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

Food Directorate / Direction des aliments

Health Canada / Santé Canada

Center for Food Safety and Applied Nutrition

Food and Drug Administration

U.S. Department of Health and Human Services

2012

Contributors

Canada	United States
Risk Assessment Team	
Mark Smith	Régis Pouillot
William Ross	Sherri Dennis
Loan Nguyen	Steve Gendel[1]
	Clarence Murray III
Risk Management Team	
Hélène Couture	Vincent Bunning
Jeff Farber	Ted Elkin
André Jean	Kathy Gombas
	John Sheehan
	Donald Zink

Risk Communication

Susan Cahill

Information Specialist

Lori Papadakis

[1] Until 2010.

Acknowledgments

The following organizations and individuals are acknowledged for their contributions to this project:

- Former FDA/CFSAN risk assessors and risk managers David Carlson, Richard C. Whiting and Robert L. Buchanan for their participation in the early stage of the project;
- Greg Paoli for the organization of an applied training in Analytica®;
- The International Dairy Foods Association (Carry Frye) for the organization of a field trip in a cheese processing facility;
- Versar, Inc (David Bottimore, Kathy Coon, Stephanie Sarraino) for their organization of the Peer review;
- József Baranyi, James S. Dickson, Cary Frye and Ralph L. Kodell for their peer review of the draft report document and the draft model;
- Angela Catford for her review and suggestions for the final draft report.

- This work was supported in part by an appointment to the Research Participation Program at the Center for Food Safety and Applied Nutrition administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the U.S. Food and Drug Administration.

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

http://www.fda.gov/food/scienceresearch/researchareas/riskassessmentsafetyassessment/

Table of Contents

List of Tables

List of Figures

Abbreviations and Acronyms

a_w	Water activity
CDC	Center 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
MC	Monte-Carlo
m.l.e.	maximum likelihood estimator
MPN	Most Probable Number
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(μ, σ) or *N*(μ, σ)	Normal (Gaussian) distribution with mean μ and standard deviation σ
ln(*x*)	Natural (base *e*) logarithm of *x*
logNormal(μ, σ) or *LN*(μ, σ)	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*
logit(*p*)	ln(*p*/(1-*p*))
Poisson(λ)	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(μ, σ, *a*, *b*)	Truncated normal distribution *i.e.* *N*(μ, σ) 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, 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 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.

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: Camembert, as an example of 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 Camembert-like cheese. A fully quantitative approach is taken and mathematical / probabilistic modeling is employed to estimate the risk per serving of Camembert-like 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

[2] Available at http://www.fda.gov/food/scienceresearch/researchareas/riskassessmentsafetyassessment/

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").

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 baseline model is first developed and studied. This baseline model serves as a reference to compare the risk under other alternative scenarios.

Baseline model

The baseline model considers the manufacture of soft-ripened cheese (Camembert-like) made from pasteurized milk, using the stabilized cheese process (Kosikowski and Mistry 1987; Lawrence *et al.* 1987). 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 Camembert. 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.

Camembert 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 i 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 i: 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				United States			
	Elderly	Pregnant women	IC*	General	Elderly	Pregnant women	IC*	General
L. monocytogenes contamination in farm	A	A	A	A	B	B	B	B
L. monocytogenes 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
L. monocytogenes 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.

Alternative scenarios

Alternative scenarios deal with the manufacture of Camembert-like cheese made from raw milk, using traditional process (Sanaa *et al.* 2004). 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 aging is modeled using environmental parameters of "traditional process" (Sanaa *et al.* 2004), in contrast to the "stabilized process" used for industrialized cheeses in the 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.08.044[3] and 21 CFR 133.182(a)[4], respectively). For raw-milk cheeses, two cases are illustrated: Farmstead operations, where milk is collected for cheese-making from a single herd, on the farm where the cheese-making operation resides; and artisanal-scale operations, where milk for cheese-making is collected from 2 farms and pooled.

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 a treatment procedure that reduces the bacterial load in milk by 3 $\log_{10}$ (*i.e.* an average 1,000-fold reduction in the concentration);
- 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 composite sample of 25 g from 5 cheeses of every cheese lot, 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

[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

assessment would inform. For example, the risk assessment does not consider the availability of a specific milk mitigation alternative that achieves a 3 $\log_{10}$ reduction in *L. monocytogenes* concentration in bulk milk, nor the feasibility of testing some or all bulk milk prior to cheese making nor the cost of testing some or all cheese lots.

