551	0.00169	0.272	2.91	2.62	3.21
United States	Median	0.0410	0.0102	0.224	-0.282	-0.390	-0.121
United States	Mean	0.0474	0.0118	0.227	1.60	1.21	1.98
United States	Std. Dev.	0.0258	0.00791	.0633	2.34	2.04	2.61
United States	Quantile 1%	0.0056	4.14×10 ⁻⁴	0.117	-1.49	-1.64	-1.33
United States	Quantile 2.5%	0.00821	9.00×10 ⁻⁴	0.131	-1.30	-1.44	-1.16
United States	Quantile 5%	0.0117	0.00164	0.144	-1.15	-1.27	-1.00
United States	Quantile 10%	0.0168	0.00302	0.160	-0.965	-1.08	-0.826
United States	Quantile 25%	0.0264	0.00574	0.189	-0.652	-0.762	-0.519
United States	Quantile 50%	0.0410	0.0102	0.224	-0.282	-0.390	-0.121
United States	Quantile 75%	0.0626	0.0157	0.262	.142	-0.015	1.36
United States	Quantile 90%	0.0845	0.0223	0.299	1.71	.395	2.39
United States	Quantile 95%	0.0996	0.0270	0.332	2.34	1.04	2.69
United States	Quantile 97.5%	0.112	0.0315	0.367	2.63	2.22	2.92
United States	Quantile 99%	0.127	0.0372	0.409	2.91	2.62	3.21

LB: Lower Bound of the CI95%, uncertainty dimension; UB: Upper Bound

Table 25: Estimates for the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Raw-milk cheese, artisanal-scale case, uncertainty considered.

Country	Summary statistics	Prevalence Median	Prevalence LB CI95%	Prevalence UB CI95%	Conc. (cfu/ml, log ₁₀ statistics) Median	Conc. (cfu/ml, log ₁₀ statistics) LB CI95%	Conc. (cfu/ml, log ₁₀ statistics) UB CI95%
Canada	Median	0.0463	0.00125	0.372	-0.582	-0.694	-0.369
Canada	Mean	0.0496	0.00135	0.373	1.29	0.923	1.64
Canada	Std. Dev.	0.0175	6.85×10 ⁻⁴	.0515	2.01	1.73	2.26
Canada	Quantile 1%	0.0128	1.90×10 ⁻⁴	0.308	-1.86	-2.01	-1.72
Canada	Quantile 2.5%	0.0152	2.74×10 ⁻⁴	0.318	-1.66	-1.81	-1.53
Canada	Quantile 5%	0.0188	3.88×10 ⁻⁴	0.326	-1.50	-1.63	-1.36
Canada	Quantile 10%	0.0223	6.12×10 ⁻⁴	0.336	-1.30	-1.42	-1.16
Canada	Quantile 25%	0.0305	8.75×10 ⁻⁴	0.353	-0.968	-1.08	-0.822
Canada	Quantile 50%	0.0463	0.00125	0.372	-0.582	-0.694	-0.369
Canada	Quantile 75%	0.0646	0.00172	0.394	-0.116	-0.294	1.30
Canada	Quantile 90%	0.0755	0.00223	0.422	1.47	0.137	2.08
Canada	Quantile 95%	0.0856	0.00258	0.438	2.03	0.818	2.37
Canada	Quantile 97.5%	0.0950	0.00292	0.453	2.32	1.96	2.58
Canada	Quantile 99%	0.107	0.00338	0.470	2.58	2.33	2.84
United States	Median	0.0803	0.020	0.397	-0.569	-0.686	-0.363
United States	Mean	0.0917	0.0234	0.399	1.28	0.916	1.64
United States	Std. Dev.	0.0482	0.0155	0.103	2.00	1.74	2.27
United States	Quantile 1%	0.0111	8.28×10 ⁻⁴	0.219	-1.85	-2.00	-1.70
United States	Quantile 2.5%	0.0163	0.00180	0.243	-1.66	-1.79	-1.51
United States	Quantile 5%	0.0233	0.00327	0.266	-1.49	-1.62	-1.35
United States	Quantile 10%	0.0333	0.00604	0.293	-1.29	-1.42	-1.15
United States	Quantile 25%	0.0522	0.0115	0.341	-0.959	-1.08	-0.812
United States	Quantile 50%	0.0803	0.0203	0.397	-0.569	-0.686	-0.363
United States	Quantile 75%	0.121	0.0311	0.455	-0.114	-0.293	1.31
United States	Quantile 90%	0.162	0.0441	0.508	1.47	0.143	2.11
United States	Quantile 95%	0.189	0.0532	0.554	2.04	0.854	2.37
United States	Quantile 97.5%	0.211	0.0619	0.599	2.32	1.94	2.60
United States	Quantile 99%	0.237	0.0730	0.651	2.59	2.33	2.86

LB: Lower Bound of the CI95%, uncertainty dimension; UB: Upper Bound

7.2. Cheese Processing

In this exposure assessment, cheese processing was considered to begin at the bulk tank at a processing facility and included the following steps (Figure 15):

- Mitigation applied to the raw milk (if any),
- Cheese formation,
- Ripening,
- Aging.

Packaging occurs at the end of the ripening period. No contamination or redistribution of bacteria happens following the packaging.

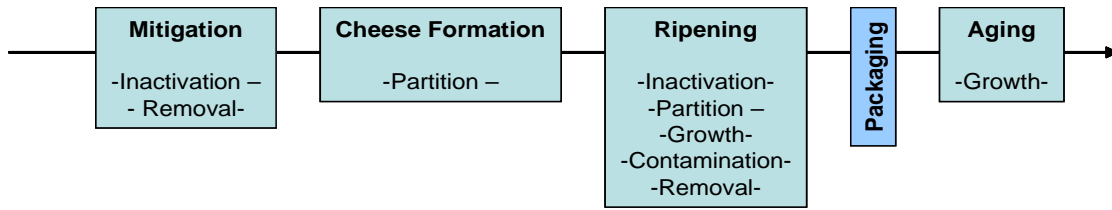


Figure 15: Schematic view of cheese processing and associated basic processes.

7.2.1. Mitigation

Inactivation

This step modeled the effect of treatment of the raw milk before it was used for cheese-making. Three mitigations were tested in this report:

- full pasteurization, *i.e.*, a pasteurization that was assumed to be fully effective. In this case, it was assumed that no *L. monocytogenes* present in the milk at the start of cheese-making survived to contaminate the cheese. In the absence of relevant data, process failures were not considered in this report. The term "pasteurization" mean the process of heating every particle of milk or milk product, in properly designed and operated equipment, to one of the time-temperature couples provided by FDA (2009, p.82);
- a process assumed to provide an expected 3 log reduction of *L. monocytogenes*.
- no treatment.

The general inactivation model was described in section 6.2.

Removal

In addition to the mitigation step, an optional testing procedure, corresponding to a Nauta's "removal process" ((Nauta 2008), see section 6.5), can be included to model the effect of removal of batches of milk as the result of milk testing before (*i.e.* during dairy silo storage) or after any other mitigations are applied. We assume that each batch detected as positive is removed from the production. We assume that the detection test is applied to a 25 ml milk sample (Latorre et al. 2009) and that the probability for the test to detect a single *L. monocytogenes* cell is $\eta = 0.75$, as used in FSIS (2003).

7.2.2. Cheese Formation

Cheese Formation: Partition from Milk to Cheese

Cheese formation results from the partitioning of milk from a silo into individual cheeses. The cheese formation step of the baseline models assumed that 2.2 l of milk are used to make a 250 g cheese (Sanaa et al. 2004). It was assumed that any *L. monocytogenes* cells present would be partitioned proportionately (see section 6.3).

The volumes of raw milk used at smaller commercial facilities making raw-milk cheese are linked to the distributions of milking herd sizes (section 7.1.1.) and the amount of variation in the milk volume that a herd contributes to a milk collection stands in relation to variability in the herd size and the among-herd variability that Albert *et al.* (2005) described,

$$Normal\left(\frac{NU}{350}, \frac{1250Y\sqrt{N}}{5000 \times 350}\right)$$

liters per day where N is the herd size and Y is the mean annual animal yield reported for the United States and Canada (AAFC 2006; USDA 2011). Large commercial cheese-making facilities making pasteurized-milk cheese use large volume dairy silos –25,000 to 150,000 liters—or draw milk for cheese-making from large volume dairy silos.

Curd Formation: Partition between Curd and Whey

Draining of whey will result in the loss of some *L. monocytogenes* cells if any are present. Based on data from Ryser and Marth (1987), Sulzer and Busse (1991), Bemrah *et al.* (1998) and Sanaa *et al.* (2004), 90% of any bacteria present are trapped in the curd while 10% are lost in the whey, through a partition process with a probability 0.9 to be trapped in the curd (see section 6.3).

The microbiological literature suggests that contamination can occur after mitigations are applied, during processing steps that still involve bulk product rather than individual cheeses. However, the literature does not describe the frequency and level of contamination that would inform model inputs for that source of contamination.

7.2.3. Ripening

Environmental Contamination

The process for modeling environmental contamination was described previously in section 6.4. The exposure assessment module assumed that this environmental contamination occurs randomly during the ripening period at a time point $T|Y=y \sim Uniform(1, y)$ days before packaging for ripening period length Y .

Initial Decline: Inactivation

Published data have shown that during the first day of ripening, there is an initial decline in the number of bacteria (see Figure 16 and section 6.1). This is treated as an inactivation process in the model (see section 6.2) leading to a 1-log reduction in the number of bacteria (Ryser and Marth 1987; Sanaa et al. 2004; Ryser 2007; Liu and Puri 2008).

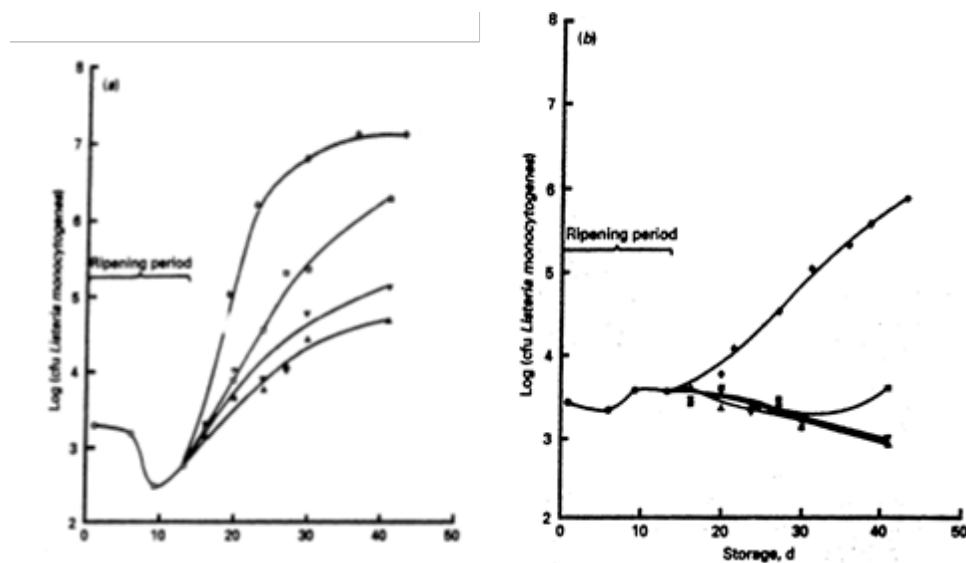


Figure 16: Example growth of *L. monocytogenes* in Camembert.

Left: surface, Right: Interior. Camembert were stored at 3, 6, 10 or 15°C (Back et al. 1993). Note that classical ripening is performed at $\approx 10^\circ\text{C}$.

Partitioning between Interior (“Core”) and Exterior (“Rind”) of the Cheese

Bacterial growth rates differ between the interior and exterior of a cheese (see Figure 16). This is primarily due to the fact that, during cheese ripening, the pH in the rind increases earlier than in the core (Ryser and Marth 1987; Back et al. 1993). Therefore, the model treated bacterial growth in each compartment separately. Assuming that a single wheel of soft-ripened cheese is a 10.8

cm diameter \times 3.2 cm high cylinder and that the rind is 0.1 cm thick (Leclercq-Perlat et al. 2006; Picque et al. 2006; Liu et al. 2007), 9.7% of the curd of the cheese will form the rind and 91.3% will become the core. The model uses a binomial process to simulate randomly partitioning the bacteria in the cheese into the two compartments.

Growth During Ripening

A lag in the growth is observed when the contamination originates from the environment. We use the theory of the “work to be done” (see section 6.1). We have

$$\lambda_T = K_\xi \times GT_T$$

where GT_T is the number of generation time in the environment T and K_ξ is a function of the physiological state ξ of the cells before transfer to the cheese.

Figure 10 (page 58) shows the number of generations done during ripening according to the model of Augustin *et al.* (2005), as a function of time, in the rind and in the core, for classical and stabilized cheeses. Table 26, further, provides the number of generations that *would have occurred* during the ripening phase, according to the time of contamination, from $t = 0$ to $t = 12$ days. If the contamination had occurred at the beginning of the ripening ($t = 0$), 3.3 generations would have occurred in cheese made with the classical process and 8.8 generations would have occurred in cheeses made with the stabilized process by the end of 12 days ripening.

Table 26: Number of generations done at the end of the ripening phase according to the time of contamination.

Time of contamination (d)	0	1	2	3	4	5	6	7	8	9	10	11	12
Classical	3.3	3.3	3.3	3.2	3.1	2.9	2.6	2.2	1.8	1.4	0.95	0.48	0.009
Stabilized	8.8	8.1	7.4	6.6	5.9	5.2	4.4	3.7	2.9	2.1	1.4	0.70	0.013

The “Stabilized” category is considered only for large, commercial, industrial operations, for cheese made from pasteurized milk. Ripening time lengths vary, from approximately 6 days to 15 days, but most often at approximately 10 d.

It is assumed that contamination during ripening occurs between 1 day after the beginning of cheese ripening and the end of cheese ripening. Growth of that contamination occurs during ripening according the results in Table 26. Residual lag time in aging for contamination

introduced during ripening is developed using the “work to be done” concept, as (Albert et al. 2005)

$$\lambda_{aging} = \max \left(0, \lambda_{theor_aging} \times \left[1 - \frac{nbG}{K_{\xi}} \right] \right)$$

For example, consider a particular case where $K_{\xi} = 5$, lag equivalent to 5 generation times. If the contamination occurred at $t = 2$ days after the start of cheese ripening, then, from Table 26, the work done in initial ripening is equivalent to 3.3 generations, for cheeses made with the classical process. The remaining lag during the aging phase is equivalent to $5 - 3.3 = 1.7$ generations. In a stabilized cheese, the work done would be 7.4 generations, larger than the example K_{ξ} , and one sees 2.4 generations of growth during ripening and no lag during aging.

The actual lag time is then a function of K_{ξ} and the growth rate, this latter parameter being a complex function of the cheese environment. Overall, for the classical ripening cheese-making, the median of the actual lag time distribution was 34 days, with 25th percentile point 13.8 days and 75th percentile 113 days. For stabilized cheeses, the median of the actual lag time was 14.1 days, with 25th percentile point 5.1 days and 75th percentile 64 days.

Removal

Removal of cheeses from the product pathway as the result of testing at the end of ripening was not considered to be part of the baseline models. However, an option to add this mitigation was included to facilitate testing alternative intervention scenarios (see section 6.5). This option assumed that $\nu = 5 \times 5$ g of cheese issued from $n = 5$ cheeses of the same lot (*i.e.*, having passed the same process, notably issued from the same batch of milk, facing the same mitigation and the same distribution of environmental contamination) were tested using a test having a probability of $\eta = 0.75$ (as used in (FSIS 2003, p. 22)) to detect a single cell. Any positive result would lead to removal of the lot.

Packaging

We consider that packaging in itself has no impact on the bacterial population in a particular cheese. However, the model does assume that additional contamination cannot occur following this step.

7.2.4. Aging

The growth of *L. monocytogenes* can occur during the aging step. This growth was modeled as previously described in the section 6.1.

Duration of Aging at the Plant

Cheeses Made from pasteurized Milk

The length of the aging period in the plant was determined by expert elicitation (IDFA 2008). In one manufacturer, the minimum time was 7 days, the maximum 21 days and the most likely time was 14 days. At a second one, these values were 3, 5 and 4 days, respectively. For a cheese from a lot at random, aging time was modeled using a mixture of two triangular distributions, with an equal probability of choosing either distribution. This can be expressed as:

$$\begin{aligned}t_{aging} &= \pi X + (1 - \pi)Y \\ \pi &\sim \text{Bernoulli}(0.5) \\ X &\sim \text{Triangular}(7, 14, 21) \\ Y &\sim \text{Triangular}(3, 4, 5)\end{aligned}$$

Cheeses Made from non-Pasteurized Milk

The pasteurized-milk cheese baseline model assumes that the milk used for cheese-making has undergone a full pasteurization. However, to facilitate testing of alternate scenarios, the model can also simulate the consequences of using raw or unpasteurized milk. In that case, the aging period lengthens to accommodate a total length of 60 days (the regulatory standard in both the U.S. (21 CFR 133.182(a)) and Canada (Food and Drugs Act B.08.030, B.08.043, B.08.044) from the beginning of manufacture until the beginning of retail display.

Temperature During the Aging Period in the Plant

The in-plant temperature experienced during aging was also determined through expert elicitation (IDFA 2008). The temperature during aging was modeled as a mixture of two triangular distributions, one with a minimum of 35°F (1.7°C), maximum of 40°F (4.4°C) and most likely temperature of 37°F (2.8°C) and the other with 37°F (2.8°C), 40°F (4.4°C) and 38°F (3.3°C), respectively, for these values, with an equal probability of choosing either distribution. This can be expressed as:

$$T_{aging} = (\pi X + (1 - \pi)Y - 32) \times 5/9$$

$$\pi \sim \text{Bernoulli}(0.5)$$

$$X \sim \text{Triangular}(35, 37, 40)$$

$$Y \sim \text{Triangular}(37, 38, 40)$$

where the result is expressed in °C. Because both triangular distributions are above the 35°F (2°C) minimum required in both the U.S. and Canada for cheeses made from raw milk and held for at least 60 days, no adjustment of the model was needed for scenarios involving raw milk.

7.3. Transport, Marketing, and Retail

Bacterial growth may occur during transport and at retail (Figure 17). This is modeled as described previously (see the section 6.1). The specific time and temperature parameters used in the baseline models are described here.

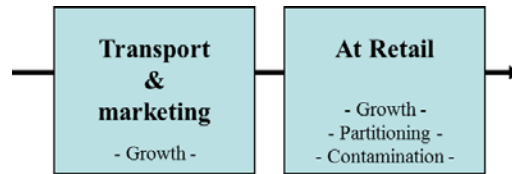


Figure 17: Schematic view of the transport, marketing and retail steps and associated basic processes.

7.3.1. Transport and Marketing Step

Time of Transport and Marketing

The duration of transport and marketing was determined by expert elicitation (IDFA 2008). The minimum, most likely and maximum time in storage between the end of aging until the cheese reached the retail store, including time in distribution centers, were estimated to be as 1, 5 and 10 days, respectively. This variability in transport and marketing stage length was modeled as:

$$t_m \sim \text{Triangular}(1, 5, 10)$$

Temperature of Transport and Marketing

Temperature during transport and marketing was determined by expert elicitation (IDFA 2008). The minimum, most likely and maximum temperature experienced by cheese during this step were estimated as 35°F (1.7°C), 40°F (4.4°C) and 50°F (10.0°C), respectively. Converting the temperatures to degree Celsius, this was modeled as:

$$T_m \sim 5/9 \times (\text{Triangular}(35, 40, 50) - 32)$$

7.3.2. Retail

Time at Retail

The duration of storage at retail was determined by expert elicitation (CFSAN 2008). The minimum, most likely and maximum time that soft-ripened cheese is displayed in the retail display cabinet were estimated as <1 day, 5 days and 14 days, respectively. This was modeled as:

$$m_{tr} \sim Uniform(0,1)$$

$$t_r \sim Triangular(m_{tr}, 5, 14)$$

Temperature at Retail

The temperature of the cheese while on display at retail was taken from measurements collected by the EcoSure network of auditors (EcoSure 2008). A description of the study design and the raw data are available on the FoodRisk.org website.¹³ Briefly, trained shoppers were asked to purchase products at retail and to measure the temperatures of these products at the store. A number of different products were purchased and tested, including cottage cheese, yogurt, and pre-packaged lunch meat or sliced meat. The data collected for semi-solid cottage cheese displayed in dairy cases at supermarkets were used to derive the parameters for this model, because this is the tested product that was most similar to soft-ripened cheese. To evaluate product temperature in the display cases, the shoppers were asked to insert a pre-calibrated thermometer into the product immediately after removing it from the display case (EcoSure 2008). The summary statistics for the cottage cheese dataset are shown in Table 27.

Table 27 Summary statistics for storage temperature (°F) for retail semi-solid cottage cheese dairy product, supermarket.

N	Mean	Var.	Min.	Perc 1%	Perc 5%	Perc 10%	Perc 25%	Perc 50%	Perc 75%	Perc 90%	Perc 95%	Perc 99%	Max
751	39.3	17.8	22	30	32	34	37	39	41	44	46	50	64

(EcoSure 2008).

Between store temperature variability was modeled using a lognormal distribution, ignoring the Ecosure (2008) design. The temperatures were converted to T_r (°C) from T_{rF} (°F) and the distribution was modeled as:

¹³ <http://foodrisk.org/exclusives/EcoSure> assessed 4/11/2012.

$$T_r = \frac{5}{9}(T_{rF} - 32)$$

$$\ln(T_{rF}) \sim \text{TruncatedNormal}(\mu, \sigma, 28.5, 60.8)$$

where $\text{TruncatedNormal}(\mu, \sigma, 28.5, 60.8)$ is the normal distribution with a mean μ , a standard deviation σ , truncated at $[28.5; 60.8]^\circ\text{F}$ (i.e. $[-1.94; 16]^\circ\text{C}$); μ and σ were estimated from raw data using a maximum likelihood method.

The description of the sampling design provided with this data set (EcoSure 2008) is somewhat brief. This prevents both accurate extrapolation from the sampling data to the corresponding target population and evaluation of the associated uncertainties. Therefore, an equal (unknown) weight was assumed for each observation. Moreover, to evaluate the uncertainty of the estimates, it was assumed that the sample design was as follows: stratification by U.S. state; a random city \times location (within state) selection with equal probabilities, and a random store type and participant (within state \times city \times location) selection with equal probabilities. The simple random sample standard error for the $\hat{\mu}$ parameter estimate was scaled by the square root of the design effect, and the effective degrees of freedom (DEFF, number of independently selected clusters, number of strata) was used in place of the number of observations in setting the shape and scale parameter for the sampling distribution for the $\hat{\sigma}^2$ parameter estimate. Using these assumptions, values for the maximum likelihood estimates and their associated uncertainty are shown in Table 28.

Table 28: Specification of the temperature T_{rF} ($^\circ\text{F}$) at retail.

<i>DEFF</i>	Maximum likelihood estimate	Uncertainty distribution ($^\circ\text{F}$)
420	$\mu = 3.6647$ $\sigma^2 = 0.01143$	$\mu \sim \text{Normal}(3.665, 0.005203^2)$ $\sigma^2 \sim [\text{Gamma}(210, [2.394]^{-1})]^{-1}$ $\rho(\mu, \sigma^2) = 0.000022$

T_{rF} is specified using $\ln(T_{rF}) \sim \text{TruncatedNormal}(\mu, \sigma^2, 28.5, 60.8)$.

Note that no specific information is available on temperature at retail for artisanal or farmstead cheeses.

Contamination at Retail

The microbiological literature suggests that contamination can occur at retail, such as when larger cheeses are cut into portions and repackaged. However, the literature does not describe the

frequency and level of contamination that would inform model inputs for that source of contamination. The soft-ripened cheeses that are this report's main focus are rarely repackaged at retail.

7.4. Home

The in home stage of the model considers two factors, *i.e.*, the conditions encountered during home storage and consumption patterns (Figure 18). Only the potential bacterial growth that might occur in the refrigerator and at room temperature in the home was considered in the model (no cross contamination). Modeling of consumption involved partitioning a whole cheese into individual servings. Both of these factors required establishing specifications for serving size distributions, preferably for each specific population (countries, subpopulations).

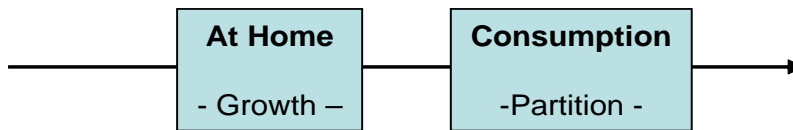


Figure 18: Schematic view of the home and consumption steps and associated basic processes.

7.4.1. Serving Size

Canada

Serving size data for Canadian populations were obtained from the nutrition component of the Canadian Community Health Survey, Cycle 2.2 (Statistics Canada 2004). After having defined the population groups using survey respondents' age and gender information, each respondent's Brie and Camembert consumption over all eating episodes during the 1st food recall day were aggregated into an individual's single-day total. The variability of single-day consumption (serving sizes) among individuals within a population group was described by combining the fraction of non-eaters (individuals who eat exactly 0 g of soft cheese), with a lognormal distribution describing the single-day consumption variability among eaters (individuals who eat more than 0 g of soft cheese) using maximum likelihood methods. The estimates that resulted are shown in Table 29. A non-parametric bootstrap (bootstrap survey weights) procedure was used to estimate the sampling distribution of $(\hat{\mu}, \hat{\sigma}^2)$ in that logNormal distribution.

Table 29: Brie and Camembert serving size distributions for Canadian population.

Population group	DRI age-sex groups	Resp.	Fraction eaters Est. (\pm s.e.) [95% c.i.] (bootstrap)	logNormal distribution eaters $\hat{\mu}$ ln(g)	logNormal distribution eaters $\hat{\sigma}^2$ ln(g)
Elderly	M >70, F >70	4,130	0.0057 (\pm 0.0015) [0.0032, 0.0089]	3.04 (\pm 0.293)	1.00 (\pm 0.280)
Pregnant women	F 19-30, F 31-50	4,772	0.012 (\pm 0.0032) [0.0032, 0.0064]	3.05 (\pm 0.283)	0.823 (\pm 0.278)
General & Immunocompromised	Children \geq 1, Adults \leq 70	29,278	0.0093 (\pm 0.0013) [0.0070, 0.012]	3.24 (\pm 0.140)	0.845 (\pm 0.134)

(Statistics Canada 2004).

United States

Serving size data for the U.S. population were derived from the National Health and Nutrition Examination Survey (NHANES) III (2003-2004). The observed quantiles for single-day consumption of Brie and Camembert (food codes 14103010, 14103020) are shown in Table 30. The data did not permit differentiation of consumption patterns for all subpopulations, due to a low sample size. This empirical cumulative distribution was used to draw random serving size values using a linear extrapolation between specified quantiles (see `CumDist` function, Analytica software, (Lumina Decision Systems 2010)).

Table 30: Parameters of the empirical cumulative distribution used to describe the serving size, U.S.

Percentile	Serving Size (g)	Percentile	Serving Size (g)	Percentile	Serving Size (g)
0	0.00	39 to 41	17.00	75 to 76	41.79
1	1.46	42 to 45	18.00	77 to 80	48.00
2	7.51	46 to 50	19.13	81 to 85	51.00
3 to 25	9.00	51 to 52	26.56	86 to 89	60.00
26 to 27	9.56	53 to 55	28.35	90 to 91	90.12
28	10.45	56 to 57	30.04	92 to 94	133.52
29 to 32	13.37	58 to 62	34.00	95 to 96	141.75
33 to 36	15.00	63 to 70	36.00	97 to 100	161.56
37 to 38	15.38	71 to 74	38.25	-	-

The data are issued from the NHANES III (2003) source.

Simulated Serving Sizes

The resulting serving size distributions were truncated to lie between 10g and 225g. Table 31 shows summary statistics for the serving size distributions for Canada and U.S. used in the model.

Table 31: Serving size (g) distribution summary statistics, soft-ripened cheese, Canada and U.S.

Country	Group	Uncertainty consideration	Mean	5 th Percentile	50 th Percentile	95 th Percentile
Canada	Elderly	Point estimate	40	11	28	115
Canada	Elderly	Uncertainty	38 [28; 50]	11 [11; 14]	28 [20; 37]	100 [69; 150]
Canada	Pregnant women	Point estimate	37	11	27	100
Canada	Pregnant women	Uncertainty	38 [22; 53]	11 [11; 13]	27 [18; 37]	100 [44; 150]
Canada	General and Immunocompromised	Point estimate	42	12	30	117
Canada	General and Immunocompromised	Uncertainty	43 [36; 50]	11 [12; 12]	31 [26; 36]	120 [89; 140]
U.S.	All	Point estimate	47	13	36	150

Notes: *Uncertainty* reports the median [2.5%, 97.5%] points from the uncertainty distribution for the summary statistic. Specification for U.S. populations includes *Point estimate* only, not distinguishing among population groups.

7.4.2. Home Storage

Data

Data on home storage practices were taken from a study performed in 2005 by RTI International *et al.* (2005) using a web panel of 2,060 U.S. adults (Cates *et al.* (2007), Kosa *et al.* (2007a), Kosa *et al.* (2007b)). The complete protocol, questionnaire and survey data are available on the FoodRisk.org website.¹⁴ Participants were asked to complete a questionnaire on storage times for 10 categories of refrigerated ready-to-eat foods and leftovers, refrigerator thermometer use, refrigerator temperature, and knowledge and use of open date statements. The storage time component included questions about storage times for both unopened and opened packages.

Storage Time at Home

The home storage time model assumed that the RTI questionnaire structure completely captured consumer eating behavior for soft cheeses. That is, consumers either *i*) keep a soft cheese package at room temperature and eat the entire package on 1 occasion; or, they store the package in the refrigerator and then, eat from the package on *ii*) one occasion; or, *iii*) more than one occasion. If the contents of a package are consumed over multiple occasions, the total storage time consists of the time the package is unopened, plus the time between opening and the last eating occasion. The RTI data were used to infer distributions for the frequency of occurrence of the three situations *i*, *ii* and *iii* described above, the time the product is unopened in the

¹⁴ <http://www.foodrisk.org/exclusives/index.cfm> assessed 4/11/2012.

refrigerator, the time the open product spends in the refrigerator between the 1st and last occasion, and the number of and the time between successive consumption occasions from opened packages (totaling the time between opening and the last eating occasion from the package). The time a package remained unopened in the refrigerator was assumed to follow a Weibull distribution; the time between opening a package and the last consumption was modeled using an Erlang distribution with a scale parameter that reflected the varying, among cheeses, number of eating occasions per cheese. The number of eating occasions was modeled using a Poisson distribution. For products that are not stored in the refrigerator (*i.e.*, those eaten at the first occasion), the time the products stayed at room temperature was modeled using a uniform distribution, on 0 to 0.5 days.

We infer the distribution for the number of servings from a single cheese using the serving size distribution. The number of those servings on an eating occasion, at random, X , has distribution represented by a Binomial distribution, that is, $X | N$ servings, M occasions $\sim \text{Binomial}(N-M+1, [1+M]^{-1})$.

Table 32 shows the derived consumer storage practices, Table 33 shows the parameter estimates for the distribution of storage times until a package is first opened, and Table 34 shows the parameter estimates for the distributions for the number of consumption occasions and the distribution for the time between successive consumption occasions.

Table 32: Soft cheese storage attributes, fraction of cheeses consumed with listed characteristic.

Characteristic	Elderly Resp.	Elderly Mean (\pm se)	Pregnant women Resp.	Pregnant women Mean (\pm se)	General population Resp.	General population Mean (\pm se)
Stored in refrigerator	138	0.990 (\pm 0.0098)	101	0.988 (\pm 0.012)	141	0.992 (\pm 0.0085)
Stored in refrigerator & eaten	137	1.0	100	1.0	140	1.0
Stored in refrigerator after opening	138	0.964 (\pm .098)	101	0.928 (\pm 0.037)	141	0.936 (\pm 0.022)
Stored in refrigerator after opening & eaten	91	0.944 (\pm .028)	71	0.876 (\pm 0.058)	108	0.915 (\pm 0.028)

Table 33: Weibull distribution for time the product is unopened until the 1st consumption (d). m.l.e. (\pm se) [95% ci] (Wald-type)

Population group	α (shape)	λ (scale)	$\rho(\hat{\lambda}, \hat{\theta})$
Elderly	0.95 \pm 0.084 [0.83, 1.2]	5.6 \pm 0.60 [4.5, 6.9]	0.36
Pregnant women	0.80 \pm 0.079 [0.64, 0.95]	5.9 \pm 0.87 [4.2, 7.6]	0.21
General & Immunocompromised	0.83 \pm 0.067 [0.70, 0.97]	5.0 \pm 0.60 [3.9, 6.2]	0.31

Table 34: Consumption occasions (Poisson λ) and time (d) between successive occasions from opened package (Exponential θ). m.l.e. (\pm se) [95% ci] (Wald-type)

Population group	λ	θ	$\rho(\hat{\lambda}, \hat{\theta})$
Elderly	1.2 \pm 0.85 [-0.51, 2.8]	8.2 \pm 3.1 [2.1, 14.3]	-0.95
Pregnant women	2.5 \pm 0.95 [0.62, 4.4]	6.7 \pm 2.3 [2.2, 11.3]	-0.93
General & Immunocompromised	2.7 \pm 1.0 [0.72, 4.7]	3.3 \pm 1.1 [1.1, 5.5]	-0.96

The simulation outputs (storage time to serving at random, in days) are shown in Table 35. Note that while the RTI 2005 dataset was used for both the U.S. and Canadian populations for storage attributes and storage time distributions, the time to a serving at random distribution differs between the two countries because of the different serving size distributions (Table 31). Among populations within a country, the time to a serving at random distributions differ because of different storage attribute and storage time distributions (Canada and U.S.) (Table 32-Table 34) and because of different serving size distributions (Table 31, Canada).

Table 35: Time (d) to serving at random.

Variability: 10,000 replicates, Monte-Carlo simulation. For uncertainty replicates: entries are median [2.5%, 97.5%] over 100 uncertainty replicates.

Country	Population	Uncertainty consideration	Mean	5 th % Percentile	Median	95 th % Percentile
Canada	Elderly	Point estimate	13	.47	9.4	37
Canada	Elderly	Uncertainty	13 [10; 15]	.44 [0.21; 0.92]	9.8 [7.6; 12]	36 [28; 42]
Canada	Pregnant women	Point estimate	15	.40	12	42
Canada	Pregnant women	Uncertainty	16 [10; 19]	.33 [0.13; 1.4]	8.6 [13; 17]	39 [27; 48]
Canada	General and Immunocompromised	Point estimate	10	.43	8.5	27
Canada	General and Immunocompromised	Uncertainty	9.2 [7.5; 11]	.30 [0.50; 0.90]	7.8 [6.3; 9.0]	23 [19; 26]
United States	Elderly	Point estimate	12	.49	9.1	36
United States	Elderly	Uncertainty	12 [10; 14]	.43 [0.22; 0.87]	7.4 [9.3; 11]	34 [28; 40]
United States	Pregnant women	Point estimate	14	.35	11	39
United States	Pregnant women	Uncertainty	13 [9.8; 17]	.31 [0.13; 0.91]	10 [7.9; 14]	34 [25; 45]
United States	General and Immunocompromised	Point estimate	10	.41	8.2	26
United States	General and Immunocompromised	Uncertainty	8.8 [7.4; 10]	.49 [0.28; 0.88]	7.3 [6.2; 8.5]	22 [19; 26]

Storage Temperature at Home

Room Temperature

The distribution of room temperatures in the home was modeled using a uniform distribution on 15 to 30°C.

Refrigerator Temperature

The distribution of home refrigerator temperatures was inferred from the RTI data. Reported temperatures below 28°F (-2.2°C) were suggested to be erroneous and were discarded from the data obtained from the online dataset. Maximum likelihood methods were used to estimate the parameters of a Laplace distribution for refrigerator temperatures for each population group, following Pouillot *et al.* (2010). The values for the location μ and the scale λ parameters of the derived Laplace distribution are shown in Table 36. The sampling distributions for these parameters' estimates were obtained by non-parametric bootstrap from the raw data.

The Laplace distribution was truncated on the [28.5; 60.8]°F interval (*i.e.* [-1.94; 16]°C).

Table 36: Parameter estimates for fitted Laplace distributions for refrigerator storage temperature (°C).

Population group	Parameter	Estimate		$\rho(\hat{\mu}, \hat{\lambda})$
		m.l.e. (\pm se)	[95% ci]	
Elderly	μ (location)	4.2 \pm 0.057	[4.1, 4.3]	0.060
Elderly	λ (scale)	2.0 \pm 0.072	[1.9, 2.1]	NA
Pregnant women	μ	4.2 \pm 0.16	[3.9, 4.5]	0.074
Pregnant women	λ	2.9 \pm 0.19	[2.5, 3.2]	NA
General and Immunocompromised	μ	4.0 \pm 0.079	[3.8, 4.1]	-0.0061
General and Immunocompromised	λ	2.5 \pm 0.091	[2.3, 2.7]	NA

NA Not Applicable

Simulated Temperature

Table 37 summarizes the properties of the temperature distributions (in °C) used in the exposure assessment.

Table 37: Temperature (°C), serving at random.
 Entries Mean [2.5%, 97.5%] uncertainty replicates.

Country	Population	Uncertainty consideration	Mean	5 th %ile	Median	95 th %ile
Canada	Elderly	Point estimate	4.5	0.3	4.5	9.1
Canada	Elderly	Uncertainty	4.7 [4.4; 5.3]	0.35 [0.087; 0.60]	4.3 [4.2; 4.4]	9.6 [8.7; 16.0]
Canada	Pregnant women	Point estimate	4.8	-0.22	4.3	11
Canada	Pregnant women	Uncertainty	4.6 [4.4; 4.8]	-0.44 [-0.20; 0.18]	4.6 [4.4; 4.8]	12 [10; 17]
Canada	General and Immunocompromised	Point estimate	4.5	-0.13	4.1	10
Canada	General and Immunocompromised	Uncertainty	4.1 [3.9; 4.3]	-0.13 [-0.33; 0.025]	4.1 [3.9; 4.3]	10 [9.5; 13]
U.S.	Elderly	Point estimate	4.5	0.29	4.2	9.1
U.S.	Elderly	Uncertainty	4.7 [4.4; 5.4]	0.35 [0.094; 0.61]	4.3 [4.2; 4.4]	9.6 [8.7; 16]
U.S.	Pregnant women	Point estimate	4.8	-0.21	4.4	11
U.S.	Pregnant women	Uncertainty	5.1 [4.7; 5.8]	-0.20 [-0.46; 0.18]	4.6 [4.4; 4.8]	12 [10; 17]
U.S.	General and Immunocompromised	Point estimate	4.5	-0.15	4.1	9.9
U.S.	General and Immunocompromised	Uncertainty	4.6 [4.3; 5.1]	-0.33 [-0.14; 0.03]	4.1 [3.9; 4.3]	10 [6.4; 13]

Contamination at Home

The microbiological literature suggests that contamination can occur in the consumer’s refrigerator when the cheese is stored, open, over several eating occasions. However, the literature does not describe the frequency and level of contamination that would inform model inputs for that source of contamination.

7.5. *L. monocytogenes* Ingested in a Serving

All servings from non *L. monocytogenes* contaminated cheeses contain, by definition, 0 *L. monocytogenes* cells. For *L. monocytogenes* contaminated cheeses, consumption was modeled as a partition process (see section 6.3). Each contaminated cheese was considered as if it were a stack of contaminated and uncontaminated grams of “core” and “rind” cheese (see section 6.1). A serving of *C* grams was considered to be a random sample of these grams amongst the grams that constitute the cheese, using a hypergeometric distribution to describe variability among servings and among cheeses. The *L. monocytogenes* present in these *C* grams were the ingested dose. Variability in the proportion of rind and core in a serving was not modeled.

This simulation process respects the clustering of *L. monocytogenes* among contaminated and uncontaminated cheeses and the clustering within contaminated cheeses.

8. Risk Characterization (Method)

The risk characterization is the final component of the risk assessment. Risk characterization integrates the hazard characterization and the exposure assessment to synthesize the probability and severity of adverse health effects in a particular population of consumers. In this risk assessment, the output of the risk characterization is the probability of invasive listeriosis following the consumption of a random serving of cheese by an individual in a considered subpopulation and country. Using a second-order Monte-Carlo simulation framework, the variability and uncertainty of the risk characterization outputs are estimated as a reflection of the variability and uncertainty of the model inputs. In addition, a sensitivity analysis is used to explore the impact of the uncertainty and variability of inputs on the risk outputs.

8.1. Output of the Risk Characterization

The main output that will be used to assess the risk of invasive listeriosis from soft-ripened cheese consumption in Canada and the U.S. is the probability of invasive listeriosis following the consumption of a random serving of cheese by an individual of the considered subpopulation. We will simplify this output to the: **risk per serving** in the particular country (Canada, U.S.) for the considered population (Elderly, Immunocompromised, Pregnant, General). This output is of interest because the expected number of cases of invasive listeriosis in a particular population during a specific period of time is proportional to the mean risk per serving. The average number of cases in $N_{c,p}$ servings is $C_{c,p} = N_{c,p} \times \bar{R}_{s_{c,p}}$, where $N_{c,p}$ is the number of servings consumed by population p in country c during this period and $\bar{R}_{s_{c,p}}$ is the mean risk per serving for this population p during this period of time¹⁵. For any risk mitigation strategy (indexed 1) that does not impact the number of servings consumed in a population, the proportion of avoided cases compared to the baseline (pasteurized-milk cheese baseline or raw-milk cheese baseline -- indexed 0) is then equal to:

$$\frac{C_1}{C_0} = \frac{\bar{R}_1}{\bar{R}_0}.$$

¹⁵ under the assumption of a binomial result for the number of cases in $N_{c,p}$ servings.

Other risk characterization outputs of interest are:

- the **risk per contaminated serving**, $R_{cs,p}$, that is the probability of illness following the consumption of a random **contaminated** serving by an individual in population p (Elderly, Immunocompromised, Pregnant or General) in the country c . A contaminated serving is defined as a serving including one or more cells of *L. monocytogenes*;
- the **prevalence of contaminated servings**, P_s , that is, the probability that a random serving of cheese contains one or more cells of *L. monocytogenes*.

Recall that all of these outputs are distributions that describe how the risk output varies over a reference population of interest. For simplicity, we will provide some statistics characterizing these distributions such as the mean, the standard deviation and some quantiles.

The number of cases per year will not be provided due to the unknown number of servings in the population.

8.2. Estimator for the Risk Outputs

The risk outputs of interest cannot be extracted directly from the literature but, rather, are synthesized by using a set of mathematical models and equations that link several input parameters to the risk outputs (see Appendix, section “Model Documentation”). Stochastic, uncertain inputs then yield stochastic, uncertain outputs whose distributions can be evaluated either analytically or by simulation.

Because the overall integration of the model to derive the final distribution of each of the risk outputs is analytically intractable, a Monte-Carlo simulation was used. Monte-Carlo simulation is a simulation sampling method: input parameters’ values are sampled from their input distributions, thus simulating the action of sampling from the inputs’ variability distributions, subject to our uncertainty. The modeled risk output calculated using those inputs propagates the inputs’ variability and acts as a sample from the risk output’s probability distribution, subject to our uncertainty about the inputs.

This computer-intensive framework allows a random sample from the (analytically intractable) distribution of the risk output to be obtained. Summary statistics that we produce from the simulated risk output Monte-Carlo sample converge to the corresponding summary statistics from the risk output's distribution in large enough simulations. Summary statistics about how those summary statistics change across the uncertainty about inputs, converge to an expression of our uncertainty about the risk output's distribution in large enough simulations.

The estimator's specification is generally completed by referring to the Monte-Carlo simulation size (below), sampling method, and randomization method. The estimators' characteristics, convergence properties and standard errors are examined in the Appendix (section "Simulation Estimator Characteristics for the Risk Outputs").

8.3. Variability / Uncertainty

8.3.1. Contrasting Variability and Uncertainty

When we account fully for how managers make risk decisions, how we treat variability and uncertainty should differ.

“Uncertainty forces decision makers to judge how *probable* it is that risks will be overestimated or underestimated for every member of the exposed population, whereas variability forces them to cope with the *certainty* that different individuals will be subjected to risks both above and below any reference point one chooses” (National Research Council 1994, p. 237)

In National Research Council's sense (1994) and under *Codex alimentarius* commission conventions, we should reserve *variability* to refer to how the risk output *varies*, over some well-defined reference population and we should reserve *uncertainty* to refer to our cumulative knowledge or lack knowledge about that variability.

Variability Sources

Variability represents the heterogeneity of the risk within a particular population. In the present application, it is linked to the variability in the exposure, *i.e.* the heterogeneity of the number of cells in a serving chosen at random. Some examples of elements of variability that are considered in this model are the location to location variability of environmental contamination as inferred

from Gombas *et al.* (2003), the variability in the ability of a *Listeria* population to grow in a cheese at random (linked to strain to strain variability and to cheese to cheese variability), the specific ability of a population of *Listeria* to grow (linked to variability in time and temperature of storage), and the variability in the number of cells per serving when a portion, which varies in size, is taken from a whole wheel of soft-ripened cheese. Such heterogeneity in the exposure leads to heterogeneity in the risk per serving: the risk per serving varies over a reference population of servings.

Uncertainty Sources

Uncertainty about how the risk per serving varies arises from our lack of perfect knowledge, and it may be related to the model used to characterize the risk, the parameters used to provide values for the model, or both. In some cases, we can reduce uncertainty by obtaining better information, but this may not always be possible. Having uncertain results implies that one might make a less-than-optimal risk decision because one may expect one outcome but something quite different might actually occur (Thompson 2002).

Sources of uncertainty include model uncertainty, data uncertainty and estimator uncertainty.

Model uncertainty includes

- how one represents, summarizes or simplifies physical phenomena;
- how one represents methods to sample information from physical phenomena; that is, the umbrella of model uncertainty includes the basic notion of how one infers from sample to sampling population and how one extrapolates from sampling population to reference population (the population that the risk assessment is interested in); and,
- how we represent the sampling distribution for the model's basic outputs.

Data uncertainty includes

- inference from small samples via a particular model to the sampling population from which the data come; and,
- lack of clear definition of the sampling population and lack of clear description for how the data were sampled from that sampling population.

Estimator uncertainty arises since simulations generate only simulation sample estimates of the summary statistics of risk outputs' distributions that we use to summarize the risk output distribution.

8.3.2. Implementing Variability and Uncertainty Separation

Indeed, the whole model is a mathematical combination of model inputs. Most of the inputs are not known perfectly; rather, quantifiable uncertainty is associated with the “best estimate” of these parameters. Similar to how a Monte-Carlo simulation transfers the variability in model inputs to model outputs, it is also possible to transfer the uncertainty associated with each input, so that the simulation produces also a measure of the amount of uncertainty around the risk outputs' variability. A second-order Monte-Carlo simulation (Frey 1992) was built to enable measurement of the uncertainty of the summary statistics for each of the risk output's distributions. The simplified process is:

- 1) to derive a (parametric or empirical) distribution of uncertainty for each uncertain parameter;
- 2) to draw one value for each of these uncertain parameters from these distributions;
- 3) to derive a typical 1-dimensional Monte-Carlo simulation using these values, considered as if fixed. This simulation leads to a distribution (of variability) of the risk output conditional on the set of particular values of the uncertain parameters. Various statistics (mean, quantiles) are evaluated from the empirical distribution to characterize this variability distribution;
- 4) to loop on the 2nd and 3rd steps a large number of times (say n_u).

At the end of the process, n_u typical 1-dimensional Monte-Carlo simulations have been performed, leading to n_u sets of distributions for each of the risk outputs and n_u sets of their summary statistics, *i.e.* n_u means, n_u quantile 0.01, ..., n_u quantile 0.99.

We summarize the result of the second-order Monte Carlo simulation using the median, 0.025th and 0.975th quantile (uncertainty distribution) of the n_u estimations of the summary statistics of the risk outputs' variability distributions. That gives a credible interval (uncertainty interval) for

each risk output summary statistic: a credible interval for the mean of the risk per serving variability distribution; a credible interval for the 95th percentile of the risk per serving variability distribution; etc.

Summary statistics (uncertainty) about how the risk outputs' distributions (and so, those distributions' summary statistics) change across the uncertainty about inputs converge to an expression of our uncertainty about the risk output's distribution in large enough simulations. Thus, this second-order Monte-Carlo simulation allows evaluation of the uncertainty around estimates of the risk or any other output. For example, we illustrate with Figure 19: we describe how the risk per serving *varies* (black), uncertainty about the whole distribution (light grey) and the uncertainty (blue) about a particular reference point (solid, vertical line) in how the risk per serving varies over some reference population. Note nevertheless that largely only a part of the overall uncertainty is measured here, *i.e.* a part of the data uncertainty.

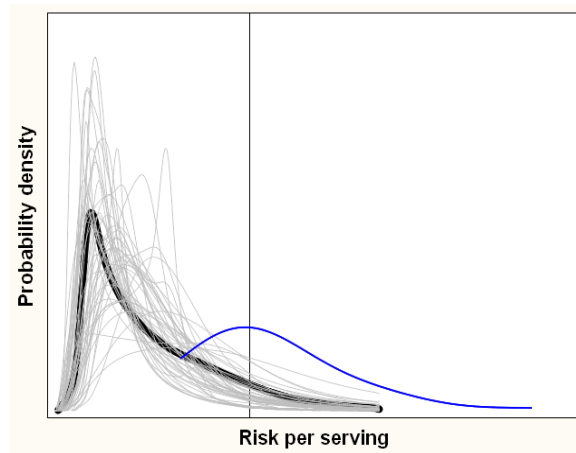


Figure 19: Illustration of second-order Monte-Carlo results.

8.3.3. Relative Sizes of Variability and Uncertainty in modeled Risk Outputs

It is useful to measure and compare the contributions of uncertainty and variability to the final risk outputs. To accomplish this, Ozkaynak *et al.* (2009) proposed some metrics to compare the order of magnitude of the uncertainty compared to the variability (Figure 20). Given

- A, the median (uncertainty distribution) of the n_u medians (variability distribution);
- B, the median (uncertainty distribution) of the n_u 95th percentiles of variability;

- C, the 95th percentile (uncertainty distribution) of the n_u medians (variability distribution);
- D, the 95th percentile (uncertainty distribution) of the n_u 95th percentiles of variability

Ozkaynak *et al.* (2009) proposed as measures of the variability and uncertainty:

- the Variability ratio = B/A ,
- the Uncertainty Ratio = C/A and,
- the Overall Uncertainty Ratio = D/A .

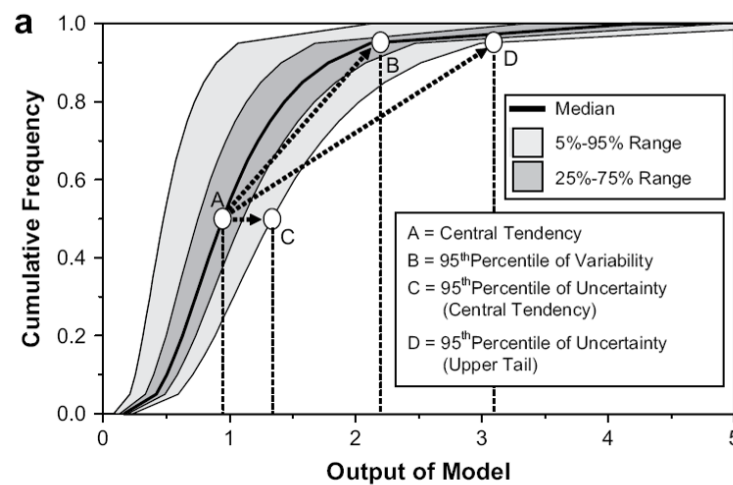


Figure 20: Illustration of the measure of variability and uncertainty (Ozkaynak *et al.* 2009).

8.4. Sensitivity Analysis

The model used in this risk assessment is complex. Sensitivity analysis is thus a key element of the process to study it. While the major interest of the model is the evaluation of the impact of specific risk mitigation strategies on risk, it is of interest to identify and prioritize key sources of variability and uncertainty, in order to further inform decision-making. Indeed, risk inputs whose variability markedly affects the risk outputs may be considered as potential candidates for mitigating the risk. On the other hand, risk inputs whose uncertainty markedly affects the uncertainty about the risk output distribution are candidates for acquiring additional information to reduce uncertainty. Two separate analyses were conducted as part of the sensitivity analysis of this risk assessment model: changing one factor at a time and rank correlation. Additional

methods (ANOVA and variance-based method) (Mokhtari and Frey 2005; Ellouze et al. 2010) were tested and gave similar results as the one presented in this report.

8.4.1. Changing one Factor at a Time

One way to study the model is to evaluate the change in the output following the change in one input. In order to study the model, some artificial scenarios will be tested to evaluate their impact on the risk per serving for a specific country and a particular subpopulation (say: Canada, Elderly). The tested scenarios are:

- Environmental contamination: from pasteurized-milk cheese baseline contamination level (section 6.4, Table 20-Table 21) to exactly 1, 10, 100, 1,000, 10,000, 100,000 cfu/cheese; and from pasteurized-milk cheese baseline prevalence (section 6.4, Table 18) to prevalences less than the 20% point of that distribution;
- Growth characteristics: from pasteurized-milk cheese baseline (section 6.1, Table 12-Table 13) to EGR_{20s} equal to 0, $\frac{1}{2}$ baseline, $2 \times$ baseline; from baseline (section 6.1, Table 14) to maximum population densities equal to the baseline $-1 \log_{10}$ or to the baseline $+1 \log_{10}$;
- Transport and Marketing temperature: pasteurized-milk cheese baseline -1°C , pasteurized-milk cheese baseline $+1^\circ\text{C}$;
- Temperature at Retail: pasteurized-milk cheese baseline -1°C , pasteurized-milk cheese baseline $+1^\circ\text{C}$;
- Home storage temperature: pasteurized-milk cheese baseline -1°C , pasteurized-milk cheese baseline $+1^\circ\text{C}$;
- Home storage duration: maximum duration of storage at home of 28 days compared to 56 days.

8.4.2. Rank Correlation

The second method used is an evaluation of the Spearman's rank correlation between inputs to the model and outputs of the model (Frey and Patil 2002). This method's output is frequently displayed as a "tornado chart." While frequently used in risk assessment, this sensitivity analysis also remains rough (Borgonovo 2006). Exploring interactions that could occur in the model is

difficult and the method is insensitive to several important types of dependence between output and inputs: non-linearity and thresholds.