For the baseline model 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 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 servings[5]:

- 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)”).

[5] 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”.

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 i, Table ii). The major outputs of the baseline model 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 listeriosis per serving for the baseline case. A sensitivity analysis of the baseline model is performed by changing one parameter at a time or using classical Spearman's rank correlation coefficients.

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 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, testing every raw-milk cheese lot is the only alternative that leads 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 ii). This result 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 $\log_{10}$ reduction of milk contamination before the cheese processing would reduce the predicted mean risk by a factor of approximately 7-10 compared to the baseline raw-milk cheese scenario. 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 ii: 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 in the conclusions.

Alternative	Relative risk compared to Baseline, Pasteurized-milk cheese		Relative risk compared to Baseline, Raw-milk cheese	
	Canada	**United States**	**Canada**	**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 *L. monocytogenes* contamination in incoming milk is applied	7.4	11	0.14 = 1/7.2	0.10 = 1/10
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 composite 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 cheese (Camembert-like cheese or cheeses with similar characteristics). Notably, the growth function parameterization relies on the more extensive growth information available for Camembert cheese and these results do not apply to other cheeses with different growth characteristics.

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). Too, that study provides incomplete information about the prevalence of contaminated lots and contaminated cheeses within contaminated lots.

The values, but also the relative impact of risk mitigations, could be impacted by the choice of the dose-response shape. Indeed, the dose-response model used is linear at low doses, while

other concave (convex) models could place more emphasis on higher (lower) doses and change some of the general conclusions of this report.

More generally, 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 model. 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.

Predictive modeling was used to model the growth of *L. monocytogenes* in soft-ripened cheeses between the point of retail and the point of consumption and the exposure assessment depended on information derived from those models. It is known that models may overestimate growth in food, and so reliance on such a model can result in an overestimation of the risk (FAO/WHO 2004). There is notably a lack of information on *L. monocytogenes* growth in naturally contaminated cheese, as well as information on the growth of *L. monocytogenes* in the presence of natural cheese flora.

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.

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. Additional data and models would be needed to provide corresponding estimates for soft-unripened cheese. Keeping in mind the limitations, 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 $\log_{10}$ 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. Outbreaks and Recalls 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 1). 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 1: 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.

During approximately the same time period, there have been 20 listeriosis outbreaks linked to cheese consumption worldwide (Table 2). The majority of these outbreaks were associated with fresh-soft or soft-ripened cheeses, and about half involved cheese made from unpasteurized milk.

Table 2: Cheese Associated *Listeria* Outbreaks, until 2008.

Year	Location	Implicated Cheese	No. of Illnesses (Deaths)	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[3]	(Jensen *et al.* 1994; Norton and Braden 2007)
1995	France	Brie de Meaux	37 (11)	Yes	(Goulet *et al.* 1995; Norton and Braden 2007)
1996	Belgium	Camembert	1	Likely	(Gilot *et al.* 1997)
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)
2003	Italy	Gorgonzola	1	No	(Gianfranceschi *et al.* 2006)
2005	U.S. (TX)	Queso fresco	12	Yes	(CDC 2005)
2005	Switzerland	Tomme	10 (3)	Likely	(Bille *et al.* 2006)
2006	U.S. (OR)	Unspecified	3	No	(CDC 2012)
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	41 (NS)	No	(MAPAQ 2010)
2008	Chile	Brie	91(5)	NS	(Promed 2008)

1. The number of cases associated with a particular food is not always clear in the publications.
2. "Outbreaks" with a single case were included when there was a clear microbiological link between the implicated food and clinical isolates.
3. NS – Not Stated

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.

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 do not contain specific standards of identity for Camembert, but do 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, but do define Camembert as having less than 56% moisture and more than 22% 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 3) 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 3: 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

Brie and Camembert are soft-ripened cheeses 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.