Considering both the variability and uncertainty, the following impacts of parameters on the final outputs were explored:

- i) impact of variable parameters on outputs: for a specific input-output pair, one Spearman's rank correlation may be estimated for each of the N_u simulations, leading to N_u Spearman's rank correlations. The median, the 0.025th quantile, and the 0.975th quantile of these N_u values may then be used as an estimate and credible interval of the Spearman's rank correlation for that pair;
- ii) impact of uncertain parameters on outputs: in the uncertainty dimension, one can estimate the Spearman's rank correlation between uncertain parameters (N_u values) and some statistics evaluated in the variability dimension, such as the mean or specific quantiles of the risk outputs' variability.

9. Results of the Model Application Examples

9.1. Results of the Pasteurized-Milk Cheese Baseline Model

The baseline model for pasteurized-milk cheese uses two major inputs/assumptions that distinguish it from other examples that we report:

- The milk for cheese-making is “fully” pasteurized, meaning that no raw milk source *L. monocytogenes* survives the pasteurization process;
- No testing procedures are implemented.

All the other inputs are set as described in the previous sections:

- Contamination with *L. monocytogenes* occurs during the ripening process; the level of contamination and the frequency of contaminated cheeses are inferred from Gombas *et al.* (2003) (section 6.4);
- Growth rate, lag time and maximum population density distributions among *L. monocytogenes* in cheeses (section 6.1);
- Storage time and storage temperature distributions among cheeses during transport and marketing and during retail, and among cheese servings during home storage (section 7);

- Serving size distributions (section 7.4.1.); and,
- Dose-response functions (section 5).

9.1.1. Organization

Risk Outputs

Exposure assessment outputs of interest describe the distribution for the number of *L. monocytogenes* in soft-ripened cheese servings. We report that result in two parts:

- distribution of the number of *L. monocytogenes* in contaminated servings; and,
- prevalence of contaminated servings, that is, prevalence of servings that contain 1 or more *L. monocytogenes*.

Risk characterization outputs of interest describe how the probability of illness (invasive listeriosis) varies:

- from consuming a contaminated serving among the contaminated servings that subpopulations consume; and,
- from consuming a serving (contaminated or not contaminated) among the servings that subpopulations consume.

Tabled Results Structure

The exposure assessment and risk characterization outputs vary among the individual units (cheeses, servings, individuals) in well-defined populations. We make *well-defined populations* more precise, in the context of reporting the risk outputs, in sections below. We organize the risk outputs' results into tables, with features set both to meet the management charge and to help in reporting risk outputs' variability. Each table contains results for a single risk output. Table columns separate results for populations (country × subpopulation) and table rows report summary statistics from the distribution (variability) of that risk output. Mean, median and variance do not adequately describe the shape of distributions as skewed as these risk outputs' distributions are; so, tables' summary statistics report several percentiles, including ones in the lower and upper tails, as well. Results in section 9.1.2. ignore parameter uncertainty; setting parameters to their most likely values describes only variability, as appropriate to context.

Section 9.1.3. 's results account for both the parameters' description of variability and also the uncertainty that we associate with those variability descriptions, insofar as the uncertainty can be captured.

Calculation Methods

Calculated results in section 9.1.2. use the simulation model's Analytica™ implementation, 100,000 iterations, with Median Latin Hypercube sampling. This number of iterations is sufficiently high to obtain good convergence for the main statistics of interest (see Appendix, section "Simulation Estimator Characteristics for the Risk Outputs"). Section 9.1.3. 's calculated results use the Analytica™ implementation of the simulation model, $N_v = 25,000$ samples in the variability dimension and $N_u = 200$ iterations in the uncertainty dimension, with Median Latin Hypercube sampling (variability dimension) and a hybrid of Median Latin Hypercube and Simple Monte Carlo sampling (uncertainty dimension). This number of iterations is sufficiently high to obtain good convergence for the main statistics of interest in both variability and uncertainty dimensions (see Appendix, section "Simulation Estimator Characteristics for the Risk Outputs").

9.1.2. No Uncertainty Considered

Number of L. monocytogenes Cells per Serving of Contaminated Cheese at the Time of Consumption

For cheeses made from fully pasteurized milk, we assumed for the purpose of this model that *L. monocytogenes* contamination comes only from the cheese processing environment. We modeled this contamination to occur after the cheeses have been formed. Therefore, all contamination remains in the cheese exterior (rind) where growth lag time and growth rates appropriate to *L. monocytogenes* in the cheese rind are applied. Environmental contamination from this source is at relatively low levels, initially 1-31 *L. monocytogenes* cfu per contaminated cheese, and occurs infrequently among all cheeses (section 6.4). Growth occurs when conditions permit; growth amounts are governed by time and temperature during cheese aging, transport and marketing, retail display and home storage (section 6.1). Contaminated servings happen only when the servings come from contaminated cheeses and the number of *L. monocytogenes* in a contaminated serving varies with the number of *L. monocytogenes* in the contaminated cheese

and with the serving size. The number of *L. monocytogenes* in a contaminated serving is the “dose” that an individual is exposed to, one of the inputs to the dose-response function. The complementary portion of the full exposure assessment output result is the prevalence of *L. monocytogenes* contaminated servings.

The number of *L. monocytogenes* in a contaminated serving at random varies among the servings that individuals in different populations (country × subpopulation) eat, since

- The number of *L. monocytogenes* in a contaminated cheese varies among contaminated cheeses
 - initial levels of contamination vary among contaminated cheeses;
 - lag times, growth rates and maximum densities vary among contaminated cheeses; and,
 - storage time and temperature from contamination to consumption vary among contaminated cheeses;
- The distribution of *L. monocytogenes* in a contaminated cheese is different from subpopulation to subpopulation due to different home storage conditions (section 7.4.2.); and,
- Serving sizes are different for individuals from Canada and from the U.S. and for individuals from different subpopulations in Canada (section 7.4.1.).

Table 38 shows summary statistics for the variability distribution of the number of *L. monocytogenes* per contaminated soft-ripened cheese serving. The median, mean, standard deviation and several percentiles capture common measures of central tendency, dispersion and distribution shape.

There is very high variability in the number of *L. monocytogenes* at the time of consumption amongst contaminated servings:

- 90% of contaminated servings have less than approximately 5,000 cfu/serving (Canadian, Elderly population);
- few servings are heavily contaminated, for example, at levels that reach the maximum population density of *L. monocytogenes*.

The minimum number of *L. monocytogenes* in a contaminated serving is logically 1. The median is 16-21 cfu per serving, depending on the country and the subpopulation. The mean number of *L. monocytogenes* in a contaminated serving lies between 736,000 ($5.9 \log_{10}$) and 2,642,000 ($6.4 \log_{10}$) cfu/serving, depending on the country and the subpopulation, at a point near the distributions' 97.5th percentiles. About 1%-5% of contaminated servings, varying with population, contain levels that exceed $5 \log_{10}$ *L. monocytogenes*. Distributions as skewed as these are common for *L. monocytogenes* risk assessments (FAO/WHO 2004).

The *L. monocytogenes* in contaminated servings distributions are different among populations (country \times subpopulation), not at low percentiles but at high percentiles, since serving size distributions; and home storage conditions' time and temperature –and so, growth at home— vary among populations. The distributions synthesized for the Immunocompromised and General populations use identical components –*L. monocytogenes* environmental contamination; time and temperature during cheese aging, transport, retail and home; serving sizes—and so, are identical.

The number of *L. monocytogenes* that contaminate a contaminated serving varies among the servings within the same population, reflecting how the initial *L. monocytogenes* environmental contamination levels; the growth conditions' storage time and temperature; and the *L. monocytogenes* growth lag time and *L. monocytogenes* growth rates vary among contaminated cheeses and how serving sizes vary among individuals in the same population.

Comparing the *L. monocytogenes* per contaminated cheese distribution at the time of contamination (≤ 31 bacteria, cf. Table 20, p. 69) and *L. monocytogenes* per serving distribution at the time of consumption (Table 38) points to the frequency of and amount of bacterial growth that follows contamination as key factors leading to the number of bacteria in a contaminated cheese at consumption, and thence to the risk (next section). When no growth occurs, the *L. monocytogenes* consumed in a contaminated serving remains small; only when growth occurs, and then, only when there is considerable growth, might the *L. monocytogenes* consumed in a contaminated serving be large.

Table 39 shows the summary statistics from the distribution of *L. monocytogenes* concentration (cfu/g) in contaminated cheeses at several points along the process pathway, confirming that bacterial growth, and particularly the growth during home storage, is a major influence on the distribution mean. Table 39's results for the amount of change in the *L. monocytogenes* concentration distribution from *Initial contamination* to *After Retail* are common to all population groups. The amount of change from *After Retail* to *After Home Storage* in Table 39 is particular to the Canadian, Elderly population's storage and consumption characteristics. This population-country is used as an example, but is indicative, also, of the magnitude of changes in *L. monocytogenes* concentrations in other population groups in those same process pathway steps.

Table 38: Number of *L. monocytogenes* cells per contaminated serving pasteurized-milk soft-ripened cheeses, no uncertainty considered.

Summary statistics from distributions describe variability among contaminated servings.

Canada	Elderly	Pregnant	IC*	General	U.S.	Elderly	Pregnant	IC	General
Median	17	21	16	16	Median	18	21	16	16
Mean	1,061,159	2,642,105	886,087	886,087	Mean	1,043,170	2,584,927	736,435	736,435
Std. dev	16,170,61	25,442,612	15,322,62	15,322,62	Std. dev	17,375,82	28,545,263	14,442,70	14,442,70
	5		2	2		6		0	0
1%ile	1	1	1	1	1%ile	1	1	1	1
2.5%ile	1	1	1	1	2.5%ile	1	1	1	1
5%ile	1	1	1	1	5%ile	1	1	1	1
10%ile	1	1	1	1	10%ile	1	1	1	1
25%ile	3	3	3	3	25%ile	3	3	3	3
50%ile	17	21	16	16	50%ile	18	21	16	16
75%ile	254	390	204	204	75%ile	252	357	197	197
90%ile	5,135	14,773	3,252	3,252	90%ile	4,812	10,379	3,027	3,027
95%ile	56,025	200,000	26,679	26,679	95%ile	49,358	124,014	24,187	24,187
97.5%ile	286,470	3,162,278	157,764	157,764	97.5%ile	241,020	2,354,498	135,692	135,692
99%ile	6,324,555	100,000,000	2,900,146	2,900,146	99%ile	3,850,756	100,000,000	1,949,514	1,949,514

* IC: Immunocompromised.

Table 39: *L. monocytogenes* cells per g at process pathway steps, for contaminated cheeses made from pasteurized-milk, no uncertainty considered.

Summary statistics from distributions describe variability among contaminated cheeses (*Initial contamination to After retail*) and among contaminated servings, Canada, Elderly population (*After Home Storage*).

Summary Statistics	Contaminated cheese Initial Contamination	Contaminated cheese After Aging	Contaminated cheese After Transport and Marketing	Contaminated cheese After Retail	Contaminated serving After Home Storage
Median	0.053	0.088	0.106	0.128	4.49
Mean	0.058	0.869	11.750	170.487	373,936.45
Std. dev.	0.042	27.012	837.505	18,262.269	6,078,826.56
1%ile	0.004	0.004	0.004	0.004	0.04
2.5%ile	0.004	0.004	0.004	0.004	0.07
5%ile	0.004	0.004	0.007	0.009	0.12
10%ile	0.004	0.010	0.013	0.013	0.25
25%ile	0.018	0.034	0.040	0.049	0.89
50%ile	0.053	0.088	0.106	0.128	4.49
75%ile	0.093	0.218	0.485	1.013	72.53
90%ile	0.119	0.959	2.492	6.813	1,525.79
95%ile	0.128	2.126	6.596	24.377	14,450.68
97.5%ile	0.133	4.193	16.225	77.817	94,720.30
99%ile	0.137	9.960	48.852	371.337	1,926,238.46

Prevalence of Contaminated Servings

The contaminated serving prevalence describes how often consumers are exposed to a contaminated pasteurized-milk cheese serving in this baseline application. The prevalence depends on the prevalence of contaminated cheeses and on how often a serving from a *L. monocytogenes* contaminated cheese is contaminated. This characteristic completes the description of the distribution of the number of *L. monocytogenes* in a soft-ripened cheese serving that began with Table 38.

Table 40 reports the probability that a serving at random is contaminated; its complement is the fraction of servings with exactly 0 *L. monocytogenes*. Under the baseline model illustrated here, with full pasteurization, no farm milk source *L. monocytogenes* contaminate cheeses. Contaminated cheeses contain only *L. monocytogenes* from in-plant environment sources.

In that case, all servings from cheeses that do not have any contamination have exactly 0 *L. monocytogenes*; but also, some servings with exactly 0 *L. monocytogenes* come from some cheeses that do have some environmental contamination. Contaminated cheeses yield servings with 0 *L. monocytogenes*

- more frequently from cheeses with relatively low *L. monocytogenes* load at consumption; such cheeses are ones with a relatively small amount of contamination at source and in which no or very little growth occurs;
- less frequently from cheeses with high *L. monocytogenes* contamination than from cheeses with relatively low *L. monocytogenes* contamination.

The prevalence of *L. monocytogenes* contaminated cheeses varies, reference the findings in Gombas *et al.* (2003), specifically among different geographical areas, or, more generally or as an extrapolation, among the different conditions that occur among the cheeses observed at retail within those geographical areas. In this model, cheese prevalence does not vary among populations (country \times subpopulation) within a geographic area, by assumption. However, growth to different *L. monocytogenes* levels in a contaminated cheese (different growth conditions during home storage) and different serving size distributions lead to differences in sampling distributions among populations for the *L. monocytogenes* in a contaminated serving,

- between countries, for which individuals' serving size distributions are different;
- among subpopulations in Canada, for which individuals' serving size distributions are different;
- among subpopulations in either country; although the same storage time and temperature distributions lead to the same amounts of growth from the same initial contamination levels to the same levels at the beginning of home storage, different home storage time and home temperature distributions lead to different amounts of growth from the beginning of home storage to the time of consumption; and,
- among individuals in the same subpopulation; that home storage times to consumption vary and that home storage temperatures vary among cheeses (individuals' cheeses) leads to varying amounts of growth from the beginning of home storage to the time of consumption.

The mean contaminated servings prevalence in the pasteurized-milk cheese baseline is about 6-7 per 1,000 servings in Canada and in the U.S. (Table 40). By construction in this baseline model, it is almost identical in Canada and in the U.S., and comparable to what was observed by Gombas *et al.* (2003). Indeed, the same back-calculation from Gombas *et al.* (2003) data was

used for both countries. From contamination at the end of cheese processing to consumption, the only basic process that impacts this prevalence is a partitioning process, from the cheese to the serving. No bacterial inactivation process and no removal process apply in this part of the pathway model in this baseline representation.

Contaminated serving prevalence varies over approximately 2 orders of magnitude from its distribution's 1% point to its distribution's 99% point, for example, from approximately 0.02% (2 per 10,000 servings) to approximately 2.7% (2.7 per 100 servings) (Table 40, Canada, Elderly population) in these results. Contaminated serving prevalence varies more among the servings within the same population than the serving prevalence distribution varies among different populations, between countries or among subpopulations within country (Table 40, between columns, same summary statistic (row)).

Note that this risk output will mathematically have an important impact on the final predicted risk.

Table 40: Prevalence of contaminated servings of cheeses made from pasteurized-milk, no uncertainty considered.

Summary statistics from distributions describe variability among contaminated servings prevalence.

Canada	Elderly	Pregnant	IC*	General	US	Elderly	Pregnant	IC	General
Median	0.47%	0.47%	0.49%	0.49%	Median	0.49%	0.49%	0.49%	0.49%
Mean	0.64%	0.63%	0.65%	0.65%	Mean	0.66%	0.66%	0.66%	0.66%
Std. dev.	0.58%	0.58%	0.58%	0.58%	Std. dev.	0.59%	0.59%	0.59%	0.59%
1%ile	0.02%	0.02%	0.02%	0.02%	1%ile	0.02%	0.02%	0.02%	0.02%
2.5%ile	0.04%	0.04%	0.04%	0.04%	2.5%ile	0.04%	0.04%	0.04%	0.04%
5%ile	0.06%	0.06%	0.06%	0.06%	5%ile	0.06%	0.06%	0.06%	0.06%
10%ile	0.10%	0.10%	0.10%	0.10%	10%ile	0.10%	0.10%	0.10%	0.10%
25%ile	0.22%	0.21%	0.23%	0.23%	25%ile	0.23%	0.23%	0.23%	0.23%
50%ile	0.47%	0.47%	0.49%	0.49%	50%ile	0.49%	0.49%	0.49%	0.49%
75%ile	0.89%	0.88%	0.91%	0.91%	75%ile	0.92%	0.92%	0.92%	0.92%
90%ile	1.41%	1.39%	1.43%	1.43%	90%ile	1.45%	1.45%	1.45%	1.45%
95%ile	1.80%	1.77%	1.81%	1.81%	95%ile	1.84%	1.84%	1.84%	1.84%
97.5%ile	2.19%	2.16%	2.20%	2.20%	97.5%ile	2.22%	2.22%	2.22%	2.22%
99%ile	2.66%	2.65%	2.69%	2.69%	99%ile	2.71%	2.71%	2.71%	2.71%

* IC: Immunocompromised.

Risk per Contaminated Serving

The distribution for the risk per contaminated serving, which expresses the probability of invasive listeriosis from eating a *L. monocytogenes* contaminated soft-ripened cheese serving, is

synthesized by applying the FAO/WHO (2004) dose-response function (section 5) to the *L. monocytogenes* dose in a contaminated serving (Table 38). This is a direct mapping of the number of *L. monocytogenes* in a contaminated serving to the probability of illness from consuming that number of *L. monocytogenes*, via the dose-response function. The probability of invasive listeriosis from a serving with 0 *L. monocytogenes* is, logically, identically 0.

Differences in the risk per contaminated serving (Table 41) among populations (country × subpopulation) accrue

- Between countries, from differences in distributions of the number of *L. monocytogenes* in a contaminated serving (Table 38);
- Within country, between susceptible subpopulations (Elderly, Pregnant, Immunocompromised) and non-susceptible (General) from differences in the probability of invasive listeriosis from consuming the same number of *L. monocytogenes* (dose-response model *r*-parameter) and from differences in distributions of the number of *L. monocytogenes* in a contaminated serving (any column within Table 38); and,
- Within subpopulations, from varying number of *L. monocytogenes* in a contaminated serving.

The mean risk per contaminated serving, among the contaminated servings eaten by individuals in the same population varies as

- 1.1×10^{-6} , 2.8×10^{-6} , 9.4×10^{-7} among the susceptible populations (Elderly, Pregnant, Immunocompromised) in Canada and 2.1×10^{-8} in the General population in Canada; and,
- 1.1×10^{-6} , 2.7×10^{-6} , 7.8×10^{-7} among the susceptible populations (Elderly, Pregnant, Immunocompromised) in the U.S. and 1.7×10^{-8} in the General population in the U.S.

The risk per contaminated serving varies among contaminated servings consumed within the same subpopulation by about $6.3 \log_{10}$ to $8.0 \log_{10}$ from its distribution's 1% point to its distribution's 99% point. The range is wider for the Pregnant women subpopulation and narrower for the other subpopulations. The median risk per contaminated serving is relatively low; from Table 38's results, the median risk is linked to exposure to 16 to 21 *L. monocytogenes*

cells. All populations' risk per contaminated serving distributions are highly skewed, with a median risk approximately 5 log₁₀ lower than the mean risk. This reflects the highly skewed distribution of the number of *L. monocytogenes* per contaminated serving. From this result, and recalling that the expected number of cases is proportional to the mean risk per serving, one can conclude that the number of cases is linked to the very few highest exposures.

The higher risk per contaminated serving for the Elderly, Pregnant women and Immunocompromised populations compared to the General population is expected; at the same *L. monocytogenes* dose it is due entirely to differences in the dose-response model for those populations. Indeed, the FAO/WHO dose response model is almost linear for the levels of exposure to *L. monocytogenes*, with a slope equal to 2.4×10^{-14} for the General population and 1.1×10^{-12} for the other subpopulations, that is, a -1.7 log₁₀ offset. Differences in the distribution of *L. monocytogenes* in contaminated servings among subpopulations (Table 38) have much less influence on Table 41's differences in the risk per contaminated serving among subpopulations. Among the contaminated servings eaten by individuals in the same subpopulation, higher risk per contaminated serving is always associated with higher numbers of *L. monocytogenes* in a contaminated serving; lower risk per contaminated serving is always associated with lower numbers of *L. monocytogenes* in a contaminated serving.

Table 41: Risk of invasive listeriosis per contaminated serving, of cheeses made from pasteurized-milk, no uncertainty considered.

Summary statistics from distributions describe variability among the risk per contaminated serving.

Canada	Elderly	Pregnant	IC*	General	US	Elderly	Pregnant	IC	General
Median	1.80×10^{-11}	2.23×10^{-11}	1.70×10^{-11}	3.79×10^{-13}	Median	1.91×10^{-11}	2.23×10^{-11}	1.70×10^{-11}	3.79×10^{-13}
Mean	1.12×10^{-06}	2.80×10^{-06}	9.39×10^{-07}	2.10×10^{-08}	Mean	1.11×10^{-06}	2.74×10^{-06}	7.81×10^{-07}	1.75×10^{-08}
Std. dev.	1.71×10^{-05}	2.70×10^{-05}	1.62×10^{-05}	3.63×10^{-07}	Std.dev.	1.84×10^{-05}	3.02×10^{-05}	1.53×10^{-05}	3.42×10^{-07}
1%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	1%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
2.5%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	2.5%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
5%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	5%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
10%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	10%ile	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
25%ile	3.18×10^{-12}	3.18×10^{-12}	3.18×10^{-12}	7.11×10^{-14}	25%ile	3.18×10^{-12}	3.18×10^{-12}	3.18×10^{-12}	7.11×10^{-14}
50%ile	1.80×10^{-11}	2.23×10^{-11}	1.70×10^{-11}	3.79×10^{-13}	50%ile	1.91×10^{-11}	2.23×10^{-11}	1.70×10^{-11}	3.79×10^{-13}
75%ile	2.69×10^{-10}	4.14×10^{-10}	2.16×10^{-10}	4.83×10^{-12}	75%ile	2.67×10^{-10}	3.78×10^{-10}	2.09×10^{-10}	4.67×10^{-12}
90%ile	5.44×10^{-09}	1.57×10^{-08}	3.45×10^{-09}	7.71×10^{-11}	90%ile	5.10×10^{-09}	1.10×10^{-08}	3.21×10^{-09}	7.17×10^{-11}
95%ile	5.94×10^{-08}	2.12×10^{-07}	2.83×10^{-08}	6.32×10^{-10}	95%ile	5.23×10^{-08}	1.31×10^{-07}	2.56×10^{-08}	5.73×10^{-10}
97.5%ile	3.04×10^{-07}	3.35×10^{-06}	1.67×10^{-07}	3.74×10^{-09}	97.5%ile	2.55×10^{-07}	2.50×10^{-06}	1.44×10^{-07}	3.22×10^{-09}
99%ile	6.70×10^{-06}	1.06×10^{-04}	3.07×10^{-06}	6.87×10^{-08}	99%ile	4.08×10^{-06}	1.06×10^{-04}	2.07×10^{-06}	4.62×10^{-08}

* IC: Immunocompromised.

Risk per Serving at Random

The risk per serving at random combines the previous estimate (risk per contaminated serving) and the prevalence of contaminated servings, at the mean of the contaminated servings prevalence distribution. These results apply to a serving at random from among all servings consumed by individuals in the population, subpopulation by subpopulation.

The mean risk per serving (Table 42) varies as

- 7.2×10^{-9} , 1.8×10^{-8} , 6.1×10^{-9} among the susceptible populations (Elderly, Pregnant women, Immunocompromised, respectively) in Canada and 1.4×10^{-10} in the non-susceptible population (General) in Canada; and,
- 7.3×10^{-9} , 1.8×10^{-8} , 5.2×10^{-9} among the susceptible populations (Elderly, Pregnant women, Immunocompromised, respectively) in the U.S. and 1.2×10^{-10} in the non-susceptible population (General) in the U.S.

These mean values correspond to one case of invasive listeriosis per

- 138 Million servings in the Elderly population, 56 Million servings in the Pregnant women population, 163 Million servings in the Immunocompromised population and 7,290 Million servings in the General population, in Canada; and,
- 136 Million servings for the Elderly population, 55 Million servings for the Pregnant women population, 193 Million servings for the Immunocompromised population and 8,644 Million for the General population, in the U.S.

The risk per serving at random varies among servings consumed within the same subpopulation by about $6 \log_{10}$ to $8 \log_{10}$ from its distribution's 1% point to its distribution's 99% point. The range is wider for the Pregnant women subpopulation and narrower for the other subpopulations. The median risk per serving at random is relatively low. All populations' risk per serving distribution is highly skewed, with median risk approximately $5 \log_{10}$ lower than the mean risk. This reflects the highly skewed risk per contaminated serving distributions in Table 41.

The mean per serving risk is >150 times higher for Pregnant women than for the U.S. General population, as a reference, and the mean per serving risk for the Elderly and Immunocompromised populations is approximately 50 times higher than for the U.S. General population (Table 43).

Table 42: Risk of invasive listeriosis per serving pasteurized-milk soft-ripened cheeses, no uncertainty considered.

Summary statistics from distributions describe variability among the risk per serving at random.

Canada	Elderly	Pregnant	IC*	General	US	Elderly	Pregnant	IC	General
Median	1.16×10^{-13}	1.41×10^{-13}	1.11×10^{-13}	2.48×10^{-15}	Median	1.26×10^{-13}	1.48×10^{-13}	1.12×10^{-13}	2.51×10^{-15}
Mean	7.22×10^{-09}	1.77×10^{-08}	6.13×10^{-09}	1.37×10^{-10}	Mean	7.33×10^{-09}	1.82×10^{-08}	5.17×10^{-09}	1.16×10^{-10}
Std. dev.	1.10×10^{-07}	1.71×10^{-07}	1.06×10^{-07}	2.37×10^{-09}	Std. dev.	1.22×10^{-07}	2.00×10^{-07}	1.01×10^{-07}	2.27×10^{-09}
1%ile	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	1%ile	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
2.5%ile	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	2.5%ile	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
5%ile	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	5%ile	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
10%ile	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	10%ile	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
25%ile	2.04×10^{-14}	2.01×10^{-14}	2.08×10^{-14}	4.64×10^{-16}	25%ile	2.11×10^{-14}	2.11×10^{-14}	2.11×10^{-14}	4.71×10^{-16}
50%ile	1.16×10^{-13}	1.41×10^{-13}	1.11×10^{-13}	2.48×10^{-15}	50%ile	1.26×10^{-13}	1.48×10^{-13}	1.12×10^{-13}	2.51×10^{-15}
75%ile	1.73×10^{-12}	2.62×10^{-12}	1.41×10^{-12}	3.16×10^{-14}	75%ile	1.77×10^{-12}	2.51×10^{-12}	1.38×10^{-12}	3.09×10^{-14}
90%ile	3.50×10^{-11}	9.92×10^{-11}	2.25×10^{-11}	5.03×10^{-13}	90%ile	3.38×10^{-11}	7.29×10^{-11}	2.13×10^{-11}	4.75×10^{-13}
95%ile	3.81×10^{-10}	1.34×10^{-09}	1.85×10^{-10}	4.13×10^{-12}	95%ile	3.47×10^{-10}	8.71×10^{-10}	1.70×10^{-10}	3.80×10^{-12}
97.5%ile	1.95×10^{-09}	2.12×10^{-08}	1.09×10^{-09}	2.44×10^{-11}	97.5%ile	1.69×10^{-09}	1.65×10^{-08}	9.53×10^{-10}	2.13×10^{-11}
99%ile	4.31×10^{-08}	6.72×10^{-07}	2.01×10^{-08}	4.49×10^{-10}	99%ile	2.71×10^{-08}	7.03×10^{-07}	1.37×10^{-08}	3.06×10^{-10}

* IC: Immunocompromised.