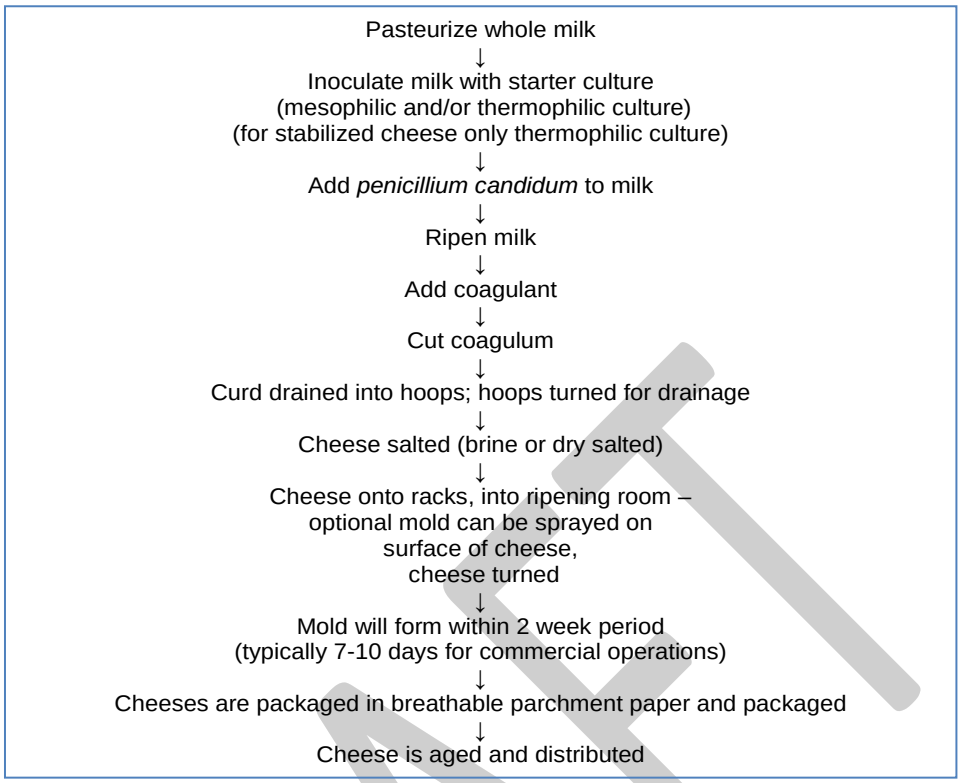

Figure 1: General flow chart for commercial production of Camembert.

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 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 a baseline model 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 model.

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.

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 Camembert 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 baseline model was developed using parameters and input values obtained from the published literature, industry sources, public submissions, and specific expert elicitation. This baseline model was 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 model.

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).

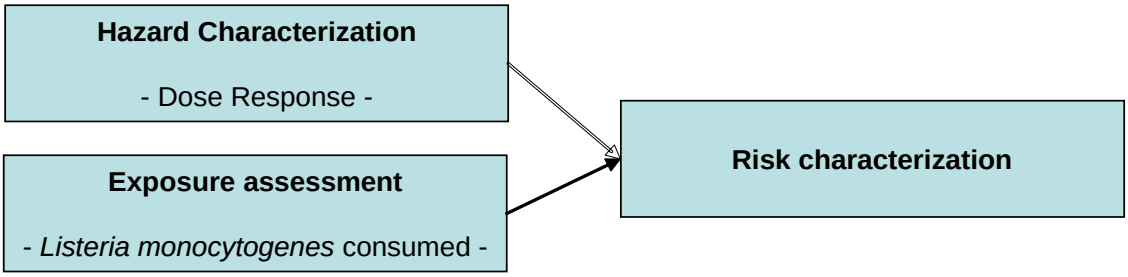

Figure 2: The 3 modules of the model.

The exposure assessment module contained a series of stages corresponding to the major stages in the process of producing, distributing, and consuming Camembert. 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 the baseline model.

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 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.