Table 43: Relative mean risk of invasive listeriosis per serving of cheese made from pasteurized milk, at random, no uncertainty considered.

Population Group	Elderly	Pregnant women	Immunocompromised	General
Canada	62.4	153.4	53.0	1.186
United States	63.3	157	44.7	1.00 (reference)

9.1.3. Uncertainty Considered

Section 9.1.3. 's results account for both the parameters' description of variability and also for the uncertainty that we associate with those variability descriptions, insofar as that uncertainty can be captured from the existing literature. To do so, we use the uncertainty distributions of parameters' and model inputs' descriptions of variability, as detailed in the methods sections. Uncertainty in those components is propagated through to the risk outputs using a second order Monte-Carlo simulation. The pasteurized-milk cheese baseline model that applies to section 9.1.2. 's results also applies to this section's results.

Risk per Serving

Table 44 and the Table 45 report results for the risk of invasive listeriosis per serving at random from soft-ripened cheeses made from pasteurized milk in Canada and in the U.S., respectively. Summary statistics (median, mean, standard deviation and some percentiles, in row) describe how the risk per serving varies among servings within subpopulations. As well, the tables provide point estimates (median of the uncertainty distribution) and their 95% credible interval (CI95, 2.5th and 97.5th percentiles of the uncertainty distribution), as a measure of uncertainty about each summary statistic. As an example, for the Canadian Elderly population,

- the mean risk of invasive listeriosis per serving at random is 2.9×10^{-8} (median value over uncertainty distribution for the mean risk of invasive listeriosis) with a credible interval [1.0×10^{-9} , 4.0×10^{-7}] (2.5th and 97.5th percentiles of the uncertainty distribution for the mean risk of invasive listeriosis); those interval endpoints are 28 times less and 14 times more than the median value of 2.9×10^{-8} ;
- the median risk is 1.8×10^{-13} [1.2×10^{-14} , 2.2×10^{-12}]; and,
- the 99th percentile is 7.0×10^{-7} [7.8×10^{-9} , 1.0×10^{-5}].

The relationship between the 95% credible interval endpoints and the median (uncertainty) for the risk per serving summary statistics is approximately the same for the other subpopulations, as well. The uncertainty distribution for each summary statistic (mean, median, percentiles) in Table 44 and the Table 45 is positively skewed. (Recall Figure 19, page 104). Uncertainty distributions are more highly skewed for the percentiles in the upper tail of the variability distribution than for the percentiles in the lower tail of the variability distribution and we see even more highly skewed uncertainty distributions for the median and quartiles of the variability distribution. Discussions about the size of the risk output and comparisons of the distribution summary statistics among subpopulations apply to these results, as well.

Table 44: Risk of invasive listeriosis per serving at random, of cheese made from pasteurized milk, among subpopulations in Canada.

Results of second-order Monte Carlo simulation describe uncertainty about summary statistics from distributions that describe variability among the risk per serving at random.

Summary statistics	Elderly Median	Elderly CI95 LB	Elderly CI95 UB	Pregnant Median	Pregnant LB CI95	Pregnant UB CI95	IC* Median	IC* LB CI95	IC* UB CI95	General Median	General LB CI95	General UB CI95
Median	1.78×10^{-13}	1.17×10^{-14}	2.25×10^{-12}	2.04×10^{-13}	1.42×10^{-14}	3.15×10^{-12}	1.59×10^{-13}	9.92×10^{-15}	1.94×10^{-12}	4.55×10^{-15}	6.25×10^{-16}	4.29×10^{-14}
Mean	2.95×10^{-08}	1.04×10^{-09}	4.04×10^{-07}	4.41×10^{-08}	1.23×10^{-09}	7.13×10^{-07}	2.06×10^{-08}	7.95×10^{-10}	3.75×10^{-07}	4.98×10^{-10}	3.79×10^{-11}	1.02×10^{-08}
Std. Dev.	2.92×10^{-07}	1.22×10^{-08}	4.14×10^{-06}	3.35×10^{-07}	9.73×10^{-09}	6.09×10^{-06}	2.58×10^{-07}	9.91×10^{-09}	5.06×10^{-06}	6.50×10^{-09}	5.78×10^{-10}	1.16×10^{-07}
1%ile	1.12×10^{-14}	5.45×10^{-16}	1.07×10^{-13}	1.11×10^{-14}	5.50×10^{-16}	1.02×10^{-13}	1.14×10^{-14}	5.44×10^{-16}	1.06×10^{-13}	2.98×10^{-16}	3.56×10^{-17}	2.86×10^{-15}
2.5%ile	1.12×10^{-14}	5.45×10^{-16}	1.07×10^{-13}	1.11×10^{-14}	5.50×10^{-16}	1.02×10^{-13}	1.14×10^{-14}	5.44×10^{-16}	1.06×10^{-13}	2.98×10^{-16}	3.56×10^{-17}	2.86×10^{-15}
5%ile	1.12×10^{-14}	5.45×10^{-16}	1.07×10^{-13}	1.11×10^{-14}	5.50×10^{-16}	1.02×10^{-13}	1.14×10^{-14}	5.44×10^{-16}	1.06×10^{-13}	2.98×10^{-16}	3.56×10^{-17}	2.86×10^{-15}
10%ile	1.35×10^{-14}	7.76×10^{-16}	1.71×10^{-13}	1.34×10^{-14}	7.65×10^{-16}	1.75×10^{-13}	1.38×10^{-14}	7.72×10^{-16}	1.76×10^{-13}	3.67×10^{-16}	4.41×10^{-17}	4.22×10^{-15}
25%ile	3.55×10^{-14}	2.17×10^{-15}	3.54×10^{-13}	3.31×10^{-14}	2.29×10^{-15}	3.57×10^{-13}	3.55×10^{-14}	2.16×10^{-15}	3.83×10^{-13}	9.26×10^{-16}	1.32×10^{-16}	8.79×10^{-15}
50%ile	1.78×10^{-13}	1.17×10^{-14}	2.25×10^{-12}	2.04×10^{-13}	1.42×10^{-14}	3.15×10^{-12}	1.59×10^{-13}	9.92×10^{-15}	1.94×10^{-12}	4.55×10^{-15}	6.25×10^{-16}	4.29×10^{-14}
75%ile	3.16×10^{-12}	1.85×10^{-13}	4.23×10^{-11}	5.05×10^{-12}	2.99×10^{-13}	1.96×10^{-10}	2.19×10^{-12}	1.43×10^{-13}	2.88×10^{-11}	6.33×10^{-14}	7.10×10^{-15}	6.50×10^{-13}
90%ile	1.34×10^{-10}	4.23×10^{-12}	2.90×10^{-09}	3.99×10^{-10}	1.12×10^{-11}	2.10×10^{-08}	6.09×10^{-11}	3.26×10^{-12}	2.12×10^{-09}	1.50×10^{-12}	1.31×10^{-13}	3.57×10^{-11}
95%ile	1.43×10^{-09}	3.72×10^{-11}	2.76×10^{-08}	7.74×10^{-09}	1.37×10^{-10}	1.39×10^{-06}	5.84×10^{-10}	3.24×10^{-11}	2.38×10^{-08}	1.61×10^{-11}	1.33×10^{-12}	4.74×10^{-10}
97.5%ile	2.25×10^{-08}	2.51×10^{-10}	1.09×10^{-06}	2.68×10^{-07}	2.05×10^{-09}	9.71×10^{-06}	4.65×10^{-09}	1.92×10^{-10}	7.10×10^{-07}	1.27×10^{-10}	6.71×10^{-12}	8.27×10^{-09}
99%ile	6.99×10^{-07}	7.83×10^{-09}	1.04×10^{-05}	1.48×10^{-06}	3.86×10^{-08}	2.41×10^{-05}	2.27×10^{-07}	4.11×10^{-09}	7.36×10^{-06}	6.18×10^{-09}	1.19×10^{-10}	2.13×10^{-07}

*IC: Immunocompromised; LB: Lower bound of the CI95%, uncertainty dimension; UB: Upper bound

Table 45: Risk of invasive listeriosis per serving at random, for cheese made from pasteurized milk, among subpopulations in the U.S.

Results of second-order Monte Carlo simulation describe uncertainty about summary statistics from distributions that describe variability among the risk per serving at random.

Summary statistics	Elderly Median	Elderly CI95 LB	Elderly CI95 UB	Pregnant Median	Pregnant LB CI95	Pregnant UB CI95	IC* Median	IC* LB CI95	IC* UB CI95	General Median	General LB CI95	General UB CI95
Median	1.86×10^{-13}	1.29×10^{-14}	2.49×10^{-12}	2.15×10^{-13}	1.41×10^{-14}	2.71×10^{-12}	1.67×10^{-13}	1.06×10^{-14}	1.97×10^{-12}	4.78×10^{-15}	6.20×10^{-16}	4.27×10^{-14}
Mean	2.93×10^{-08}	7.52×10^{-10}	4.05×10^{-07}	5.08×10^{-08}	1.59×10^{-09}	7.62×10^{-07}	2.07×10^{-08}	7.35×10^{-10}	3.62×10^{-07}	5.39×10^{-10}	3.68×10^{-11}	1.08×10^{-08}
Std. Dev.	3.11×10^{-07}	1.06×10^{-08}	4.50×10^{-06}	4.39×10^{-07}	1.47×10^{-08}	7.61×10^{-06}	2.71×10^{-07}	9.64×10^{-09}	5.01×10^{-06}	7.11×10^{-09}	5.38×10^{-10}	1.17×10^{-07}
1%ile	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	3.03×10^{-16}	3.63×10^{-17}	2.80×10^{-15}
2.5%ile	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	3.03×10^{-16}	3.63×10^{-17}	2.80×10^{-15}
5%ile	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	1.16×10^{-14}	5.59×10^{-16}	1.08×10^{-13}	3.03×10^{-16}	3.63×10^{-17}	2.80×10^{-15}
10%ile	1.51×10^{-14}	7.82×10^{-16}	1.74×10^{-13}	1.56×10^{-14}	7.82×10^{-16}	1.91×10^{-13}	1.36×10^{-14}	7.82×10^{-16}	1.74×10^{-13}	3.86×10^{-16}	4.52×10^{-17}	3.47×10^{-15}
25%ile	3.84×10^{-14}	2.23×10^{-15}	4.43×10^{-13}	3.88×10^{-14}	2.35×10^{-15}	4.43×10^{-13}	3.63×10^{-14}	2.22×10^{-15}	3.81×10^{-13}	9.44×10^{-16}	1.21×10^{-16}	8.94×10^{-15}
50%ile	1.86×10^{-13}	1.29×10^{-14}	2.49×10^{-12}	2.15×10^{-13}	1.41×10^{-14}	2.71×10^{-12}	1.67×10^{-13}	1.06×10^{-14}	1.97×10^{-12}	4.78×10^{-15}	6.20×10^{-16}	4.27×10^{-14}
75%ile	3.15×10^{-12}	1.87×10^{-13}	4.21×10^{-11}	4.08×10^{-12}	2.72×10^{-13}	8.40×10^{-11}	2.17×10^{-12}	1.41×10^{-13}	2.87×10^{-11}	6.21×10^{-14}	7.27×10^{-15}	6.28×10^{-13}
90%ile	1.27×10^{-10}	4.17×10^{-12}	2.42×10^{-09}	2.43×10^{-10}	8.65×10^{-12}	8.38×10^{-09}	5.74×10^{-11}	3.08×10^{-12}	1.92×10^{-09}	1.36×10^{-12}	1.28×10^{-13}	3.36×10^{-11}
95%ile	1.44×10^{-09}	3.43×10^{-11}	2.69×10^{-08}	4.58×10^{-09}	1.18×10^{-10}	2.40×10^{-07}	4.96×10^{-10}	2.78×10^{-11}	2.19×10^{-08}	1.50×10^{-11}	1.16×10^{-12}	3.92×10^{-10}
97.5%ile	1.78×10^{-08}	2.24×10^{-10}	7.17×10^{-07}	1.41×10^{-07}	1.42×10^{-09}	7.70×10^{-06}	4.20×10^{-09}	1.83×10^{-10}	6.44×10^{-07}	1.11×10^{-10}	5.76×10^{-12}	9.35×10^{-09}
99%ile	7.07×10^{-07}	7.51×10^{-09}	1.13×10^{-05}	1.52×10^{-06}	3.88×10^{-08}	2.35×10^{-05}	1.90×10^{-07}	3.59×10^{-09}	7.58×10^{-06}	4.55×10^{-09}	1.18×10^{-10}	2.21×10^{-07}

*IC: Immunocompromised; LB: Lower bound of the CI95%, uncertainty dimension; UB: Upper bound

Relative Influence of Variability and Uncertainty on the Risk per Serving Output

Figure 20 marks the points A-D used to calculate Ozkaynak *et al.*'s (2009) Variability Ratio (B÷A), Uncertainty Ratio (C÷A) and Overall Uncertainty Ratio (D÷A) on the distribution function for the risk per serving at random for the Canadian Elderly population.

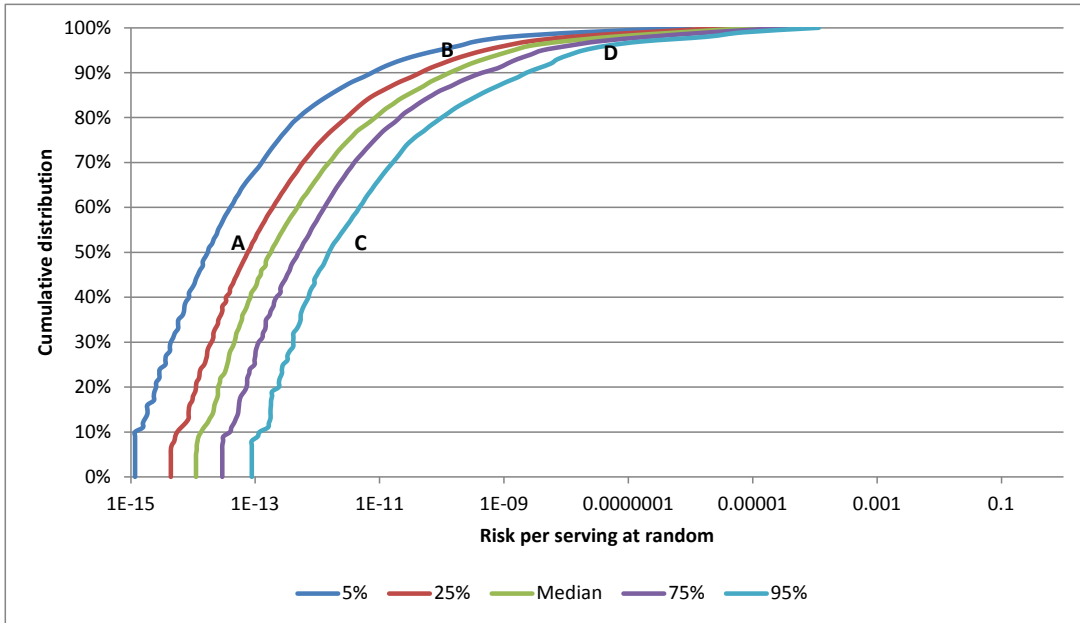


Figure 21: Distribution for risk of invasive listeriosis per soft-ripened cheese serving at random, Elderly population, Canada.

The Variability Ratio, here 8,005, measures the distance between the median and the 95th percentile. The Uncertainty Ratio, here 9, measures the distance between the median and the upper limit of its 90% credible interval. The Overall Uncertainty Ratio, here 107,933, measures the distance between the median and the upper limit of the 90% credible interval of the 95th percentile. From these statistics, one may conclude that the variability largely overwhelms the (considered) uncertainty in this model.

9.2. Sensitivity Analysis: Changing one Parameter at a Time

Changing one parameter at a time acts as a form of a sensitivity analysis to evaluate the model behavior. The parameters that are changed and the specific changes to their values do not reflect any risk mitigation (see, for that purpose, section 10).

We evaluate $dMean(Parameter)$, the change in the mean risk output with reference to a change in a particular model input *Parameter* from a baseline model setting, and $dMedian(Parameter)$, the change in the median risk output with reference to a change in a particular model input *Parameter* from a baseline model setting, as the ratios:

$$dMean = \frac{[\text{Mean evaluated using an alternative model}]}{[\text{Mean evaluated using the baseline model}]},$$

$$dMedian = \frac{[\text{Median evaluated using an alternative model}]}{[\text{Median evaluated using the baseline model}]}$$

In order to better understand the meaning of these measures of change in the context of such highly skewed risk output distributions, we note that a large value of $dMean(Parameter)$ indicates that the *Parameter* has a large impact, particularly, on the highest percentiles of the risk distribution, and so, on the mean of the risk output distribution. $dMean(Parameter)$ values greater than 1 point to a *Parameter* whose change effects an increase in the mean value, relative to the mean value calculated at a baseline value of the *Parameter*. $dMean(Parameter)$ values less than 1 point to a *Parameter* whose change effects a decrease in the mean value, relative to the mean value calculated at a baseline value of the *Parameter*. A large $dMedian(Parameter)$ value indicates that the *Parameter* has a large impact on the whole risk distribution, particularly effecting a shift of location for the risk distribution.

In the following sections, we report results for these measures of change for the Risk per serving or the Risk per contaminated serving risk output, for the Canadian Elderly population relative to the pasteurized-cheese baseline model. Other outputs' or other subpopulations' results are also reported if the characteristics of the measures of change are different.

9.2.1. Environmental Contamination Prevalence and Levels

The baseline models use the environmental contamination distribution that section 6.4 derived from Gombas *et al.* (2003) data as the only source of *L. monocytogenes* that persists to consumption.

We evaluate the impact of varying the level of that contamination per cheese (from 1 cfu per contaminated cheese to 100,000 cfu per contaminated cheese) on the median and mean risk per contaminated serving. In the baseline model, environmental contamination varies from 1 to 31 *L. monocytogenes* per contaminated cheese (distribution, see Table 20, p. 69).

The median risk increases linearly with the level of environmental contamination: a 10-fold increase in environmental contamination levels increases the median risk per contaminated serving 10-fold. One *L. monocytogenes* cfu is ½ the median of the baseline model (Table 46).

The impact on the mean is smaller. For example, the mean risk per contaminated serving increases 1.4-fold when the environmental contamination increases 10-fold from 10,000 cfu/cheese to 100,000 cfu/cheese. Also, the *dMean* rate of change with increasing level of environmental contamination slows as the level of contamination increases (1-10, 10-100, ... in Table 46). This result suggests that other factors than high initial environmental contamination are needed to affect the mean risk per contaminated serving.

Table 46: Sensitivity of the risk per contaminated serving, Canadian Elderly population, to the level of environmental contamination, relative to the pasteurized-milk cheese (PMC) baseline.

Statistical summary	PMC Baseline	1 cfu/cheese	10 cfu/cheese	100 cfu/cheese	1,000 cfu/cheese	10,000 cfu/cheese	100,000 cfu/cheese
<i>dMedian</i>	1.00	0.47	1.0	7.3	74	740	7,141
<i>dMean</i>	1.00	0.51	0.9	2.6	4.4	6.5	8.8

9.2.2. Growth Characteristics

Section 6.1 captures *L. monocytogenes* growth in contaminated cheeses with the three-phase linear model. This section examines the sensitivity of risk outputs to changes to the exponential growth rate and maximum population density that parameterize the primary growth model, to the storage temperature that parameterizes the secondary growth model and to the storage time and temperature that parameterizes the amount of growth.

Exponential Growth Rate

We tested the influence of the Exponential Growth Rate (*EGR*) on the risk per serving by comparing pasteurized-milk cheese baseline model risk per contaminated serving to the risk per contaminated serving under changes to the *EGR* as

- no growth, ($EGR_{20} = 0 \log_{10}$ cfu per gram per day implies no growth at any temperature);
- lower than pasteurized-milk cheese baseline growth, dividing the baseline EGR_{20} by a factor of 2, when growth occurs;

- higher than pasteurized-milk cheese baseline growth, multiplying the baseline EGR_{20} by a factor of 2, when growth occurs.

When growth occurs as in the pasteurized-milk cheese baseline model, the mean risk per serving is >53,000 times larger than the mean risk per contaminated serving when no growth occurs (Table 47, *dMean* row). This result suggests that the risk is principally linked to the bacterial growth that occurs in stages along the process pathway.

Halving the EGR_{20} dramatically reduces the mean risk per contaminated serving, by a factor of approximately 8. On the other hand, doubling the EGR_{20} multiplies the mean risk by a factor of approximately 4. This reflects the model's representation of the non-linearity of this system, the system's asymptote at the maximum population density and interactions among EGR_{20} and other factors.

Halving the EGR_{20} has a small effect also on the median risk per contaminated serving. Doubling the EGR_{20} scales the median risk per contaminated serving to 9.5 times the pasteurized-milk cheese baseline model's median risk.

In the gamma concept predictive microbiology framework (Zwietering et al. 1996), environmental factors act independently ($0 \leq \gamma_i(x_i) \leq 1$) or with a positive synergy ($0 \leq \gamma_{\text{int}}(x_1, \dots, x_n) \leq 1$) on the EGR according to $EGR = EGR_{20} \left(\prod_i \gamma_i(x_i) \right) \gamma_{\text{int}}(x_1, \dots, x_n)$. So, to halve the EGR , modify any one or more factors such that $\gamma_{\text{int}}(x_1, \dots, x_n) \prod_i \gamma_i(x_i) = 1/2$; modifying one or more factors such that $\gamma_{\text{int}}(x_1, \dots, x_n) \prod_i \gamma_i(x_i) = 2$, doubles the EGR . The mean risk per contaminated serving changes with changing EGR_{20} , whatever method is used to effect the EGR changes.

Maximum Population Density

A 1 log₁₀ higher and a 1 log₁₀ lower maximum population density for *L. monocytogenes* in soft-ripened cheese has a large impact on the mean risk per contaminated serving (Table 47, *dMean*) and no impact on the median risk per contaminated serving (Table 47, *dMedian* = 1). Changing

the maximum population density affects only those situations where growth to high levels can occur. Those situations have a large impact on the mean risk but no impact on the median risk.

Table 47: Sensitivity of the risk per contaminated serving to growth characteristics relative to the pasteurized-milk cheese baseline.

Summary statistics	PMC Baseline	EGR_{20} 0	EGR_{20} $\frac{1}{2} \times \text{baseline}$	EGR_{20} $2 \times \text{baseline}$	Maximum population density - 1 log ₁₀	Maximum population density +1 log ₁₀
<i>dMedian</i>	1.00	0.35	0.47	9.5	1.0	1.0
<i>dMean</i>	1.00	1.9×10^{-5}	0.12	4.2	0.15	6.8

Temperature and Time of Storage

We tested the influence on the risk per contaminated serving of

- a general decrease of 1°C during transport and marketing, at retail and during storage in the home refrigerator, compared to the PMC baseline
- a general increase of 1°C during transport and marketing, at retail and during storage in the home refrigerator, compared to the PMC baseline;
- a maximum duration of home storage of 28 days (vs. 56 days in the pasteurized-milk cheese baseline).

The impact of changes to the home refrigerator temperature is the most important one: an increase of 1°C increases the mean risk per contaminated serving by a factor of 1.7 (Table 48, top). A 1°C storage temperature increase or decrease during transport and marketing storage or during retail storage increases or decreases the mean risk per contaminated serving by only a small amount.

Shortening the maximum duration of the home storage from 56 days to 28 days reduces the mean risk by a factor of approximately 2 for the Elderly population and the Pregnant women population and by a factor of 1.4 for the Immunocompromised population and the General population (Table 48, bottom). Storage times longer than 28 days happen more frequently among servings eaten by individuals in the Elderly population and in the Pregnant women population (>10%) than among servings eaten by individuals in the Immunocompromised population and the General population (<5%).

Table 48: Sensitivity of the risk per contaminated serving to the storage time and temperature relative to the pasteurized-milk cheese (PMC) baseline.

Temperature	PMC Baseline	-1°C compared to baseline	-1°C compared to baseline	-1°C compared to baseline	+1°C compared to baseline	+1°C compared to baseline	+1°C compared to baseline
		Transport & Marketing	Retail	Home	Transport & Marketing	Retail	Home
<i>dMedian</i>	1.0	0.88	0.88	0.82	1.2	1.2	1.3
<i>dMean</i>	0.94	0.90	0.88	0.53	1.0	1.0	1.7

Table : Sensitivity of the risk per contaminated serving to the storage duration relative to the pasteurized-milk cheese (PMC) baseline

Max. home storage duration	PMC Baseline	Max. home storage: (28 days vs. baseline, 56 days)	Max. home storage: (28 days vs. baseline, 56 days)	Max. home storage: (28 days vs. baseline, 56 days) IC	Max. home storage: (28 days vs. baseline, 56 days)
		Elderly	Pregnant women		General
<i>dMedian</i>	1.00	0.88	0.81	0.94	0.94
<i>dMean</i>	1.00	0.52	0.45	0.70	0.70

9.3. Sensitivity Analysis: Other Methods

Sensitivity analyses in this section follow common practices in microbiological risk assessments to identify which model components contribute more to or less to the risk outputs' variability and uncertainty.

9.3.1. Model Components' Variability

In our baseline case, with full pasteurization of the raw milk used for cheese-making, model parameters from the on farm module do not apply; they are uncorrelated with the risk per serving at random or any other risk output in this pasteurized-milk cheese baseline case. Contaminated cheese prevalence, well defined and reported in section 6.4, describes how contaminated cheese prevalence varies against the geographical area environmental contamination prevalence established. For the risk per serving at random risk output, we average over the serving prevalence distribution. As a result, serving prevalence and risk per serving at random are fully independent, uncorrelated, by construction.

These sensitivity analyses describe the relationship between the variability in inputs and the variability in risk outputs. Interpreted in context, the analyses inform about:

- those inputs to which small, medium or large changes evoke small, medium or large changes to the risk output (for model diagnostics);
- those parameters for which some control effects a desired amount of control over the risk distribution (for appropriate control points).

Spearman’s rank correlation provides one, commonly used global measure of the relationship between a model output and its model inputs. It performs well when that relationship is monotonic but less well in the presence of some curvilinear relationships, some thresholds and some asymptotes in the relationship. Its use is limited to considering only one parameter at a time. As a consequence, no interaction between parameters can be easily tested.

Table 49 uses the absolute value of the Spearman’s rank correlation coefficient between inputs (LH column) and the risk per serving at random, calculated within the Monte-Carlo simulation framework, to order the inputs from top to bottom in the table. No uncertainty is considered. Inputs with positive rank correlations are ones for which the risk per serving at random increases as the input increases. Inputs with negative rank correlations are ones for which the risk per serving at random decreases as the input increases.

The list of inputs includes both inputs that are externally specified (*Parent*) and ones that are derived from externally specified parameters by a specified functional relationship (*Child*). For example, storage times and temperatures are specified; EGR_{20} , T_{min} and K_{ξ} are specified; serving sizes are specified. On the other hand, EGR_T during aging, transport & marketing, at retail and in home refrigerator is derived –from the EGR_{20} , T_{min} and storage temperature; the number of *L. monocytogenes* in a contaminated serving is derived –from initial contamination, growth parameters, environmental parameters, serving size ... In Table 49, “specified” (or *Parent*) parameters are in bold font and left-aligned; “derived” (or *Child*) parameters, ones that are functions of *Parent* and other *Child* parameters, are in normal font and right-aligned in Table 49’s *Inputs* column.

The three “parent” parameters with the largest rank correlations in absolute value are ones that lead bacterial growth, *i.e.* the K_{ξ} parameter that relates the growth rate and the lag time, the exponential growth rate (EGR_{20}) and the minimal temperature of growth (T_{min} , negatively

correlated). The initial level of *L. monocytogenes* environmental contamination has similar but lesser influence on the risk per serving at random. Storage time and temperature parameters are less influential still. Home storage environmental conditions are more influential than other storage steps' environmental conditions.

Table 49: Spearman's rank correlations between various inputs and the risk per serving of soft-ripened cheese at random, made from pasteurized-milk, for the Elderly population, Canada.

Input		Estimate
Specified (Parent) parameters	Derived (Child) parameters	
	Number of <i>Lm</i> in contaminated servings	1.00
	Number of <i>Lm</i> in contaminated cheese after home storage	0.95
	Number of <i>Lm</i> in contaminated cheese after retail storage	0.83
	Number of <i>Lm</i> in contaminated cheese after transport and marketing storage	0.75
	Number of <i>Lm</i> in contaminated cheese after aging	0.64
Parameter K_{ξ} for <i>Lm</i> growth lag time		-0.54
	Number of <i>Lm</i> in contaminated cheese after ripening	0.50
	EGR_T during aging	0.48
	EGR_T at home storage	0.45
EGR_{20}		0.45
	EGR_T during transport and marketing	0.43
	EGR_T at retail storage	0.41
T_{min} in cheese		-0.36
Time when the environmental contamination occurs		-0.26
Number of <i>Lm</i>, Environmental contamination		0.21
	Number of grams of products without <i>Lm</i>	-0.20
Time of storage at home		0.20
Temperature of home refrigerator		0.15
Serving size		0.11
Temperature at retail		0.09
Time of aging		0.09
Time to 1st consumption		0.09
Temperature during transport and marketing		0.06
Storage time at retail		0.06
Storage time during transport and marketing		0.05
Temperature during aging		0.03
	Number of servings per package	0.00
Time of room storage at home		0.00
Temperature of room storage, at home		0.00

Notes: All growth parameters — EGR_{20} ; K_{ξ} , EGR_T at home, retail, transport & marketing, aging—refer to environmental source *L. monocytogenes* contaminating the cheese rind.

9.3.2. Model Components' Uncertainty

Table 50 shows the Spearman's rank correlations between the N_u values of the mean risk per serving at random and the N_u values of the 97.5th percentile risk per serving at random and some parameters for which we included uncertainty specifications.

Uncertainty in the dose response parameter r has a much higher impact on the uncertainty that we associate with the mean risk per serving at random than any other single parameter. The uncertainty of the 97th percentile is associated more highly with uncertainty about growth parameters, notably parameters that specify the EGR_{20} distribution.

Table 50: Spearman’s rank correlation between the mean or the 97.5th percentile of the risk per serving of soft-ripened cheese at random and some uncertain parameters.

Uncertain parameter	For Mean risk	For 97.5 th quantile of risk
Dose response r parameter	0.53	0.27
Ecosure (2007), home temperature, mean parameter	-0.26	-0.03
Ecosure (2007), retail temperature, variance parameter	-0.24	-0.15
Canada, consumption, logNormal, mean parameter	0.23	0.25
EGR_{20} , Exterior, ln sigma parameter	0.20	0.38
time to 1st consumption, alpha parameter	-0.17	0.04
Canada, consumption, logNormal, variance parameter	0.17	-0.01
Time to last consumption, between successive, theta parameter	0.15	0.13
EGR_{20} , Interior, ln lambda parameter	0.15	0.07
EGR_{20} , Interior, ln alpha parameter	-0.14	-0.05
$E[\ln Kx_i]$	0.13	0.00
Ecosure (2007), retail temperature, mean parameter	0.12	0.11
EGR_{20} , Interior, logit theta parameter	-0.12	-0.16
Temperature at home, mu parameter	-0.11	-0.15
Fraction cheeses stored in refrigerator	0.11	0.27
Time to 1 st consumption, lambda parameter	-0.09	-0.12
Ecosure (2007), home temperature, variance parameter	0.08	-0.07
Fraction cheeses stored open and eaten	0.08	0.00
$\text{Var}(T_{min})$	-0.07	-0.01
Temperature at home, sigma parameter	0.07	0.14
Max. density Lm in cheese	0.05	0.09
Time to last consumption, number occasions, lambda parameter	0.04	0.02
EGR_{20} , Exterior, ln alpha parameter	0.04	0.14
EGR_{20} , Interior, ln sigma parameter	-0.04	-0.01
EGR_{20} , Exterior, logit theta parameter	-0.04	0.00
EGR_{20} , Exterior, ln lambda parameter	-0.04	-0.13
$\text{Var}(\ln Kx_i)$	0.03	0.28
$E[T_{min}]$	-0.01	0.17
Fraction cheeses stored open	0.01	0.02

Data uncertainty is not considered for all input parameters in the model. For example, we attribute no uncertainty to the data issued from expert elicitations; we attribute no uncertainty to some serving size distributions. We do not account for uncertainty about extrapolation of information appropriate for one reference population to another reference population. For example, storage time and temperature distributions are extrapolated from U.S. transport & marketing, retail and home storage practices to Canadian practices, for which there are data gaps. We do account for model uncertainty in representations of some data sets by empirical distributions or analytical distributions.

10. Results of the Model Application Alternatives

10.1. *Raw-milk Cheese Baseline and Alternatives for Raw-milk Cheese*

In the pasteurized-milk cheese baseline model, the milk for cheese-making is “fully” pasteurized, meaning that no raw milk source *L. monocytogenes* survive the pasteurization process. In-plant environmental contamination provides the only exposure route. In this section, we consider milk that is not fully pasteurized. Exposure to *L. monocytogenes* contamination comes from both milk-source *L. monocytogenes* and in-plant environmental contamination.

Soft-ripened cheeses made from unpasteurized-milk are processed using a “traditional” (*i.e.* “non stabilized”) process while soft-ripened cheeses made from pasteurized-milk are processed using a “stabilized” (Kosikowski and Mistry 1987; Lawrence et al. 1987) process. Cheese processing also differs for the aging time at the manufacturer. Current regulations in Canada under the Food and Drugs Act (B.08.030, B.08.043, B.08.044) allow for the sale of raw-milk cheeses if the cheeses are stored for 60 days or more from the beginning of the manufacturing process, and at a temperature of at least 2°C (35°F). In the U.S., similar requirements exist (21 CFR 133.182(a)). The risk assessment model for raw-milk cheese accounts for those regulatory requirements by specifying that the sum of the aging time at the cheese manufacturer and the time during transport and marketing equals 60 days. The temperature during the aging period is greater than 2°C (35°F) in the pasteurized-milk cheese baseline model; it is unchanged in these alternatives for raw-milk cheese. Here, as elsewhere in this report, we adhere to regulatory definitions for pasteurized milk.

Section 7.1 (and Appendix, section “On Farm”) describe the model and assumptions used to evaluate the prevalence and level of *L. monocytogenes* contamination of milk from the farm. They demonstrate differences in *L. monocytogenes* bulk milk prevalence and levels between two illustrative cases: farmstead-scale operations, where milk for cheese-making is collected from 1 herd of size 7 to 112 cows; and, artisanal-scale operations, where milk for cheese-making is drawn from the milk from 2 herds of size 7 to 112 cows (D'Amico and Donnelly 2010). The *L. monocytogenes* bulk milk prevalence is higher for the artisanal-scale operations case than for

the farmstead-scale operations case while the level of *L. monocytogenes* contamination in contaminated milk is nearly the same (Table 22 and Table 23 in section 7.1).

The raw-milk cheese baseline is defined from raw-milk cheeses made from farmstead-scale operations. We evaluate the artisanal-scale operation scenario in this risk assessment as an illustrative case. We do not treat it as a baseline for application of the mitigations later considered in this report, though risk managers using this report may choose to do so if desired.

Reporting Outputs of Interest

The output of interest for this section is the risk per serving at random, that is, the probability of illness (invasive listeriosis) for a soft-ripened cheese serving at random. We organize the risk outputs' results into tables, with features set both to meet the management charge and to help in reporting risk outputs' variability. Table columns separate results for populations (country × subpopulation) and table rows report summary statistics from the distribution (variability) of that risk output. Mean, median and variance do not adequately describe the shape of distributions as skewed as these risk outputs' distributions are; so, tables' summary statistics report several percentiles, including ones in the lower and upper tails, as well.

Results in the first part of section 10.1.1. ignore parameter uncertainty; setting parameters to their most likely values describes only variability, as appropriate to context. Results in the second part of section 10.1.1. account for both the parameters' description of variability and also the uncertainty that we associate with those variability descriptions, insofar as the uncertainty can be captured.

We evaluate the change in the mean risk per serving at random and the median risk per serving at random with reference to a change in a particular alternative from baseline models setting using the *dMean* and *dMedian* statistics as in section 9. *dMean* and *dMedian* are evaluated as the ratios:

$$dMean = \frac{[\text{Mean evaluated using an alternative model}]}{[\text{Mean evaluated using the baseline model}]},$$

$$dMedian = \frac{[\text{Median evaluated using an alternative model}]}{[\text{Median evaluated using the baseline model}]}$$

A large value of *dMean* indicates a large impact of the alternative on the highest percentiles of the risk per serving at random distribution, and so, on the mean of the risk per serving at random distribution. *dMean* values greater than 1 point to alternatives whose changes effect an increase in the mean value, relative to the mean value calculated at the baseline value. *dMean* values less than 1 point to alternatives whose changes effect a decrease in the mean value, relative to the mean value calculated in the baseline model. A large *dMedian* value indicates that the alternative has a large impact on the whole risk distribution, particularly effecting a shift of location for the risk distribution. Note that the mean risk per serving at random is linearly linked to the expected number of cases in the population.

The change in the mean risk per serving at random and the change in the median risk per serving at random are evaluated relative to the pasteurized-milk cheese baseline, as previously, and relative to a raw-milk cheese baseline.

Calculation Methods

Calculated results use the simulation model’s Analytica™ implementation, 100,000 iterations, with Median Latin Hypercube sampling or use the Analytica™ implementation of the simulation model, $N_v = 25,000$ samples in the variability dimension and $N_u = 200$ iterations in the uncertainty dimension, with Median Latin Hypercube sampling (variability dimension) and a hybrid of Median Latin Hypercube and Simple Monte Carlo sampling (uncertainty dimension). This number of iterations is sufficiently high to obtain good convergence for the main statistics of interest in both dimensions (see Appendix, section “Simulation Estimator Characteristics for the Risk outputs”).

10.1.1. Baseline for Raw-Milk Cheese

No Uncertainty Considered

For populations in Canada, mean risk per raw-milk soft-ripened cheese serving at random varies as 3.8×10^{-7} , 9.2×10^{-7} , 4.2×10^{-7} among the susceptible populations (Elderly, Pregnant women, Immunocompromised, respectively) and 9.5×10^{-9} in the non-susceptible population (General) (Table 51). These values correspond to one case of invasive listeriosis per 2,600,000 servings eaten by individuals in the Elderly population, 1,100,000 servings in the Pregnant women population, 2,400,000 servings in the Immunocompromised population and 105 Million servings in the General population.

For populations in the U.S., the mean risk per raw-milk soft-ripened cheese serving at random varies as 8.2×10^{-7} , 1.8×10^{-6} , 8.1×10^{-7} among the susceptible Elderly, Pregnant women and Immunocompromised populations, respectively, and 1.8×10^{-8} in the non-susceptible General population. These values correspond to one case of invasive listeriosis per 1,200,000 servings eaten by individuals in the Elderly population, 570,000 servings in the Pregnant women population, 1,200,000 servings in the Immunocompromised population and 55 Million servings in the General population in the U.S.

The median risk is much lower than the mean, ranging from 8.9×10^{-13} to 4.0×10^{-11} per serving at random among the Canadian populations and from 1.9×10^{-12} to 1.3×10^{-10} per serving at random in the U.S. populations. This reflects the asymmetric distribution of the risk in the population of servings: some rare events with high probability of illness considerably influence the mean value, a phenomenon that we observed also in the results for the pasteurized-milk cheese (section 9).

Table 51: Risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw milk , from farmstead-scale operations under the current 60-day aging regulation.

Summary statistics from distributions describe variability among the risk per serving.

Canada	Elderly	Pregnant	IC*	General	U.S.	Elderly	Pregnant	IC	General
Median	4.40×10^{-11}	5.63×10^{-11}	3.96×10^{-11}	8.85×10^{-13}	Median	9.97×10^{-11}	1.25×10^{-10}	8.39×10^{-11}	1.88×10^{-12}
Mean	3.82×10^{-07}	9.23×10^{-07}	4.24×10^{-07}	9.50×10^{-09}	Mean	8.19×10^{-07}	1.75×10^{-06}	8.13×10^{-07}	1.82×10^{-08}
Std. dev.	5.76×10^{-06}	9.67×10^{-06}	6.70×10^{-06}	1.51×10^{-07}	Std. dev.	1.17×10^{-05}	1.99×10^{-05}	1.38×10^{-05}	3.10×10^{-07}
1%ile	3.14×10^{-14}	3.13×10^{-14}	3.15×10^{-14}	7.03×10^{-16}	1%ile	5.12×10^{-14}	5.12×10^{-14}	5.12×10^{-14}	1.14×10^{-15}
2.5%ile	3.14×10^{-14}	3.13×10^{-14}	3.15×10^{-14}	7.03×10^{-16}	2.5%ile	1.02×10^{-13}	1.02×10^{-13}	1.02×10^{-13}	2.29×10^{-15}
5%ile	6.28×10^{-14}	6.26×10^{-14}	6.30×10^{-14}	1.41×10^{-15}	5%ile	2.05×10^{-13}	2.05×10^{-13}	1.54×10^{-13}	3.43×10^{-15}
10%ile	2.20×10^{-13}	2.19×10^{-13}	2.21×10^{-13}	4.93×10^{-15}	10%ile	5.64×10^{-13}	6.15×10^{-13}	5.64×10^{-13}	1.26×10^{-14}
25%ile	2.17×10^{-12}	2.41×10^{-12}	2.14×10^{-12}	4.79×10^{-14}	25%ile	5.28×10^{-12}	5.84×10^{-12}	4.87×10^{-12}	1.09×10^{-13}
50%ile	4.40×10^{-11}	5.63×10^{-11}	3.96×10^{-11}	8.85×10^{-13}	50%ile	9.97×10^{-11}	1.25×10^{-10}	8.39×10^{-11}	1.88×10^{-12}
75%ile	2.55×10^{-09}	3.28×10^{-09}	2.06×10^{-09}	4.61×10^{-11}	75%ile	5.12×10^{-09}	6.75×10^{-09}	4.10×10^{-09}	9.17×10^{-11}
90%ile	3.45×10^{-08}	6.26×10^{-08}	2.90×10^{-08}	6.49×10^{-10}	90%ile	6.88×10^{-08}	1.18×10^{-07}	5.64×10^{-08}	1.26×10^{-09}
95%ile	1.63×10^{-07}	5.08×10^{-07}	1.35×10^{-07}	3.03×10^{-09}	95%ile	3.12×10^{-07}	8.34×10^{-07}	2.49×10^{-07}	5.57×10^{-09}
97.5%ile	8.21×10^{-07}	6.08×10^{-06}	6.82×10^{-07}	1.52×10^{-08}	97.5%ile	1.62×10^{-06}	8.42×10^{-06}	1.14×10^{-06}	2.54×10^{-08}
99%ile	6.28×10^{-06}	2.19×10^{-05}	6.31×10^{-06}	1.41×10^{-07}	99%ile	1.29×10^{-05}	3.61×10^{-05}	1.03×10^{-05}	2.29×10^{-07}

* IC: Immunocompromised.

For the Elderly population in Canada, the mean risk of invasive listeriosis from consuming a raw-milk soft-ripened cheese serving at random from farmstead-scale operations is 53 times higher than the mean risk for pasteurized-milk cheese (Table 52) and the mean risk is 52, 69 and 69 times higher for the Pregnant women, the Immunocompromised and the General population in Canada, respectively. In the United-States, the mean risk of invasive listeriosis from consuming a raw-milk soft-ripened cheese serving at random from artisanal-scale operations is 112, 96, 157 and 157 times higher than the mean risk following the consumption of pasteurized-milk cheese for the Elderly, Pregnant women, Immunocompromised and General population, respectively. The median risk per serving at random is larger than the median risk per pasteurized-milk cheese serving by a factor ranging from 357 to 399 in Canada and 746 to 844 in the U.S. That is, the whole distribution of the risk, not only a few high values, is shifted to higher values of the probability of illness compared to pasteurized-milk cheese baseline.

Table 52: Risk of invasive listeriosis per serving: soft-ripened cheese made from raw milk baseline (farmstead-scale operations under the current 60-day aging regulation) vs. soft-ripened cheese made from pasteurized milk baseline.

Canada	Elderly	Pregnant	IC*	General	U.S.	Elderly	Pregnant	IC*	General
<i>dMedian</i>	381	399	358	357	<i>dMedian</i>	788	844	746	746
<i>dMean</i>	53	52	69	69	<i>dMean</i>	112	96	157	157

*IC: Immunocompromised.

The higher risk of invasive listeriosis from consumption of raw-milk cheese is linked:

- to the higher predicted prevalence of contaminated cheeses and servings:

- in the baseline model for fully pasteurized-milk cheese, the prevalence of contaminated cheese was predicted to be approximately 0.7%, all from in-plant environment source *L. monocytogenes*;
- in the case of farmstead raw-milk soft-ripened cheese, the prevalence of contaminated cheeses is predicted to be 3.2% (Canada) and 4.7% (U.S.) (prevalence distribution means), from *L. monocytogenes* contaminated bulk raw milk and in-plant environment contamination;
 - at farm bulk milk prevalence and levels consistent with the available literature, 2.2% (Canada) and 3.7% (U.S.) of cheeses made from raw milk are predicted to contain *L. monocytogenes* at the end of cheese production (prevalence distribution means, Table 22);
 - *L. monocytogenes* in those contaminated cheeses are predicted to grow to high enough levels to evoke the risk results in Table 41;
- to the higher predicted level of contamination of *L. monocytogenes* in contaminated cheeses:
 - *L. monocytogenes* in the raw bulk milk are not inactivated by pasteurization and some are predicted to survive the other barriers in the cheese-making process;
 - the median number of *L. monocytogenes* in a contaminated raw-milk cheese serving at time of consumption is predicted to be approximately 1,400 for the Elderly population in Canada and approximately 1,900 for the Elderly population in the U.S.; for soft-ripened cheese servings made from pasteurized milk, the median number of *L. monocytogenes* in a contaminated serving at time of consumption is predicted to be 12;
 - the median risk per raw-milk cheese serving at random is larger than the baseline case's median risk per fully pasteurized-milk cheese serving, because the prevalence of contaminated servings is larger than in the pasteurized-milk cheese baseline case —*L. monocytogenes* from the raw milk— and because the number of *L. monocytogenes* in a contaminated cheese is larger than in the pasteurized-milk cheese baseline case; servings with very small numbers of *L. monocytogenes* do occur, but much less often;

- and, to a lesser degree, to the 60 day aging regulation that allows *L. monocytogenes* to grow, when conditions permit, during a longer period of time, even for those contaminated soft-ripened cheeses with only in-plant environment *L. monocytogenes* contamination.

The higher mean and median risks per serving at random predicted in the U.S. compared to ones predicted for Canada are due to the higher prevalence of contamination in farm bulk tank surveys for the U.S. (see Table 22, section 7.1, estimated mean: 2.4% in Canada vs. 4.2% in the United States).

Uncertainty Considered

Table 53 and Table 54 report results for the risk of invasive listeriosis per serving from raw-milk soft-ripened cheeses made in farmstead-scale operations when uncertainty is considered (method sections). As an example, for the Canadian Elderly population (Table 53),

- the mean risk of invasive listeriosis per serving at random is 7.9×10^{-7} (median value over uncertainty distribution for the mean risk of invasive listeriosis) with a credible interval [2.4×10^{-8} , 2.7×10^{-5}] (2.5th and 97.5th percentiles of the uncertainty distribution for the mean risk of invasive listeriosis); those interval endpoints are 33 times less and 34 times more than the median (uncertainty) value of 7.9×10^{-7} for the mean risk per serving;
- the median risk is 5.1×10^{-11} [3.8×10^{-13} , 5.1×10^{-9}]; and,
- the 99th percentile of the distribution is 1.3×10^{-5} [3.9×10^{-7} , 4.8×10^{-4}].

For this risk output, the Variability Ratio (Ozkaynak et al. 2009), which measures the distance between the median and the 95th percentile, is approximately 6,700. The Uncertainty Ratio, which measures the distance between the median and the upper limit of its 90% credible interval, is approximately 52. The Overall Uncertainty Ratio, which measures the distance between the median and the upper limit of the 90% credible interval of the 95th percentile, is approximately 116,900. From these statistics, one may conclude that the variability in the risk output largely overwhelms the uncertainty in the risk output accounted for in this model.

Table 53: Risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw-milk, farmstead-scale operations, under the current 60-day aging regulation, among subpopulations in Canada.

Results of second-order Monte Carlo simulation describe uncertainty about summary statistics from distributions that describe variability among the risk per serving at random.

Summary statistics	Elderly Median	Elderly LB CI95	Elderly UB CI95	Pregnant Median	Pregnant LB CI95	Pregnant UB CI95	IC* Median	IC* LB CI95	IC* UB CI95	General Median	General LB CI95	General UB CI95
Median	5.14×10 ⁻¹¹	3.80×10 ⁻¹³	5.09×10 ⁻⁰⁹	6.61×10 ⁻¹¹	5.76×10 ⁻¹³	5.98×10 ⁻⁰⁹	4.69×10 ⁻¹¹	3.58×10 ⁻¹³	3.70×10 ⁻⁰⁹	1.07×10 ⁻¹²	1.02×10 ⁻¹⁴	8.36×10 ⁻¹¹
Mean	7.86×10 ⁻⁰⁷	2.36×10 ⁻⁰⁸	2.65×10 ⁻⁰⁵	1.50×10 ⁻⁰⁶	3.19×10 ⁻⁰⁸	5.04×10 ⁻⁰⁵	7.62×10 ⁻⁰⁷	2.64×10 ⁻⁰⁸	2.00×10 ⁻⁰⁵	1.65×10 ⁻⁰⁸	5.55×10 ⁻¹⁰	5.73×10 ⁻⁰⁷
Std. Dev.	1.05×10 ⁻⁰⁵	3.73×10 ⁻⁰⁷	3.38×10 ⁻⁰⁴	1.30×10 ⁻⁰⁵	5.28×10 ⁻⁰⁷	5.00×10 ⁻⁰⁴	1.05×10 ⁻⁰⁵	3.60×10 ⁻⁰⁷	3.29×10 ⁻⁰⁴	2.40×10 ⁻⁰⁷	9.20×10 ⁻⁰⁹	7.68×10 ⁻⁰⁶
1%ile	5.27×10 ⁻¹⁴	3.87×10 ⁻¹⁵	1.64×10 ⁻¹²	5.30×10 ⁻¹⁴	4.11×10 ⁻¹⁵	1.62×10 ⁻¹²	5.39×10 ⁻¹⁴	4.12×10 ⁻¹⁵	1.33×10 ⁻¹²	1.43×10 ⁻¹⁵	6.30×10 ⁻¹⁷	5.40×10 ⁻¹⁴
2.5%ile	7.24×10 ⁻¹⁴	3.87×10 ⁻¹⁵	4.08×10 ⁻¹²	6.99×10 ⁻¹⁴	4.11×10 ⁻¹⁵	3.58×10 ⁻¹²	7.55×10 ⁻¹⁴	4.12×10 ⁻¹⁵	3.59×10 ⁻¹²	1.73×10 ⁻¹⁵	6.30×10 ⁻¹⁷	1.16×10 ⁻¹³
5%ile	1.38×10 ⁻¹³	5.38×10 ⁻¹⁵	9.16×10 ⁻¹²	1.50×10 ⁻¹³	5.29×10 ⁻¹⁵	9.35×10 ⁻¹²	1.38×10 ⁻¹³	5.55×10 ⁻¹⁵	8.34×10 ⁻¹²	2.93×10 ⁻¹⁵	1.05×10 ⁻¹⁶	2.61×10 ⁻¹³
10%ile	3.37×10 ⁻¹³	1.07×10 ⁻¹⁴	2.49×10 ⁻¹¹	3.26×10 ⁻¹³	1.05×10 ⁻¹⁴	2.92×10 ⁻¹¹	3.27×10 ⁻¹³	1.11×10 ⁻¹⁴	2.51×10 ⁻¹¹	6.21×10 ⁻¹⁵	1.23×10 ⁻¹⁶	6.95×10 ⁻¹³
25%ile	1.91×10 ⁻¹²	2.81×10 ⁻¹⁴	1.88×10 ⁻¹⁰	2.01×10 ⁻¹²	3.63×10 ⁻¹⁴	2.06×10 ⁻¹⁰	2.02×10 ⁻¹²	2.81×10 ⁻¹⁴	1.79×10 ⁻¹⁰	4.25×10 ⁻¹⁴	6.84×10 ⁻¹⁶	5.69×10 ⁻¹²
50%ile	5.14×10 ⁻¹¹	3.80×10 ⁻¹³	5.09×10 ⁻⁰⁹	6.61×10 ⁻¹¹	5.76×10 ⁻¹³	5.98×10 ⁻⁰⁹	4.69×10 ⁻¹¹	3.58×10 ⁻¹³	3.70×10 ⁻⁰⁹	1.07×10 ⁻¹²	1.02×10 ⁻¹⁴	8.36×10 ⁻¹¹
75%ile	4.28×10 ⁻⁰⁹	3.12×10 ⁻¹¹	2.19×10 ⁻⁰⁷	6.45×10 ⁻⁰⁹	4.95×10 ⁻¹¹	2.32×10 ⁻⁰⁷	3.44×10 ⁻⁰⁹	2.08×10 ⁻¹¹	1.52×10 ⁻⁰⁷	7.08×10 ⁻¹¹	7.15×10 ⁻¹³	4.57×10 ⁻⁰⁹
90%ile	6.37×10 ⁻⁰⁸	1.21×10 ⁻⁰⁹	2.55×10 ⁻⁰⁶	1.48×10 ⁻⁰⁷	1.97×10 ⁻⁰⁹	4.78×10 ⁻⁰⁶	5.81×10 ⁻⁰⁸	8.67×10 ⁻¹⁰	1.84×10 ⁻⁰⁶	1.21×10 ⁻⁰⁹	2.98×10 ⁻¹¹	1.09×10 ⁻⁰⁷
95%ile	3.44×10 ⁻⁰⁷	6.10×10 ⁻⁰⁹	1.26×10 ⁻⁰⁵	1.45×10 ⁻⁰⁶	1.67×10 ⁻⁰⁸	6.29×10 ⁻⁰⁵	2.94×10 ⁻⁰⁷	5.20×10 ⁻⁰⁹	8.29×10 ⁻⁰⁶	6.05×10 ⁻⁰⁹	1.62×10 ⁻¹⁰	4.22×10 ⁻⁰⁷
97.5%ile	1.77×10 ⁻⁰⁶	2.77×10 ⁻⁰⁸	7.13×10 ⁻⁰⁵	8.55×10 ⁻⁰⁶	1.13×10 ⁻⁰⁷	2.86×10 ⁻⁰⁴	1.62×10 ⁻⁰⁶	3.07×10 ⁻⁰⁸	5.01×10 ⁻⁰⁵	3.67×10 ⁻⁰⁸	9.60×10 ⁻¹⁰	2.50×10 ⁻⁰⁶
99%ile	1.29×10 ⁻⁰⁵	3.87×10 ⁻⁰⁷	4.81×10 ⁻⁰⁴	3.61×10 ⁻⁰⁵	6.72×10 ⁻⁰⁷	1.13×10 ⁻⁰³	1.15×10 ⁻⁰⁵	3.35×10 ⁻⁰⁷	3.38×10 ⁻⁰⁴	2.55×10 ⁻⁰⁷	9.16×10 ⁻⁰⁹	1.10×10 ⁻⁰⁵

*IC: Immunocompromised; LB: Lower bound of the CI95%, uncertainty dimension; UB: Upper bound

Table 54: Risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw-milk, farmstead-scale operations, under the current 60-day aging regulation, among subpopulations in the U.S.