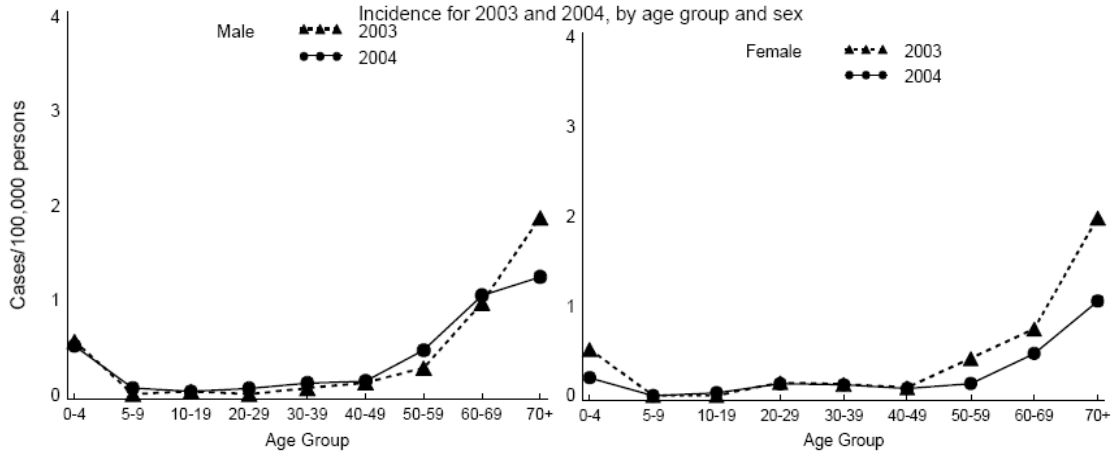

Figure 4: CDC FoodNet Data on the incidence of listeriosis by age and gender in the U.S. (CDC 2006).

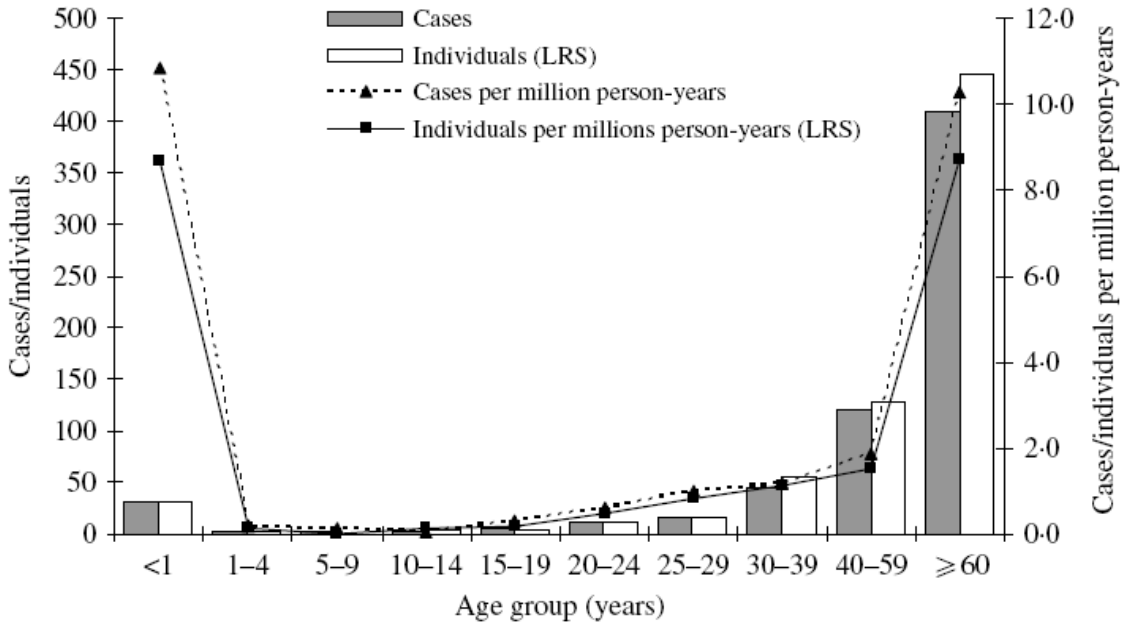

Figure 5: Canadian data on the incidence of listeriosis from the National Listeriosis Reference Service and the National Notifiable Diseases system (Clark *et al.* 2010).