Results of second-order Monte Carlo simulation describe uncertainty about summary statistics from distributions that describe variability among the risk per serving at random.

Summary statistics	Elderly Median	Elderly LB CI95	Elderly UB CI95	Pregnant Median	Pregnant LB CI95	Pregnant UB CI95	IC* Median	IC* LB CI95	IC* UB CI95	General Median	General LB CI95	General UB CI95
Median	8.74×10 ⁻¹¹	2.59×10 ⁻¹²	5.51×10 ⁻⁰⁹	1.07×10 ⁻¹⁰	2.91×10 ⁻¹²	7.37×10 ⁻⁰⁹	6.88×10 ⁻¹¹	2.32×10 ⁻¹²	5.11×10 ⁻⁰⁹	2.24×10 ⁻¹²	3.16×10 ⁻¹⁴	5.70×10 ⁻¹¹
Mean	1.24×10 ⁻⁰⁶	7.29×10 ⁻⁰⁸	3.23×10 ⁻⁰⁵	2.54×10 ⁻⁰⁶	1.73×10 ⁻⁰⁷	8.31×10 ⁻⁰⁵	1.09×10 ⁻⁰⁶	7.80×10 ⁻⁰⁸	4.17×10 ⁻⁰⁵	3.48×10 ⁻⁰⁸	1.42×10 ⁻⁰⁹	6.26×10 ⁻⁰⁷
Std. Dev.	1.94×10 ⁻⁰⁵	1.23×10 ⁻⁰⁶	5.02×10 ⁻⁰⁴	2.73×10 ⁻⁰⁵	2.18×10 ⁻⁰⁶	6.66×10 ⁻⁰⁴	1.78×10 ⁻⁰⁵	1.36×10 ⁻⁰⁶	5.56×10 ⁻⁰⁴	5.29×10 ⁻⁰⁷	3.15×10 ⁻⁰⁸	8.55×10 ⁻⁰⁶
1%ile	7.24×10 ⁻¹⁴	7.80×10 ⁻¹⁵	1.48×10 ⁻¹²	7.24×10 ⁻¹⁴	7.80×10 ⁻¹⁵	1.56×10 ⁻¹²	7.37×10 ⁻¹⁴	7.80×10 ⁻¹⁵	1.56×10 ⁻¹²	1.93×10 ⁻¹⁵	1.80×10 ⁻¹⁶	3.41×10 ⁻¹⁴
2.5%ile	1.06×10 ⁻¹³	8.35×10 ⁻¹⁵	3.12×10 ⁻¹²	1.06×10 ⁻¹³	8.35×10 ⁻¹⁵	3.12×10 ⁻¹²	1.05×10 ⁻¹³	8.35×10 ⁻¹⁵	3.13×10 ⁻¹²	2.90×10 ⁻¹⁵	2.06×10 ⁻¹⁶	7.43×10 ⁻¹⁴
5%ile	2.08×10 ⁻¹³	1.43×10 ⁻¹⁴	7.00×10 ⁻¹²	2.08×10 ⁻¹³	1.43×10 ⁻¹⁴	7.02×10 ⁻¹²	2.04×10 ⁻¹³	1.43×10 ⁻¹⁴	7.00×10 ⁻¹²	5.53×10 ⁻¹⁵	3.43×10 ⁻¹⁶	1.64×10 ⁻¹³
10%ile	4.81×10 ⁻¹³	2.87×10 ⁻¹⁴	2.59×10 ⁻¹¹	5.08×10 ⁻¹³	2.87×10 ⁻¹⁴	2.59×10 ⁻¹¹	4.70×10 ⁻¹³	2.87×10 ⁻¹⁴	2.29×10 ⁻¹¹	1.35×10 ⁻¹⁴	6.82×10 ⁻¹⁶	5.23×10 ⁻¹³
25%ile	3.76×10 ⁻¹²	1.61×10 ⁻¹³	2.27×10 ⁻¹⁰	4.00×10 ⁻¹²	1.61×10 ⁻¹³	2.41×10 ⁻¹⁰	3.57×10 ⁻¹²	1.43×10 ⁻¹³	1.89×10 ⁻¹⁰	1.05×10 ⁻¹³	2.00×10 ⁻¹⁵	3.67×10 ⁻¹²
50%ile	8.74×10 ⁻¹¹	2.59×10 ⁻¹²	5.51×10 ⁻⁰⁹	1.07×10 ⁻¹⁰	2.91×10 ⁻¹²	7.37×10 ⁻⁰⁹	6.88×10 ⁻¹¹	2.32×10 ⁻¹²	5.11×10 ⁻⁰⁹	2.24×10 ⁻¹²	3.16×10 ⁻¹⁴	5.70×10 ⁻¹¹
75%ile	6.18×10 ⁻⁰⁹	1.74×10 ⁻¹⁰	3.11×10 ⁻⁰⁷	8.88×10 ⁻⁰⁹	2.85×10 ⁻¹⁰	4.56×10 ⁻⁰⁷	4.82×10 ⁻⁰⁹	1.26×10 ⁻¹⁰	2.86×10 ⁻⁰⁷	1.57×10 ⁻¹⁰	2.34×10 ⁻¹²	3.46×10 ⁻⁰⁹
90%ile	1.01×10 ⁻⁰⁷	3.74×10 ⁻⁰⁹	3.24×10 ⁻⁰⁶	1.97×10 ⁻⁰⁷	6.95×10 ⁻⁰⁹	8.51×10 ⁻⁰⁶	8.72×10 ⁻⁰⁸	2.96×10 ⁻⁰⁹	3.32×10 ⁻⁰⁶	2.69×10 ⁻⁰⁹	7.44×10 ⁻¹¹	4.46×10 ⁻⁰⁸
95%ile	4.70×10 ⁻⁰⁷	2.04×10 ⁻⁰⁸	1.25×10 ⁻⁰⁵	2.00×10 ⁻⁰⁶	6.21×10 ⁻⁰⁸	9.62×10 ⁻⁰⁵	3.96×10 ⁻⁰⁷	1.35×10 ⁻⁰⁸	1.61×10 ⁻⁰⁵	1.13×10 ⁻⁰⁸	4.17×10 ⁻¹⁰	2.07×10 ⁻⁰⁷
97.5%ile	2.64×10 ⁻⁰⁶	9.16×10 ⁻⁰⁸	7.88×10 ⁻⁰⁵	1.33×10 ⁻⁰⁵	4.72×10 ⁻⁰⁷	7.24×10 ⁻⁰⁴	1.84×10 ⁻⁰⁶	5.83×10 ⁻⁰⁸	1.12×10 ⁻⁰⁴	5.86×10 ⁻⁰⁸	1.89×10 ⁻⁰⁹	1.42×10 ⁻⁰⁶
99%ile	1.81×10 ⁻⁰⁵	7.23×10 ⁻⁰⁷	4.79×10 ⁻⁰⁴	5.81×10 ⁻⁰⁵	2.65×10 ⁻⁰⁶	2.23×10 ⁻⁰³	1.56×10 ⁻⁰⁵	6.16×10 ⁻⁰⁷	6.96×10 ⁻⁰⁴	4.90×10 ⁻⁰⁷	1.43×10 ⁻⁰⁸	9.38×10 ⁻⁰⁶

*IC: Immunocompromised; LB: Lower bound of the CI95%, uncertainty dimension; UB: Upper bound

10.1.2. Artisanal-scale Operations

No Uncertainty Considered

If the cheeses originate from artisanal-scale operations, which draw milk for cheese-making from milk collected from 2 farms, the mean risk per raw-milk soft-ripened cheese serving at random (Table 55) varies as 6.5×10^{-7} , 1.4×10^{-6} , 6.1×10^{-7} among the susceptible populations (Elderly,

Pregnant women, Immunocompromised, respectively) and 1.4×10^{-8} in the non-susceptible population in Canada; and varies as 1.3×10^{-6} , 2.7×10^{-6} , 1.2×10^{-6} among the susceptible populations (Elderly, Pregnant women, Immunocompromised, respectively) and 2.6×10^{-8} in the non-susceptible population in the U.S.

Table 55: Risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw milk, artisanal-scale operations, under the current 60-day aging regulation, no uncertainty considered.

Summary statistics from distributions describe variability among the risk per serving at random.

Summary statistics	Canada Elderly	Canada Pregnant	Canada IC*	Canada General	Summary statistics	U.S. Elderly	U.S. Pregnant	U.S. IC	U.S. General
Median	5.70×10^{-11}	7.03×10^{-11}	5.12×10^{-11}	1.15×10^{-12}	Median	1.18×10^{-10}	1.46×10^{-10}	1.00×10^{-10}	2.24×10^{-12}
Mean	6.53×10^{-07}	1.36×10^{-06}	6.14×10^{-07}	1.38×10^{-08}	Mean	1.29×10^{-06}	2.65×10^{-06}	1.17×10^{-06}	2.63×10^{-08}
Std. dev.	1.09×10^{-05}	1.46×10^{-05}	9.94×10^{-06}	2.23×10^{-07}	Std. dev.	2.09×10^{-05}	3.16×10^{-05}	2.01×10^{-05}	4.52×10^{-07}
1%ile	5.39×10^{-14}	5.38×10^{-14}	5.42×10^{-14}	1.21×10^{-15}	1%ile	9.10×10^{-14}	9.10×10^{-14}	9.10×10^{-14}	2.03×10^{-15}
2.5%ile	5.39×10^{-14}	5.38×10^{-14}	1.08×10^{-13}	2.42×10^{-15}	2.5%ile	1.82×10^{-13}	1.82×10^{-13}	1.82×10^{-13}	4.07×10^{-15}
5%ile	1.62×10^{-13}	1.61×10^{-13}	1.63×10^{-13}	3.63×10^{-15}	5%ile	2.73×10^{-13}	2.73×10^{-13}	2.73×10^{-13}	6.10×10^{-15}
10%ile	3.78×10^{-13}	3.77×10^{-13}	3.79×10^{-13}	8.48×10^{-15}	10%ile	8.19×10^{-13}	9.10×10^{-13}	8.19×10^{-13}	1.83×10^{-14}
25%ile	2.86×10^{-12}	3.07×10^{-12}	2.76×10^{-12}	6.18×10^{-14}	25%ile	6.19×10^{-12}	6.82×10^{-12}	5.64×10^{-12}	1.26×10^{-13}
50%ile	5.70×10^{-11}	7.03×10^{-11}	5.12×10^{-11}	1.15×10^{-12}	50%ile	1.18×10^{-10}	1.46×10^{-10}	1.00×10^{-10}	2.24×10^{-12}
75%ile	3.20×10^{-09}	5.36×10^{-09}	2.61×10^{-09}	5.84×10^{-11}	75%ile	6.52×10^{-09}	9.10×10^{-09}	4.94×10^{-09}	1.10×10^{-10}
90%ile	4.52×10^{-08}	8.61×10^{-08}	4.02×10^{-08}	8.99×10^{-10}	90%ile	9.52×10^{-08}	1.62×10^{-07}	7.84×10^{-08}	1.75×10^{-09}
95%ile	2.21×10^{-07}	7.04×10^{-07}	1.92×10^{-07}	4.30×10^{-09}	95%ile	4.46×10^{-07}	1.15×10^{-06}	3.55×10^{-07}	7.93×10^{-09}
97.5%ile	1.18×10^{-06}	6.63×10^{-06}	1.01×10^{-06}	2.26×10^{-08}	97.5%ile	2.27×10^{-06}	1.06×10^{-05}	1.72×10^{-06}	3.85×10^{-08}
99%ile	1.08×10^{-05}	3.10×10^{-05}	1.08×10^{-05}	2.42×10^{-07}	99%ile	1.83×10^{-05}	5.39×10^{-05}	1.82×10^{-05}	4.07×10^{-07}

* IC: Immunocompromised.

The mean risk of invasive listeriosis from consuming a serving of soft-ripened cheese from artisanal-scale operations is much higher for raw-milk cheese than for pasteurized-milk cheese (Table 56), by 77 (Pregnant women) to 100 (General population) times in Canada and by 146 (Pregnant women) to 227 (General population) times in the U.S. for the same reasons that we described in the preceding section for farmstead-scale operations. The mean and median risks per raw-milk soft-ripened cheese serving at random from artisanal-scale operations are slightly higher than the risk per serving from farmstead-scale operations (Table 56).

Table 56: Relative risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw milk, artisanal-scale operations, under current 60 day aging regulation vs. soft-ripened cheese made from pasteurized milk baseline and vs. soft-ripened cheese made from raw milk baseline (farmstead-scale operations, under current 60 day aging regulation).

Country	Relative to pasteurized-milk cheese baseline					Relative to raw-milk cheese baseline				
		Elderly	Pregnant women	IC*	General		Elderly	Pregnant women	IC*	General
Canada	<i>dMedian</i>	493	499	462	462	<i>dMedian</i>	1.29	1.25	1.29	1.29
Canada	<i>dMean</i>	90	77	100	100	<i>dMean</i>	1.71	1.48	1.45	1.45
United States	<i>dMedian</i>	934	992	891	891	<i>dMedian</i>	1.18	1.18	1.19	1.19
United States	<i>dMean</i>	176	146	227	227	<i>dMean</i>	1.57	1.52	1.45	1.45

* IC: Immunocompromised.

These slightly higher mean and median risks for artisanal-scale operations compared to the raw-milk cheese baseline (farmstead-scale operations) reflect the slightly higher *L. monocytogenes* prevalence that results from mixing milk from 2 farms, each with the same prevalence of contaminated bulk milk and storage until processing, compared to making cheese from milk from one farm without delay. Table 23 (Section 7.1) indicates that the mean prevalence in the dairy silo bulk milk attributed to artisanal-scale operations is 4.6% in Canada and 8.2% in the U.S., compared to the 2.4% (Canada) and 4.2% (U.S.) for farmstead-scale operations. The slightly lower level of contamination in contaminated bulk milk in the artisanal-scale operations compared to the farmstead-scale operations (*e.g.* \log_{10} of the mean number of cfu/ml: 1.29 vs. 1.60 in Canada, (Table 23, Section 7.1) does not compensate for this higher level of prevalence.

Uncertainty Considered

Table 57 and Table 58 report results for the risk of invasive listeriosis per serving from raw-milk soft-ripened cheeses made, when uncertainty is considered (refer to method sections). As an example, for the Canadian Elderly population (Table 57),

- the mean risk of invasive listeriosis per serving at random is 1.3×10^{-6} (median value over uncertainty distribution for the mean risk of invasive listeriosis) with a credible interval $[1.7 \times 10^{-8}, 4.6 \times 10^{-5}]$ (2.5th and 97.5th percentiles of the uncertainty distribution for the mean risk of invasive listeriosis); those endpoints are 77 times less and 35 times more than the median (uncertainty) value of 1.3×10^{-6} for the mean risk per serving;
- the median risk is 6.2×10^{-11} [3.8×10^{-13} , 7.7×10^{-9}]; and,
- the 99th percentile of the distribution is 2.0×10^{-5} [2.8×10^{-7} , 8.2×10^{-4}].

For this risk output, the Variability Ratio (Ozkaynak et al. 2009), that measures the distance between the median and the 95th percentile is approximately 7,200. The Uncertainty Ratio, which measures the distance between the median and the upper limit of its 90% credible interval, is approximately 50. The Overall Uncertainty Ratio, which measures the distance between the median and the upper limit of the 90% credible interval of the 95th percentile, is approximately 172,000. From these statistics, one may conclude that the variability in the risk output largely overwhelms the uncertainty in the risk output accounted for in this model.

Table 57: Risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw milk, artisanal-scale operations, under the current 60-day aging regulation, among subpopulations in Canada.
Results of second-order Monte Carlo simulation describe uncertainty about summary statistics from distributions that describe variability among the risk per serving at random.

Summary statistics	Elderly Median	Elderly LB CI95	Elderly UB CI95	Pregnant Median	Pregnant LB CI95	Pregnant UB CI95	IC* Median	IC* LB CI95	IC* UB CI95	General Median	General LB CI95	General UB CI95
Median	6.16×10 ⁻¹¹	3.76×10 ⁻¹³	7.68×10 ⁻⁰⁹	7.05×10 ⁻¹¹	4.98×10 ⁻¹³	1.33×10 ⁻⁰⁸	5.17×10 ⁻¹¹	3.30×10 ⁻¹³	6.89×10 ⁻⁰⁹	2.02×10 ⁻¹²	7.01×10 ⁻¹⁵	2.31×10 ⁻¹⁰
Mean	1.29×10 ⁻⁰⁶	1.68×10 ⁻⁰⁸	4.57×10 ⁻⁰⁵	2.28×10 ⁻⁰⁶	3.26×10 ⁻⁰⁸	1.12×10 ⁻⁰⁴	1.08×10 ⁻⁰⁶	1.36×10 ⁻⁰⁸	4.90×10 ⁻⁰⁵	3.24×10 ⁻⁰⁸	3.05×10 ⁻¹⁰	1.67×10 ⁻⁰⁶
Std. Dev.	1.80×10 ⁻⁰⁵	2.42×10 ⁻⁰⁷	6.41×10 ⁻⁰⁴	2.27×10 ⁻⁰⁵	4.19×10 ⁻⁰⁷	8.85×10 ⁻⁰⁴	1.70×10 ⁻⁰⁵	3.54×10 ⁻⁰⁷	6.04×10 ⁻⁰⁴	4.78×10 ⁻⁰⁷	6.03×10 ⁻⁰⁹	2.84×10 ⁻⁰⁵
1%ile	8.12×10 ⁻¹⁴	1.82×10 ⁻¹⁵	2.24×10 ⁻¹²	8.04×10 ⁻¹⁴	1.84×10 ⁻¹⁵	2.23×10 ⁻¹²	8.10×10 ⁻¹⁴	1.83×10 ⁻¹⁵	2.24×10 ⁻¹²	3.02×10 ⁻¹⁵	1.26×10 ⁻¹⁶	8.75×10 ⁻¹⁴
2.5%ile	1.02×10 ⁻¹³	1.82×10 ⁻¹⁵	4.48×10 ⁻¹²	1.04×10 ⁻¹³	1.84×10 ⁻¹⁵	4.46×10 ⁻¹²	1.10×10 ⁻¹³	1.83×10 ⁻¹⁵	4.48×10 ⁻¹²	3.69×10 ⁻¹⁵	1.26×10 ⁻¹⁶	1.86×10 ⁻¹³
5%ile	1.84×10 ⁻¹³	3.18×10 ⁻¹⁵	1.11×10 ⁻¹¹	2.00×10 ⁻¹³	2.79×10 ⁻¹⁵	8.92×10 ⁻¹²	1.98×10 ⁻¹³	3.23×10 ⁻¹⁵	8.95×10 ⁻¹²	6.51×10 ⁻¹⁵	1.29×10 ⁻¹⁶	4.20×10 ⁻¹³
10%ile	4.35×10 ⁻¹³	6.36×10 ⁻¹⁵	2.68×10 ⁻¹¹	4.61×10 ⁻¹³	5.58×10 ⁻¹⁵	2.57×10 ⁻¹¹	4.76×10 ⁻¹³	6.74×10 ⁻¹⁵	2.80×10 ⁻¹¹	1.49×10 ⁻¹⁴	2.47×10 ⁻¹⁶	1.14×10 ⁻¹²
25%ile	2.99×10 ⁻¹²	1.68×10 ⁻¹⁴	2.17×10 ⁻¹⁰	2.93×10 ⁻¹²	1.60×10 ⁻¹⁴	2.48×10 ⁻¹⁰	3.00×10 ⁻¹²	2.17×10 ⁻¹⁴	2.32×10 ⁻¹⁰	9.12×10 ⁻¹⁴	5.26×10 ⁻¹⁶	6.88×10 ⁻¹²
50%ile	6.16×10 ⁻¹¹	3.76×10 ⁻¹³	7.68×10 ⁻⁰⁹	7.05×10 ⁻¹¹	4.98×10 ⁻¹³	1.33×10 ⁻⁰⁸	5.17×10 ⁻¹¹	3.30×10 ⁻¹³	6.89×10 ⁻⁰⁹	2.02×10 ⁻¹²	7.01×10 ⁻¹⁵	2.31×10 ⁻¹⁰
75%ile	4.57×10 ⁻⁰⁹	4.59×10 ⁻¹¹	3.04×10 ⁻⁰⁷	6.69×10 ⁻⁰⁹	6.48×10 ⁻¹¹	6.78×10 ⁻⁰⁷	3.30×10 ⁻⁰⁹	3.49×10 ⁻¹¹	3.17×10 ⁻⁰⁷	1.37×10 ⁻¹⁰	4.78×10 ⁻¹³	1.35×10 ⁻⁰⁸
90%ile	7.94×10 ⁻⁰⁸	1.02×10 ⁻⁰⁹	3.61×10 ⁻⁰⁶	1.57×10 ⁻⁰⁷	2.03×10 ⁻⁰⁹	1.38×10 ⁻⁰⁵	6.17×10 ⁻⁰⁸	9.39×10 ⁻¹⁰	4.09×10 ⁻⁰⁶	2.30×10 ⁻⁰⁹	1.47×10 ⁻¹¹	1.68×10 ⁻⁰⁷
95%ile	4.46×10 ⁻⁰⁷	6.23×10 ⁻⁰⁹	2.32×10 ⁻⁰⁵	1.68×10 ⁻⁰⁶	1.44×10 ⁻⁰⁸	1.93×10 ⁻⁰⁴	3.22×10 ⁻⁰⁷	4.69×10 ⁻⁰⁹	2.36×10 ⁻⁰⁵	1.12×10 ⁻⁰⁸	6.49×10 ⁻¹¹	7.09×10 ⁻⁰⁷
97.5%ile	2.40×10 ⁻⁰⁶	2.73×10 ⁻⁰⁸	1.46×10 ⁻⁰⁴	1.32×10 ⁻⁰⁵	1.49×10 ⁻⁰⁷	7.53×10 ⁻⁰⁴	1.55×10 ⁻⁰⁶	1.91×10 ⁻⁰⁸	1.11×10 ⁻⁰⁴	6.01×10 ⁻⁰⁸	4.36×10 ⁻¹⁰	4.16×10 ⁻⁰⁶
99%ile	2.00×10 ⁻⁰⁵	2.77×10 ⁻⁰⁷	8.24×10 ⁻⁰⁴	5.12×10 ⁻⁰⁵	4.74×10 ⁻⁰⁷	2.63×10 ⁻⁰³	1.37×10 ⁻⁰⁵	2.01×10 ⁻⁰⁷	8.17×10 ⁻⁰⁴	4.64×10 ⁻⁰⁷	5.62×10 ⁻⁰⁹	2.79×10 ⁻⁰⁵

*IC: Immunocompromised; LB: Lower bound of the CI95%, uncertainty dimension; UB: Upper bound

Table 58: Risk of invasive listeriosis per serving at random of soft-ripened cheese made from raw milk, artisanal-scale operations, under the current 60-day aging regulation, among subpopulations in the U.S.

Results of second-order Monte Carlo simulation describe uncertainty about summary statistics from distributions that describe variability among the risk per serving at random.

Summary statistics	Elderly Median	Elderly LB CI95	Elderly UB CI95	Pregnant Median	Pregnant LB CI95	Pregnant UB CI95	IC* Median	IC* LB CI95	IC* UB CI95	General Median	General LB CI95	General UB CI95
Median	1.50×10 ⁻¹⁰	3.07×10 ⁻¹²	3.64×10 ⁻⁰⁹	1.78×10 ⁻¹⁰	3.89×10 ⁻¹²	3.73×10 ⁻⁰⁹	1.20×10 ⁻¹⁰	2.58×10 ⁻¹²	2.49×10 ⁻⁰⁹	4.15×10 ⁻¹²	1.28×10 ⁻¹³	1.33×10 ⁻¹⁰
Mean	1.98×10 ⁻⁰⁶	5.41×10 ⁻⁰⁸	3.09×10 ⁻⁰⁵	4.72×10 ⁻⁰⁶	1.46×10 ⁻⁰⁷	5.81×10 ⁻⁰⁵	1.85×10 ⁻⁰⁶	5.78×10 ⁻⁰⁸	2.85×10 ⁻⁰⁵	6.24×10 ⁻⁰⁸	2.12×10 ⁻⁰⁹	1.11×10 ⁻⁰⁶
Std. Dev.	3.53×10 ⁻⁰⁵	8.82×10 ⁻⁰⁷	3.92×10 ⁻⁰⁴	5.00×10 ⁻⁰⁵	2.05×10 ⁻⁰⁶	5.58×10 ⁻⁰⁴	3.07×10 ⁻⁰⁵	9.88×10 ⁻⁰⁷	4.24×10 ⁻⁰⁴	9.93×10 ⁻⁰⁷	3.62×10 ⁻⁰⁸	1.50×10 ⁻⁰⁵
1%ile	1.26×10 ⁻¹³	6.13×10 ⁻¹⁵	1.39×10 ⁻¹²	1.31×10 ⁻¹³	6.13×10 ⁻¹⁵	1.39×10 ⁻¹²	1.31×10 ⁻¹³	6.13×10 ⁻¹⁵	1.39×10 ⁻¹²	3.93×10 ⁻¹⁵	3.74×10 ⁻¹⁶	6.59×10 ⁻¹⁴
2.5%ile	1.72×10 ⁻¹³	6.13×10 ⁻¹⁵	2.78×10 ⁻¹²	1.79×10 ⁻¹³	6.13×10 ⁻¹⁵	2.78×10 ⁻¹²	1.72×10 ⁻¹³	6.13×10 ⁻¹⁵	2.78×10 ⁻¹²	5.76×10 ⁻¹⁵	4.00×10 ⁻¹⁶	1.13×10 ⁻¹³
5%ile	3.58×10 ⁻¹³	1.25×10 ⁻¹⁴	4.24×10 ⁻¹²	3.45×10 ⁻¹³	1.25×10 ⁻¹⁴	4.24×10 ⁻¹²	3.34×10 ⁻¹³	1.23×10 ⁻¹⁴	4.24×10 ⁻¹²	1.07×10 ⁻¹⁴	7.97×10 ⁻¹⁶	2.33×10 ⁻¹³
10%ile	8.60×10 ⁻¹³	3.02×10 ⁻¹⁴	1.27×10 ⁻¹¹	8.62×10 ⁻¹³	3.05×10 ⁻¹⁴	1.27×10 ⁻¹¹	8.49×10 ⁻¹³	3.02×10 ⁻¹⁴	1.14×10 ⁻¹¹	2.56×10 ⁻¹⁴	1.60×10 ⁻¹⁵	6.14×10 ⁻¹³
25%ile	6.23×10 ⁻¹²	1.45×10 ⁻¹³	9.52×10 ⁻¹¹	6.75×10 ⁻¹²	1.88×10 ⁻¹³	1.10×10 ⁻¹⁰	6.31×10 ⁻¹²	1.44×10 ⁻¹³	8.92×10 ⁻¹¹	1.99×10 ⁻¹³	8.15×10 ⁻¹⁵	4.53×10 ⁻¹²
50%ile	1.50×10 ⁻¹⁰	3.07×10 ⁻¹²	3.64×10 ⁻⁰⁹	1.78×10 ⁻¹⁰	3.89×10 ⁻¹²	3.73×10 ⁻⁰⁹	1.20×10 ⁻¹⁰	2.58×10 ⁻¹²	2.49×10 ⁻⁰⁹	4.15×10 ⁻¹²	1.28×10 ⁻¹³	1.33×10 ⁻¹⁰
75%ile	9.64×10 ⁻⁰⁹	2.08×10 ⁻¹⁰	1.80×10 ⁻⁰⁷	1.40×10 ⁻⁰⁸	2.86×10 ⁻¹⁰	2.89×10 ⁻⁰⁷	7.92×10 ⁻⁰⁹	1.64×10 ⁻¹⁰	1.82×10 ⁻⁰⁷	2.85×10 ⁻¹⁰	3.25×10 ⁻¹²	6.64×10 ⁻⁰⁹
90%ile	1.42×10 ⁻⁰⁷	4.17×10 ⁻⁰⁹	2.67×10 ⁻⁰⁶	2.99×10 ⁻⁰⁷	1.07×10 ⁻⁰⁸	5.78×10 ⁻⁰⁶	1.15×10 ⁻⁰⁷	3.55×10 ⁻⁰⁹	2.52×10 ⁻⁰⁶	4.55×10 ⁻⁰⁹	1.34×10 ⁻¹⁰	1.12×10 ⁻⁰⁷
95%ile	7.66×10 ⁻⁰⁷	1.92×10 ⁻⁰⁸	1.36×10 ⁻⁰⁵	3.23×10 ⁻⁰⁶	7.91×10 ⁻⁰⁸	6.73×10 ⁻⁰⁵	6.30×10 ⁻⁰⁷	1.78×10 ⁻⁰⁸	1.61×10 ⁻⁰⁵	2.18×10 ⁻⁰⁸	6.73×10 ⁻¹⁰	5.48×10 ⁻⁰⁷
97.5%ile	3.88×10 ⁻⁰⁶	8.05×10 ⁻⁰⁸	7.58×10 ⁻⁰⁵	2.19×10 ⁻⁰⁵	4.69×10 ⁻⁰⁷	3.67×10 ⁻⁰⁴	3.72×10 ⁻⁰⁶	6.66×10 ⁻⁰⁸	8.70×10 ⁻⁰⁵	1.20×10 ⁻⁰⁷	2.49×10 ⁻⁰⁹	3.60×10 ⁻⁰⁶
99%ile	2.94×10 ⁻⁰⁵	8.32×10 ⁻⁰⁷	4.64×10 ⁻⁰⁴	9.64×10 ⁻⁰⁵	3.13×10 ⁻⁰⁶	1.51×10 ⁻⁰³	3.01×10 ⁻⁰⁵	6.78×10 ⁻⁰⁷	5.19×10 ⁻⁰⁴	9.96×10 ⁻⁰⁷	2.24×10 ⁻⁰⁸	2.36×10 ⁻⁰⁵

*IC: Immunocompromised; LB: Lower bound of the CI95%, uncertainty dimension; UB: Upper bound

10.1.3. Mitigations for Raw-Milk Cheese

In this section, risk results from raw-milk soft-ripened cheese made under several mitigation alternatives:

- no restriction on aging time;
- unspecified 3, 4, 5, and 6 log₁₀ *L. monocytogenes* reduction in raw milk before cheese-making;
- use of an unspecified substance that reduces surface contamination by 2 log₁₀; and,
- testing bulk milk and cheese lots.

These alternatives, applied one at a time, are examined for farmstead- and artisanal-scale operations and are compared, for the Elderly population in Canada or the U.S., to the following baselines:

- a “Pasteurized-milk cheese Baseline” (section 9); and,
- a “Raw-milk cheese Baseline” (farmstead-scale operations, with no milk pasteurization, under the current 60 day aging regulation).

Environmental contamination at the frequency and levels derived in previous sections are present in all alternatives and baseline cases examined.

Following *Codex alimentarius*, U.S. and Canadian recommendations, evaluations of the availability, feasibility and cost of mitigations are done, not as part of the risk assessment (this report), but externally to the risk assessment, as part of the risk management that the risk assessment would inform. For example, the risk assessment does not consider the availability of a specific milk mitigation alternative that achieves a 3, 4, 5, or 6 log₁₀ reduction in *L. monocytogenes* concentration in bulk milk, nor the availability of an antimicrobial substance that achieves a 2 log₁₀ reduction, nor the feasibility and cost of testing some or all bulk milk prior to cheese making, nor the feasibility and cost of testing some or all cheese lots.

No Restriction on the Aging Duration for Soft-ripened Cheeses

We simulate the effects of a storage time comparison by defining 2 soft-ripened cheese manufacturer aging time distributions

- No restrictions: unrestricted cheese manufacturer storage time is inferred from the aging time that expert elicitation gave for pasteurized-milk cheese; and,
- 60 days regulation: storage time is inferred from regulated minimum storage time and time in transport & marketing stages

and make cheeses' storage times otherwise subject to the same transport, distribution, retail and consumer storage times. We assume that all other practices for soft-ripened cheese manufacture and storage are the same, whichever aging time scenario is followed.

The mean risk of listeriosis when there is no regulatory minimum for the aging time for soft-ripened cheese is approximately one-half to two-thirds the mean risk for the baseline for raw-milk cheese, for which a minimum 60 day aging regulation is in force (Table 59, bottom rows; 0.67, farmstead-scale in Canada, 0.56 in the U.S. to 0.57 artisanal-scale operations in Canada and 0.55 in the U.S.). The mean risk and median risk remain much higher than for pasteurized-milk soft-ripened cheese (Table 59, top rows): 36 times higher in Canada and 62 times higher in the U.S. for the mean risk under farmstead-scale operations; and, 52 times higher in Canada and 97 times higher in the U.S. for artisanal-scale operations. The mean risk and median risk per serving at random from raw-milk cheeses remain higher for cheeses from artisanal-scale operations than for cheeses from farmstead-scale operations.

Table 59: Relative size of mean and median from the distribution for risk per serving at random of soft-ripened cheese made from raw milk when there is no restriction on the aging duration.

Baseline case	Statistic	Canada Farmstead	Canada Artisanal	United States Farmstead	United States Artisanal
Pasteurized-milk cheese	<i>dMedian</i>	56	70	113	135
Pasteurized-milk cheese	<i>dMean</i>	36	52	62	97
Raw-milk cheese	<i>dMedian</i>	0.15	0.14	0.14	0.14
Raw-milk cheese	<i>dMean</i>	0.67	0.57	0.56	0.55

Sixty days aging can lead to more *L. monocytogenes* growth in contaminated cheeses, where conditions permit. There are smaller median and mean probability of illness among consumer servings when there is no regulated minimum storage time rather than a minimum 60 day storage time requirement for a soft-ripened cheese that does permit growth, frequently, in both cheese rind and in cheese core, under the conditions detailed here for cheese aging storage time and temperature.

Under conditions where *L. monocytogenes* does not grow during cheese aging, the selection of either aging model is neutral; both lead to the same distribution for risk per serving at random. Under conditions where *L. monocytogenes* declines during aging, there would be lower probability of illness when there is a minimum storage time rather than no minimum, but, for soft-ripened cheese, no decrease in the *L. monocytogenes* population in contaminated cheeses is expected to occur during aging. It is important to note that this risk assessment relates only to the risk presented by *L. monocytogenes* and for soft-ripened cheese. A complete assessment of the impact of a minimum 60-day aging regulation would also consider the impact on the risk from pathogens other than *L. monocytogenes* and for cheese other than soft-ripened cheese.

Three log₁₀ Reduction

We consider a mitigation strategy that is applied to the raw milk at the beginning of cheese manufacturing that would reduce the *L. monocytogenes* population in the raw milk by three logs, which we apply as detailed in section 6.2.

Results for farmstead-scale operations (Table 60) suggest that this mitigation strategy reduces the mean risk by a factor of approximately 7-10 (1/0.14 in Canada and 1/0.10 in the U.S.), and reduces the median risk by a factor of 27-40 (1/0.036, Canada; 1/0.025, U.S.) compared to the baseline for raw milk (Table 60). In more heavily contaminated milk, milk-source

L. monocytogenes that survive the mitigation can grow to high levels and have more influence on the risk than does in plant environment-sourced *L. monocytogenes*.

This mitigation strategy leads to a mean risk of invasive listeriosis that remains high compared to the mean risk from pasteurized-milk cheeses (from 14 times higher for raw-milk cheeses from farmstead-scale operations in Canada to 29 times higher for raw-milk cheeses from artisanal-scale operations in the U.S.).

Table 60: Relative size of mean and median from the distribution for risk per serving at random of soft-ripened cheese made from raw milk when an under 3 log₁₀ reduction mitigation is applied to bulk raw milk.

Baseline case	Statistic	Canada	Canada	United States	United States
		Farmstead	Artisanal	Farmstead	Artisanal
Pasteurized-milk cheese	<i>dMedian</i>	14	19	20	29
Pasteurized-milk cheese	<i>dMean</i>	7.4	11	11	17
Raw-milk cheese	<i>dMedian</i>	0.036	0.038	0.025	0.031
Raw-milk cheese	<i>dMean</i>	0.14	0.12	0.10	0.10

Risk Management Options Besides Pasteurization¹⁶

The animal husbandry and microbiological literature attributes various strategies for mitigation of pathogen contamination of bulk milk as raw material for cheese-making, but it poorly translates those mitigations into quantitative reductions in *L. monocytogenes* prevalence/concentration. Thus, this risk assessment compares risk results that would follow from changes to the *L. monocytogenes* prevalence/concentration of bulk milk, without attributing those changes to specific mitigation strategies.

We expanded the risk assessment to include raw-milk cheese scenarios that apply a 4 log₁₀ reduction, a 5 log₁₀ reduction and a 6 log₁₀ reduction to the level of *L. monocytogenes* contamination in contaminated bulk milk destined for raw-milk cheese manufacture by (unspecified) processes to all bulk milk destined for raw-milk cheese manufacture. Results (Table 61) suggest that the mean predicted level of risk per serving of raw-milk soft-ripened cheese for the Elderly populations in Canada and in the U.S. would be slightly higher than for

¹⁶ This section was added in response to public comments to the draft version of this report.

pasteurized-milk cheese when a 5 log₁₀ reduction is applied to the farm bulk tank concentration in *L. monocytogenes*. A 6 log₁₀ reduction in concentration in the bulk tank milk would lead to a mean predicted risk equivalent to or lower than the mean predicted risk for the pasteurized-milk cheeses.

How to achieve such levels of log₁₀ reduction as systematically as through a pasteurization process, and how to control such a process, is outside the scope of this risk assessment.

Table 61: Relative size of mean and median from the distribution for risk per serving at random of soft-ripened cheese made from raw milk when an under 4 log₁₀, 5 log₁₀ and 6 log₁₀ reduction mitigation is applied to bulk tank milk, farmstead scale operations.

Alternative vs. Baseline	Baseline: Raw-milk cheese			Baseline: Pasteurized-milk cheese		
	Statistic	Canada	U.S.	Statistic	Canada	U.S.
4 log ₁₀ reduction	<i>dMedian</i>	0.003	0.002	<i>dMedian</i>	1.1	1.5
4 log ₁₀ reduction	<i>dMean</i>	0.028	0.020	<i>dMean</i>	1.7	2.0
5 log ₁₀ reduction	<i>dMedian</i>	0.002	0.001	<i>dMedian</i>	0.65	0.79
5 log ₁₀ reduction	<i>dMean</i>	0.018	0.011	<i>dMean</i>	1.1	1.2
6 log ₁₀ reduction	<i>dMedian</i>	0.001	0.001	<i>dMedian</i>	0.55	0.60
6 log ₁₀ reduction	<i>dMean</i>	0.014	0.008	<i>dMean</i>	0.84	0.80

*Antimicrobial Substance*¹⁷

An alternative mitigation suggested by some comments to the draft version of this report would be the use of an antimicrobial substance on the surface of the cheese to limit the growth of, or reduce, the *L. monocytogenes* bacterial population.

To obtain an effect of the order of magnitude needed to get to a level of risk similar to the one of the pasteurized-milk cheese baseline, we tested the impact of a hypothetical substance (an antimicrobial voluntarily added during the manufacture of the raw-milk cheese) that would reduce the *L. monocytogenes* concentration present at the surface of the cheese by 2 log₁₀ cfu, *i.e.* in the order of magnitude of what could be expected for such effect (Guenther and Loessner 2011). The mean risk of invasive listeriosis per serving at random of such raw-milk cheeses would be 50 and 86 times higher than the risk per serving at random of pasteurized-milk cheese,

¹⁷ This section was added in response to public comments on the draft version of this report.

as estimated in the pasteurized-milk cheese baseline, in Canada and the U.S., respectively (Table 62).

The availability of such an antimicrobial substance, how such a substance could be used in raw-milk soft-ripened cheese (including applicable regulatory requirements), and how such an effect could be obtained, systematically, in raw-milk soft-ripened cheese is out of the scope of this risk assessment.

Table 62: Risk of invasive listeriosis per serving of soft-ripened cheese made from raw milk. Baseline for soft-ripened cheese made from raw milk (report) vs. addition of a substance that reduces the surface contamination by 2 log₁₀.

Baseline case	Statistic	Canada	United States
Pasteurized-milk cheese	<i>dMedian</i>	96	188
Pasteurized-milk cheese	<i>dMean</i>	50	86
Raw-milk cheese	<i>dMedian</i>	0.25	0.24
Raw-milk cheese	<i>dMean</i>	0.83	0.82

10.2. Testing Bulk Milk and Cheese Lots

Testing bulk milk or cheese lots is considered as an alternative mitigation, using the model and assumptions provided in section 6.5. Milk testing can occur at various places within the process.

We considered for farmstead production (one single farm):

- Milk testing at the farm level, with one test at every milk collection; or
- Cheese lot testing.

We considered for artisanal production (two farms):

- Farm milk testing with one test at every milk collection;
- Farm milk testing with one test at every farm;
- Dairy silo testing; or,
- Cheese lot testing.

The bulk milk testing alternatives have volume tested (25 ml), single *L. monocytogenes* detection probability (0.75) and test frequency (100% of farms, milk collections, dairy silos) in common.

The cheese lot testing alternative has 100% of cheese lots tested; test applied to 25 g made of 5 g

from each of 5 cheeses at random from the lot¹⁸; and 75% single *L. monocytogenes* detection probability. In all scenarios, in-plant environmental *L. monocytogenes* contaminate approximately 2.5% of the cheeses in an environmentally-contaminated lot, as in the baseline models.

Risk results calculated for the testing scenarios assume that farms, milk collections, dairy silos or cheese lots detected positive for *L. monocytogenes* are diverted from human consumption. Risk results ignore the collateral effects on *L. monocytogenes* risk from tests for other pathogens and for milk or cheese quality.

Table 63, for the Elderly population in Canada, and Table 64, for the Elderly population in the U.S., report the change in the median and mean risk per serving at random under these testing procedures as mitigations, individually, relative to the median and mean risk for pasteurized-milk cheeses and raw-milk cheeses with no mitigations.

The impact of the testing procedure on the risk varies with the country (bulk milk prevalence), the production scale (farmstead, artisanal) and the place in the process where testing occurs. Implementing a testing procedure consistently leads to lower mean and median risks per serving at random than a baseline for raw-milk cheese that has no testing component. Cheese lot testing results in a greater reduction in the mean and median risks per serving than any of the bulk milk testing alternatives, and is the only alternative that reduces the mean and median risks per serving for raw-milk cheese below the pasteurized-milk cheese baseline scenario.

¹⁸ This protocol is considered for risk assessment purposes as representative of a typical protocol likely to be used by manufacturers. It is more sensitive (less false negative results) than sampling a single analytical portion of 25 g from the same lot. Nevertheless, it is different from, and less sensitive than the composite protocol (*i.e.* 50 g from each of 5 cheeses for each composite and two composites from the lot) recommended in the FDA Bacteriological Analytical Manual (BAM) (FDA 2011) for the detection of *Listeria monocytogenes* in cheese products that is typically used for regulatory purposes.

10.2.1. Bulk Milk Testing

Testing bulk milk does reduce the mean risk of listeriosis per serving of soft-ripened cheese made with raw milk. Nevertheless, no strategy leads to a mean risk lower than the risk linked to the consumption of pasteurized-milk soft-ripened cheese. For example, for farmstead-scale operations in Canada (Table 63),

- the mean risk per raw-milk soft-ripened cheese serving at random is approximately 24 times smaller (1/0.042) when every milk collection is tested for *L. monocytogenes*, than when no testing is done on milk used to produce raw-milk soft-ripened cheese;
- the median risk per raw-milk soft-ripened cheese serving at random is approximately 82 times smaller (1/0.012) when every milk collection is tested for *L. monocytogenes*, than when no testing is done on milk used to produce raw-milk soft-ripened cheese.

Nevertheless, for farmstead-scale operations in Canada,

- the mean risk per raw-milk soft-ripened cheese serving at random, when every milk collection is tested for *L. monocytogenes*, is still 2.2 times higher than the mean risk per pasteurized-milk soft-ripened cheese serving;
- the median risk per raw-milk soft-ripened cheese serving at random, when every milk collection is tested for *L. monocytogenes*, is still 4.6 times higher than the median risk per pasteurized-milk soft-ripened cheese serving.

Table 63: Impact of testing bulk milk or soft-ripened cheese lots on the risk per serving, relative to the risk per serving of baseline cases for Elderly population in Canada.

Stage of processing	Baseline: Pasteurized- milk cheese			Baseline: Raw-milk cheese		
		Farmstead	Artisanal		Farmstead	Artisanal
Farm level, test every milk collection	<i>dMedian</i>	4.6	4.5	<i>dMedian</i>	0.012	0.009
Farm level, test every milk collection	<i>dMean</i>	2.2	2.3	<i>dMean</i>	0.042	0.025
Farm level, test at every farm	<i>dMedian</i>	-	10.0	<i>dMedian</i>	-	0.020
Farm level, test at every farm	<i>dMean</i>	-	6.4	<i>dMean</i>	-	0.071
Dairy silo	<i>dMedian</i>	4.2	6.6	<i>dMedian</i>	0.011	0.013
Dairy silo	<i>dMean</i>	2.0	3.1	<i>dMean</i>	0.038	0.034
Cheese lots	<i>dMedian</i>	0.163	0.575	<i>dMedian</i>	0.000	0.001
Cheese lots	<i>dMean</i>	0.080	0.390	<i>dMean</i>	0.002	0.004

Table 64: Impact of testing bulk milk or soft-ripened cheese lots on the risk per serving, relative to the risk per serving of baseline cases for Elderly population in the U.S.

Stage of processing	Baseline:			Baseline:		
	Pasteurized-milk cheese	Farmstead	Artisanal	Raw-milk cheese	Farmstead	Artisanal
Farm level, test every milk collection	<i>dMedian</i>	5.3	5.2	<i>dMedian</i>	0.007	0.006
Farm level, test every milk collection	<i>dMean</i>	3.0	2.9	<i>dMean</i>	0.027	0.016
Farm level, test at every farm	<i>dMedian</i>	-	15.4	<i>dMedian</i>	-	0.016
Farm level, test at every farm	<i>dMean</i>	-	8.9	<i>dMean</i>	-	0.051
Dairy silo	<i>dMedian</i>	4.7	8.6	<i>dMedian</i>	0.006	0.009
Dairy silo	<i>dMean</i>	2.3	4.3	<i>dMean</i>	0.021	0.025
Cheese lots	<i>dMedian</i>	0.242	1.036	<i>dMedian</i>	0.000	0.001
Cheese lots	<i>dMean</i>	0.134	0.672	<i>dMean</i>	0.001	0.004

Similarly, for farmstead-scale operations in the U.S. (Table 64),

- the mean risk per raw-milk soft-ripened cheese serving at random is approximately 37 times smaller ($1/0.027$) when every milk collection is tested for *L. monocytogenes*, than when no testing is done on milk used to produce raw-milk soft-ripened cheese;
- the median risk per raw-milk soft-ripened cheese serving at random is approximately 149 times smaller ($1/0.007$) when every milk collection is tested for *L. monocytogenes*, than when no testing is done on milk used to produce raw-milk soft-ripened cheese.

For farmstead-scale operations in the U.S.,

- the mean risk per raw-milk soft-ripened cheese serving at random, when every milk collection is tested for *L. monocytogenes* is still 3.0 times higher than the mean risk per pasteurized-milk soft-ripened cheese serving;
- the median risk per raw-milk soft-ripened cheese serving at random, when every milk collection is tested for *L. monocytogenes* is still 5.3 times higher than the median risk per pasteurized-milk soft-ripened cheese serving.

The relative effect of testing farms (once) rather than testing every milk collection (Table 63, Table 64, artisanal-scale, 1st 2 sets of rows) holds when *L. monocytogenes* contamination in milk is a sporadic rather than a persistent phenomenon. The microbiological and animal husbandry literature documents both cases: where *L. monocytogenes* contaminated bulk milk was observed (detected) only sporadically among longitudinal studies at each farm of a group of farms; and, where *L. monocytogenes* bulk milk was observed (detected) persistently or sporadically among

longitudinal studies of only some farms and rarely or not at all among other farms in the same group of farms (Hassan et al. 2000; Hassan et al. 2001; Meyer-Broseta et al. 2003; Nightingale et al. 2004; Nightingale et al. 2005; D'Amico et al. 2008b).

10.2.2. Cheese Lot Testing

Pasteurized-milk Cheese

The impact of testing lots of cheeses made from pasteurized milk on the mean and median risks per serving (Table 65) is small (less than 10%), when in-plant environmental contamination occurs at relatively low frequency (2.5% of cheeses within an environmentally contaminated lot). Testing cheese lots removes cheeses that have high levels of *L. monocytogenes* contamination, thereby reducing the mean risk, but has little or no effect on the median risk.

Table 65: Risk of invasive listeriosis per serving of soft-ripened cheese made from pasteurized milk: relative risk when cheese lot testing is implemented.

Canada	Elderly	Pregnant	IC*	General	U.S.	Elderly	Pregnant	IC*	General
<i>dMedian</i>	1.1	1.1	1.1	1.1	<i>dMedian</i>	1.1	1.1	1.1	1.1
<i>dMean</i>	0.8	0.8	0.8	0.8	<i>dMean</i>	0.7	0.8	0.8	0.8

IC*: Immunocompromised.

Raw-milk Cheese

The mean and median risks per raw-milk cheese serving at random with testing cheese lots as a mitigation, under farmstead-scale operations, are smaller than the mean and median risks per serving from pasteurized-milk cheeses that are not subjected to cheese lot testing: for Canada, the mean risk is 12-fold (1/0.080) lower for raw-milk soft-ripened cheese serving at random with testing cheese lots than for pasteurized-milk cheese; it is 7.4-fold (1/0.134) lower in the United-States. Cheese lot testing *i*) detects cheeses contaminated by both milk-source *L. monocytogenes* and cheeses contaminated by in-plant environment source *L. monocytogenes*; *ii*) more frequently detects contaminated cheese lots that contain cheeses with higher levels of milk-source contamination; and, *iii*) more frequently detects contaminated cheese lots that contain cheeses with higher levels of environment source contamination and cheese lots with higher rates of contaminated cheeses in a contaminated lot.

Those results hold also for cheeses made under artisanal-scale operations, the mean risk being 2.6 times ($=1/0.390$) lower for tested raw-milk cheese than for pasteurized-milk cheese in Canada, and 1.5 times ($=1/0.672$) lower in the U.S.

A graphical illustration of the mean and median risks per serving at random according to the various alternatives is proposed Figure 22.

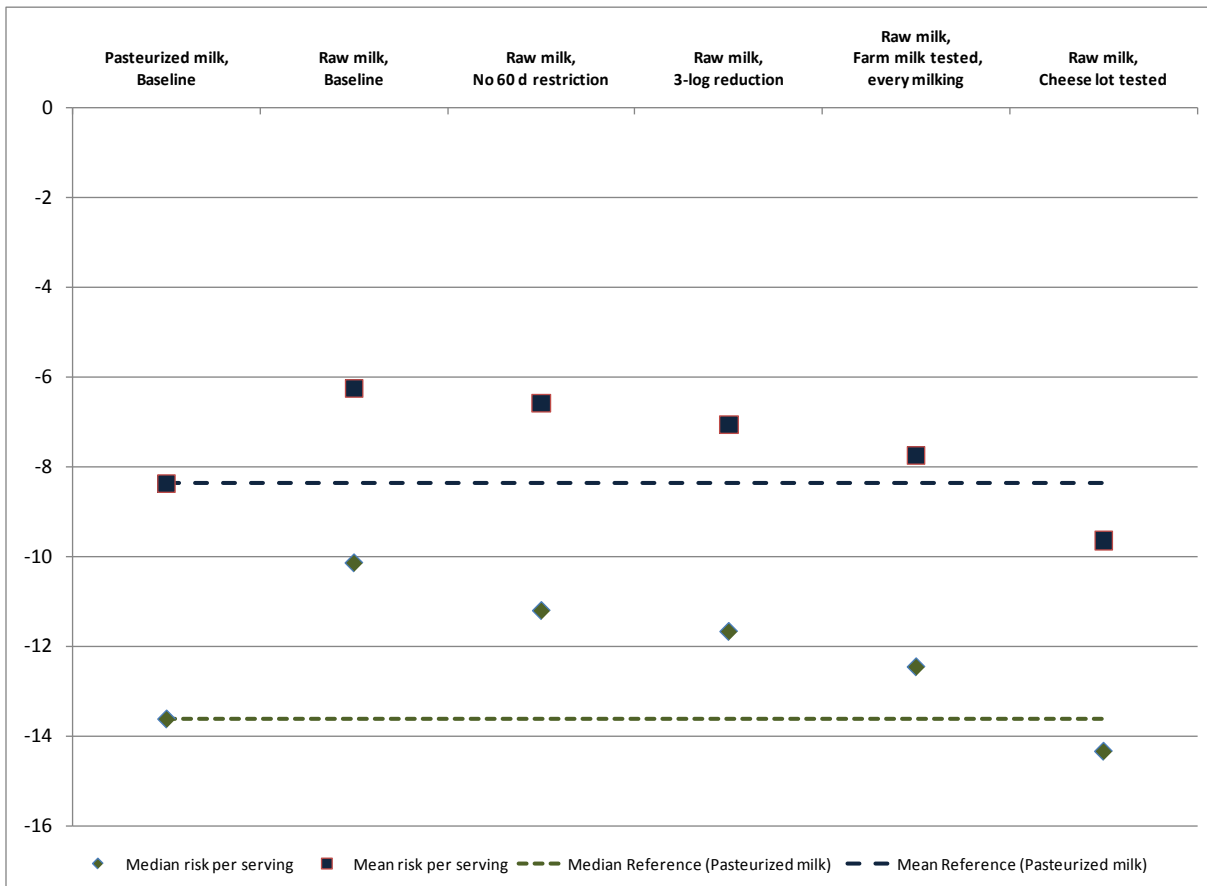


Figure 22: Log₁₀(median) (◆) and log₁₀(mean) (■) risk per serving at random for the Elderly population, Canada, comparing soft-ripened cheese made from pasteurized milk baseline, soft-ripened cheese made from raw milk baseline, farmstead raw-milk cheese without 60-day aging regulation, farmstead raw-milk cheese with a 3-log reduction of *L. monocytogenes* concentration in milk, farmstead raw-milk cheese with milk testing, farmstead raw-milk cheese with cheese lot testing. See text for details.

10.2.3. Testing Considerations

Section 10.2.1. 's bulk milk testing alternatives have several characteristics in common: volume tested (25 ml), single *L. monocytogenes* detection probability (0.75) and test frequency (100% of farms, milk collections, dairy silos). Cheese lot testing –100% of cheese lots tested; test applied

to 25 g made of 5 g from each of 5 cheeses at random from the lot; 75% single *L. monocytogenes* detection probability—is applied in the case where in-plant environmental *L. monocytogenes* contaminate 2.5% of the cheeses in an environmentally-contaminated lot.

We tested the impact of these assumptions and testing design, by evaluating the risk per serving at random from tested raw-milk cheese from artisanal-scale operations if:

- For farm milk testing:
 - o the volume tested was 125 ml, compared to 25 ml in the baseline testing case;
 - o the single *L. monocytogenes* detection probability was 0.50 or 0.90, compared to 0.75 in the baseline testing case; and,
 - o 95% of bulk milk is tested, rather than 100% in the baseline testing case.
- For cheese lot testing:
 - o the mass tested was 125 g, including 25 g from 5 cheeses, compared to 25 g (5 g × 5 cheeses) in the baseline testing case;
 - o the tested 25 g originated from 1 single cheese, compared to 5 cheeses in the baseline testing case;
 - o the single *L. monocytogenes* detection probability was 0.50 or 0.90, compared to 0.75 in the baseline testing case;
 - o in-plant environmental *L. monocytogenes* contaminates 0.5% or 1% of the cheeses in an environmentally-contaminated lot, compared to 2.5% in the baseline testing case; and,
 - o 95% of cheese lots are tested, rather than 100% in the baseline testing case.

The results (Table 66, Figure 23 and Figure 24) suggest that the efficiency of testing as a mitigation strategy is only slightly impacted by a change to many test protocol parameters. One exception is the mass of tested cheese: testing $5 \times 25\text{g} = 125\text{ g}$ is much more efficient than testing $5 \times 5\text{g} = 25\text{g}$. Whatever the change made in these parameters, the testing of dairy silo milk leads to higher mean and median risks of invasive listeriosis than cheese made from pasteurized milk with no testing. There is a predicted lower mean risk for servings of raw-milk cheese subjected to cheese lot testing than servings of pasteurized-milk cheese without testing, provided that all cheese lots are tested. Among the scenarios we evaluated, these results hold only if 100% of lots are tested: results suggest that, if 95%, rather than 100%, of lots are tested,

the impact of the testing mitigation decreases dramatically, leading to a higher mean risk than pasteurized-milk cheeses.

Table 66: Impact of parameters of testing bulk milk or soft-ripened cheese lots on the risk per serving, relative to the risk per serving of baseline testing or pasteurized-milk soft-ripened cheese for Elderly population in Canada and the U.S.

Alternative vs. Baseline Farm bulk milk testing	Baseline: Baseline Testing		Baseline: Pasteurized-milk cheese, no testing			
	Canada	U.S.	Canada	U.S.		
Baseline farm bulk testing	<i>dMedian</i>	1 (ref)	1 (ref)	<i>dMedian</i>	4.63	5.31
Baseline farm bulk testing	<i>dMean</i>	1 (ref)	1 (ref)	<i>dMean</i>	2.22	2.99
125 ml vs. 25 ml	<i>dMedian</i>	0.74	0.63	<i>dMedian</i>	3.42	3.36
125 ml vs. 25 ml	<i>dMean</i>	0.80	0.62	<i>dMean</i>	1.79	1.86
Single <i>L. monocytogenes</i> detection probability: 0.50 vs. 0.75	<i>dMedian</i>	1.30	1.43	<i>dMedian</i>	6.01	7.59
Single <i>L. monocytogenes</i> detection probability: 0.50 vs. 0.75	<i>dMean</i>	1.26	1.24	<i>dMean</i>	2.79	3.71
Single <i>L. monocytogenes</i> detection probability: 0.90 vs. 0.75	<i>dMedian</i>	0.92	0.88	<i>dMedian</i>	4.24	4.66
Single <i>L. monocytogenes</i> detection probability: 0.90 vs. 0.75	<i>dMean</i>	0.95	0.83	<i>dMean</i>	2.11	2.49
95% bulk milk tested vs. 100% bulk milk tested	<i>dMedian</i>	2.06	2.72	<i>dMedian</i>	9.56	14.42
95% bulk milk tested vs. 100% bulk milk tested	<i>dMean</i>	2.60	2.48	<i>dMean</i>	5.77	7.41
90% bulk milk tested vs. 100% bulk milk tested	<i>dMedian</i>	3.50	5.29	<i>dMedian</i>	16.20	28.09
90% bulk milk tested vs. 100% bulk milk tested	<i>dMean</i>	3.65	4.84	<i>dMean</i>	8.09	14.47

Table : Impact of parameters of testing cheese lots on the risk per serving, relative to the risk per serving of baseline testing or pasteurized-milk cheese for Elderly population in Canada and the U.S.

Alternative vs. Baseline Cheese lots testing	Baseline: Baseline Test	Canada	U.S.	Baseline: Pasteurized- milk cheese, no testing	Canada	U.S.
Baseline	<i>dMedian</i>	1 (ref)	1 (ref)	<i>dMedian</i>	0.16	0.24
Baseline	<i>dMean</i>	1 (ref)	1 (ref)	<i>dMean</i>	0.08	0.13
125 g vs. 25 g	<i>dMedian</i>	0.0005	0.0026	<i>dMedian</i>	0.0001	0.0006
125 g vs. 25 g	<i>dMean</i>	0.0038	0.0047	<i>dMean</i>	0.0003	0.0006
1 Cheese vs. 5 Cheeses	<i>dMedian</i>	0.58	0.69	<i>dMedian</i>	0.09	0.17
1 Cheese vs. 5 Cheeses	<i>dMean</i>	0.77	0.72	<i>dMean</i>	0.06	0.10
Single <i>L. monocytogenes</i> detection probability: 0.50 vs. 0.75	<i>dMedian</i>	1.28	1.38	<i>dMedian</i>	0.21	0.33
Single <i>L. monocytogenes</i> detection probability: 0.50 vs. 0.75	<i>dMean</i>	1.51	1.37	<i>dMean</i>	0.12	0.18
Single <i>L. monocytogenes</i> detection probability: 0.90 vs. 0.75	<i>dMedian</i>	0.82	0.98	<i>dMedian</i>	0.13	0.24
Single <i>L. monocytogenes</i> detection probability: 0.90 vs. 0.75	<i>dMean</i>	0.86	0.96	<i>dMean</i>	0.07	0.13
In-plant env. <i>L. monocytogenes</i> contaminates: 1% vs. 2.5%	<i>dMedian</i>	0.82	1.15	<i>dMedian</i>	0.13	0.28
In-plant env. <i>L. monocytogenes</i> contaminates: 1% vs. 2.5%	<i>dMean</i>	1.10	1.22	<i>dMean</i>	0.09	0.16
95% cheese lots tested vs. 100% cheese lots tested	<i>dMedian</i>	59	79.49	<i>dMedian</i>	9.68	19.2
95% cheese lots tested vs. 100% cheese lots tested	<i>dMean</i>	24	23.83	<i>dMean</i>	1.94	3.20
90% cheese lots tested vs. 100% cheese lots tested	<i>dMedian</i>	160	218	<i>dMedian</i>	26.1	52.7
90% cheese lots tested vs. 100% cheese lots tested	<i>dMean</i>	44.4	46.9	<i>dMean</i>	3.57	6.31

(ref): reference case.

As well, Table 66 adds some precision to common-sense qualitative statements about testing protocols and the effect of testing on risk per serving at random. Particularly, testing *i*) larger analytical samples (125 ml vs. 25 ml bulk milk; 125 g vs. 25 g cheese); *ii*) with tests with higher sensitivity (single *L. monocytogenes* detection probability 0.5, 0.75 0.9) for bulk milk or cheese lots; *iii*) higher percentages of bulk milk or cheese lots (100% vs. 90% or 95%); or, *iv*) sample made from more than 1 cheese (1 cheese vs. 5 cheeses) leads to smaller median and smaller mean risk per serving at random. As well, testing more effectively detects contaminated cheese lots that have higher within-lot contamination prevalence than contaminated cheese lots that have lower within-lot contamination prevalence.

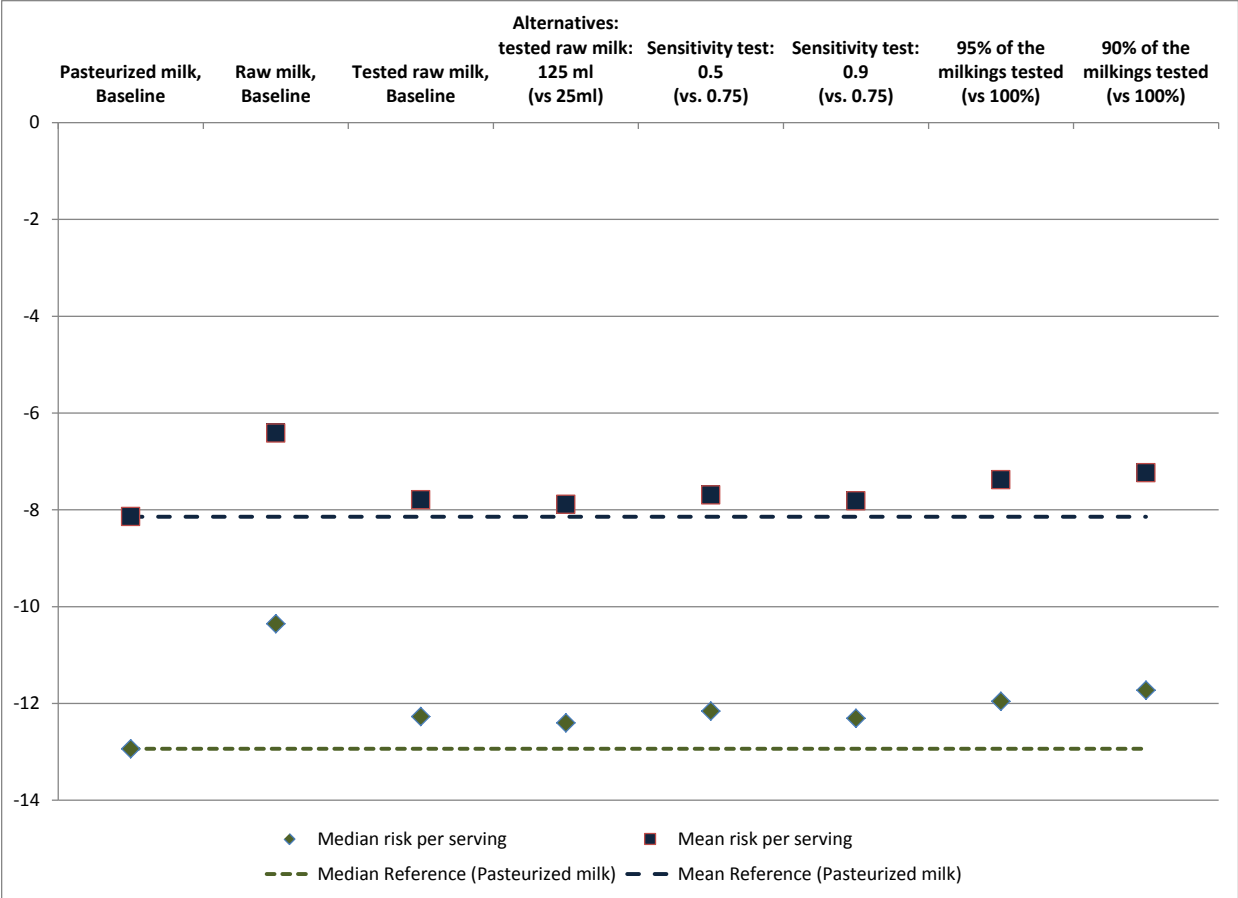


Figure 23: Log₁₀(median) (◆) and log₁₀(mean) (■) risk per serving at random for the Elderly population, Canada, comparing pasteurized-milk soft-ripened cheese baseline, farmstead raw-milk cheese with farm bulk milk tested (every milk collection) and alternatives. See text for details.

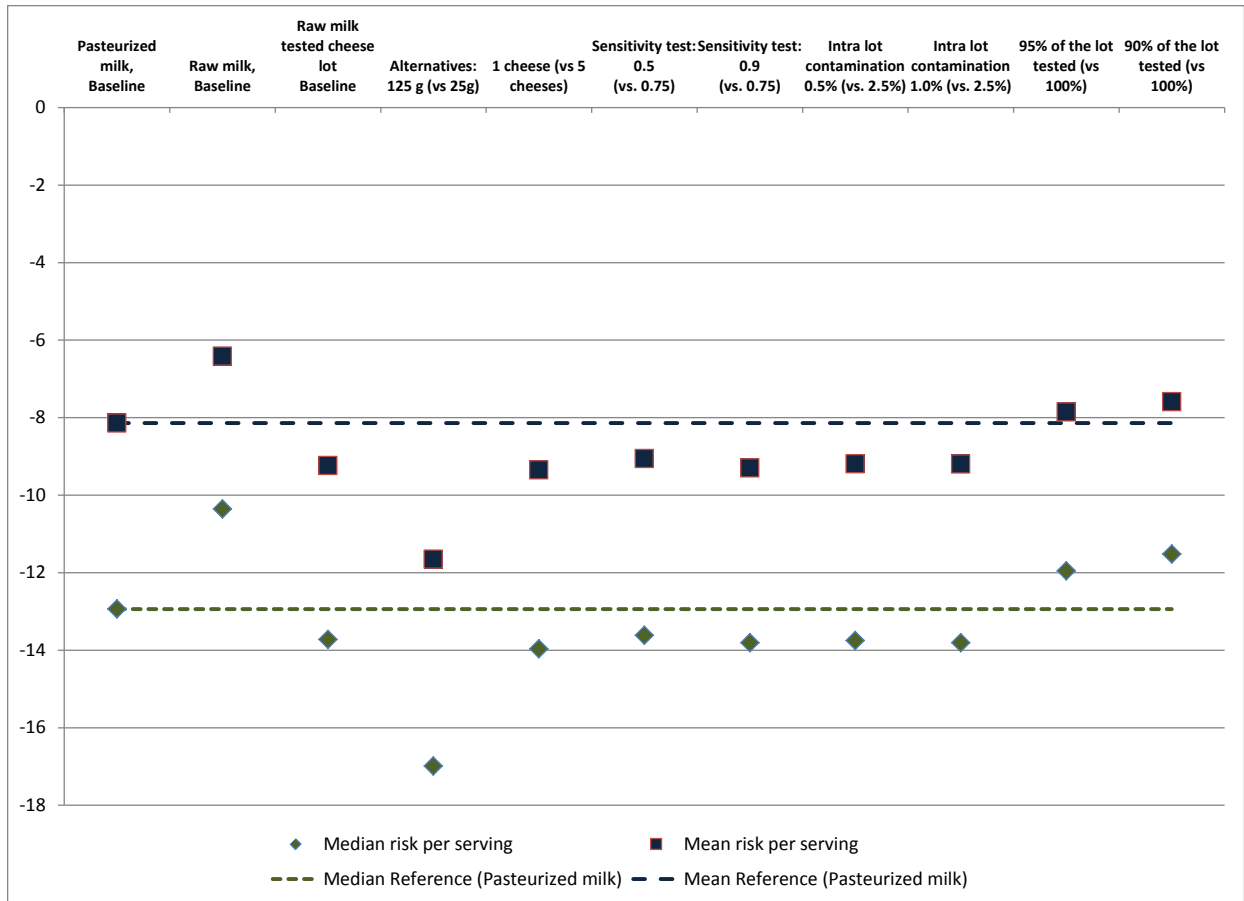


Figure 24: Log₁₀(median) (◆) and log₁₀(mean) (■) risk per serving at random for the Elderly population, Canada, comparing pasteurized-milk soft-ripened cheese baseline, farmstead raw-milk soft-ripened cheese with cheese lots tested and alternatives. See text for details.

11.Limitations, Caveats and Data Gaps

This quantitative risk assessment includes analysis of the available scientific information and data in the development of the exposure assessment of *L. monocytogenes* in soft-ripened cheese in Canada and in the U.S. and in the development of the hazard characterization’s dose-response function in susceptible and non-susceptible populations.

The model and, as a consequence, the results and conclusions of this study are limited to the pathogen, the type of cheese and the countries (Canada and U.S.) considered here. Facing a lack of available data, we did not evaluate the risk from consumption of semi-soft cheese as requested in the charge.

Cheese may be portioned before packaging, at the manufacturer or at retail, but this practice was not included in this risk assessment. All conclusions refer solely to the risk of invasive listeriosis from the presence of *L. monocytogenes* in the considered cheese: the assessment of any mitigation should consider, additionally, the potential impact of mitigations on other pathogens.

As in all risk assessments, results rely on inferences from limited data and on extrapolations

- over time; for example, from bulk tank surveys carried out in the 1990s to current day farm bulk tank characteristics and from nutrition surveys done in the early 2000s to present day;
- over space; for example, from observations on bulk tank milk concentrations reported from studies in the United States, Canada and Europe;
- from samples to sampling populations; for example from data set or results (sample) via the sample design to the sampling population; and,
- from a sampling population to the reference population of interest; for example,
 - from U.S. retail and home storage data to Canada;
 - from U.S. retail-level contamination levels and frequency to Canada;
 - from all Brie and Camembert cheese consumption to raw-milk Camembert cheese consumption;
 - from characteristics from women of child-bearing age to the same characteristics of pregnant women; and,
 - from laboratory to production-- from study populations to populations appropriate as a reference for this study.

Biases and uncertainty that those extrapolations introduce are unknown.

Indeed, it was not always possible to obtain some specific data for each country and for each subpopulation within each country. As a default, data obtained in one country were applied directly to the other one, whenever needed. Table 67 summarizes the level of variability that was distinguished for each of the major parameters of the model. Because of propagation of the variability within the model, the risk estimates are different for each subpopulation and each country. Nevertheless, only a part of the overall variability is eventually considered, due to the

lack of specific data. As an example, no difference in consumption was considered for Elderly, Pregnant women, Immunocompromised individuals or the General population in the U.S. Additional data that could better characterize these subpopulations are needed. Notably, the consumption data and the home storage conditions for people with the identified susceptibility are unknown; rather, we impute the same consumption and home storage characteristics among populations with the same or similar age-sex characteristics.

Table 67: Level of variability distinguished for process, data and estimates, according to subpopulation and country.

The same letter, same case indicates that the same data and distribution model are used for the subpopulations. Variability specifications also include full or at least partial model of data uncertainty.

Process / Data / Estimates	Canada Elderly	Canada Pregnant women	Canada IC*	Canada General	US Elderly	US Pregnant women	US IC*	US General
<i>L. monocytogenes</i> contamination in farm	A	A	A	A	B	B	B	B
<i>L. monocytogenes</i> contamination during processing	C	C	C	C	C	C	C	C
Milk and Cheese processing, transport and marketing, from farm to retail (inclusive)	D	D	D	D	D	D	D	D
<i>L. monocytogenes</i> growth model and parameters	E	E	E	E	E	E	E	E
Storage time at home	F	G	H	H	I	J	K	K
Storage temperature at home	L	M	N	N	L	M	N	N
Serving size	O	P	Q	Q	R	R	R	R
Resulting exposure assessment	S	T	U	U	V	W	X	X
Dose Response	Y	Y	Y	Z	Y	Y	Y	Z
Resulting Risk Assessment	a	b	c	d	e	f	g	h

* Immunocompromised.

The microbiological and epidemiological literature, this project's experts, industry and peer reviewers have pointed to lack of information about the non-milk contamination sources, appropriate to the type of cheeses manufactured as data gaps that a more complete model for risk assessment for soft-ripened cheeses would accommodate, notably:

- pre-process, e.g. during handling of milk, for raw-milk cheese;
- post-pasteurization, during handling of bulk milk or curds;
- during initial ripening, before packaging;
- at final packaging, either with intact, whole cheeses or when partitioning cheeses for final packaging;

- when partitioning and repackaging cheeses or when opened cheeses are on display at point of sale; and,
- during consumer storage.

Additionally, no information on the frequency of pasteurization failure is available.

However, the literature informs only anecdotally or only poorly about the frequency that such contamination occurs among marketed cheeses and how much the amount of contamination, when introduced, varies.

For example, inferences about the prevalence and level of in-plant environmental contamination in Canada and in the U.S. rely on a single study of contamination of ready-to-eat food at the retail level in U.S. (Gombas et al. 2003). It is used to infer among-cheese, in-plant environmental *L. monocytogenes* contamination; but there is incomplete information about the prevalence of contaminated lots and about the prevalence of contaminated cheeses within contaminated lots. Further, risk comparisons used the same prevalence and level of environmental contamination inferences for both industrial pasteurized-milk cheese and for farmstead or artisanal raw-milk cheese processing in the absence of information about any differences that may exist in the production methods used by large and small producers. Additional data on prevalence of *L. monocytogenes* in soft-ripened cheeses made from pasteurized milk from industrial, artisanal and farmstead-scale operations are needed to better define this environmental contamination.

Moreover, there is a notable lack of information about the differences in practices between large commercial cheese manufacturing operations and small farmstead cheese manufacturing operations. We considered specifically the “stabilization” process for commercial cheese and “traditional” process for farmstead and artisanal cheese. Nevertheless, there is a lack of information about the following:

- the time-temperature pattern and pH during the process of cheese-making; an expert elicitation provided expert opinions from two large soft-ripened cheese manufacturing facilities in the U.S., and suggests a great variability;

- how culture selection, ripening, aging, distribution, retail and home storage time and temperature characteristics and consumption characteristics differ between soft-ripened cheeses from large commercial cheese manufacturing operations and soft-ripened cheeses from smaller farmstead and artisanal cheese manufacturing operations; and
- how much annual consumption of soft cheese and raw-milk soft-ripened cheese differs among recognizable populations.

Predictive modeling was used to model the growth of *L. monocytogenes* in milk and in soft-ripened cheeses and the exposure assessment was based on information derived from those models. It is known that models may overestimate growth of *L. monocytogenes* in food, and so reliance on such a model can result in an overestimation of the risk (FAO/WHO 2004). The original meta-analysis developed for this study captures a synthesis of the information on that subject. However, the meta-analysis used here does not explicitly account for pH, water activity, lactic acid concentration and salts variability among milk and among cheeses that some models do account for; those models do not explicitly account for among strain, among *L. monocytogenes* within strain and among raw-material variability that these meta-analyses attempt to do.

This examination uses a particular dose-response model among many alternatives. No new data or model were acquired during this project: the FAO/WHO model (2004) was directly transposed. The choice of dose-response model can have an important effect on the calculated risk. For example, models that are concave at low doses (Farber et al. 1996; Bemrah et al. 1998) place more emphasis on the impact of higher doses than do those like the exponential model that are linear at low doses, while models that are convex at low doses (Williams et al. 2007) place a greater emphasis on the impact of low doses than models that are linear at low doses. Our choice of a dose-response model, then, affects how much mitigations change the risk distribution and change the risk distribution's median and mean, which we use to compare mitigations' effects. No specific, explicit consideration on the variability in the virulence of *L. monocytogenes* strains, as suggested elsewhere (Chen et al. 2006; Chen et al. 2011), was used.

More generally, there is considerable uncertainty in the dose-response model. The sensitivity analysis shows that, within the small part of the overall uncertainty that is considered here, the uncertainty surrounding the r parameter of the exponential dose-response dominates the uncertainty that we attribute to the risk results. A part of this uncertainty is naturally discarded, within this risk assessment, when alternatives are compared to the baseline models. The use of relative risk, as a metric to estimate risk mitigation strategies, may indeed be less sensitive to the specific choice of dose response, as long as its general shape is correct (considered here as linear on almost all the range of exposure). Nevertheless, the absolute values obtained in this risk assessment should not be compared with other results obtained using a different dose-response model without some caution.

Only a small part of the overall uncertainty is considered in this study, while it is recognized that there are many other types of uncertainty in risk assessments. Uncertainty includes data uncertainty (measurement errors, sampling errors, systematic errors), model uncertainty (uncertainty due to necessary simplification of real-world processes, mis-specification of the model structure, model misuse, use of inappropriate surrogate variables, use of simplifying assumptions about appropriate methods of inference from data in the microbiological literature to the real-world phenomena that they stand for), estimator uncertainty (derivation of risk outputs by simulation methods, in simulations of finite sizes) and scenario uncertainty (descriptive errors, aggregation errors, errors in professional judgment, incomplete analyses) (US EPA 1997). While our results suggest that the sources of uncertainty that we have considered and accounted for are less important than the sources of variability, absolute values should be considered only with some caution.

Additional technical discussions on limitations and caveats are provided in the corresponding appendix, specific to context: basic representation of the basic processes; basic representation of the cheese-making process; growth models; environmental contamination models; bulk tank milk prevalence models; bulk tank milk concentration models; dose-response models and alternatives; simplifying assumptions made for processes; design information in the literature; among unit (bulk milk, cheese, servings) variability; measures of importance and specification of uncertainty.

We conclude that no critical data gaps impair the conclusions of this risk assessment. Some additional data would decrease the uncertainty in the estimate, but we conclude that this risk assessment is valid, considering its measures of uncertainty, its limitations, and its caveats, as specified.

This risk assessment answers the management charge for soft-ripened cheese (see Appendix, section “Charge developed by the Risk Manager Team”) set by the FDA and the Health Canada risk managers. The results inform risk managers about managing risk of invasive listeriosis from the consumption of soft-ripened cheese. Nonetheless, its choices for baselines and simplifications for risk managers depart from some real-world scenarios. For example, a straightforward baseline that incorporates “full pasteurization” for pasteurized-milk cheeses sets aside the effects of pasteurization process failures, whose consequences have been already examined in the epidemiological literature.

The model (and its Analytica™ implementation) is available for studying other scenarios, and could be updated with other data on soft-ripened cheeses whenever available, including, but not limited to:

- specifying cheese contamination characteristics at retail (Gombas et al. 2003);
- specifying cheese processing characteristics and *L. monocytogenes* growth characteristics appropriate to other cheeses;
- specifying alternative aging, transport and marketing, retail and consumer storage time and temperature characteristics;
- specifying contamination amounts, frequency and occurrence for environmental contamination at several contamination points; and,
- using alternative dose-response models.

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