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(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:

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

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

$$\pi(d; r) = \Pr(ill \mid d, r) = 1 - \exp(-rd),\ r > 0,\ d > 0$$

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 4 (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 0.025^{th} and 0.975^{th} 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 0.025^{th} and 0.975^{th} quantiles are 9.83×10^{-16} and 3.42×10^{-13}, respectively (1,000,000 Random Monte Carlo iterations).

Table 4: Uncertainty distributions for *r* parameter values.

Non-susceptible		Susceptible	
r	Pr(*r*)	*r*	Pr(*r*)
$[0, 1.54\times10^{-14}]$	0.395	$[0, 5.57\times10^{-13}]$	0.3576
$[1.54\times10^{-14}, 3.70\times10^{-14}]$	0.1922	$[5.57\times10^{-13}, 1.13\times10^{-12}]$	0.1596
$[3.70\times10^{-14}, 5.85\times10^{-14}]$	0.0982	$[1.13\times10^{-12}, 1.70\times10^{-12}]$	0.076
$[5.85\times10^{-14}, 8.00\times10^{-14}]$	0.0652	$[1.70\times10^{-12}, 2.27\times10^{-12}]$	0.0684
$[8.00\times10^{-14}, 1.02\times10^{-13}]$	0.0464	$[2.27\times10^{-12}, 2.83\times10^{-12}]$	0.0498
$[1.02\times10^{-13}, 1.23\times10^{-13}]$	0.0342	$[2.83\times10^{-12}, 3.40\times10^{-12}]$	0.0192
$[1.23\times10^{-13}, 1.45\times10^{-13}]$	0.0256	$[3.40\times10^{-12}, 3.97\times10^{-12}]$	0.033
$[1.45\times10^{-13}, 1.66\times10^{-13}]$	0.0256	$[3.97\times10^{-12}, 4.54\times10^{-12}]$	0.0384
$[1.66\times10^{-13}, 1.88\times10^{-13}]$	0.0196	$[4.54\times10^{-12}, 5.11\times10^{-12}]$	0.0298
$[1.88\times10^{-13}, 2.09\times10^{-13}]$	0.0186	$[5.11\times10^{-12}, 5.68\times10^{-12}]$	0.0204
$[2.09\times10^{-13}, 2.31\times10^{-13}]$	0.0128	$[5.68\times10^{-12}, 6.25\times10^{-12}]$	8.40×10^{-3}
$[2.31\times10^{-13}, 2.52\times10^{-13}]$	0.0104	$[6.25\times10^{-12}, 6.82\times10^{-12}]$	0.013
$[2.52\times10^{-13}, 2.74\times10^{-13}]$	7.60×10^{-3}	$[6.82\times10^{-12}, 7.39\times10^{-12}]$	0.0164
$[2.74\times10^{-13}, 2.95\times10^{-13}]$	8.20×10^{-3}	$[7.39\times10^{-12}, 7.96\times10^{-12}]$	0.0206
$[2.95\times10^{-13}, 3.17\times10^{-13}]$	7.80×10^{-3}	$[7.96\times10^{-12}, 8.53\times10^{-12}]$	0.0194
$[3.17\times10^{-13}, 3.38\times10^{-13}]$	6.60×10^{-3}	$[8.53\times10^{-12}, 9.10\times10^{-12}]$	0.0142
$[3.38\times10^{-13}, 3.60\times10^{-13}]$	6.40×10^{-3}	$[9.10\times10^{-12}, 9.67\times10^{-12}]$	0.0166
$[3.60\times10^{-13}, 3.81\times10^{-13}]$	4.80×10^{-3}	$[9.67\times10^{-12}, 1.02\times10^{-11}]$	0.0128
$[3.81\times10^{-13}, 4.03\times10^{-13}]$	3.80×10^{-3}	$[1.02\times10^{-11}, 1.08\times10^{-11}]$	0.0102
$[4.03\times10^{-13}, 4.25\times10^{-13}]$	2.80×10^{-3}	$[1.08\times10^{-11}, 1.14\times10^{-11}]$	8.00×10^{-3}
$[4.25\times10^{-13}, 4.46\times10^{-13}]$	2.60×10^{-3}	$[1.14\times10^{-11}, 1.19\times10^{-11}]$	6.20×10^{-3}
$[4.46\times10^{-13}, 4.68\times10^{-13}]$	2.80×10^{-3}	$[1.19\times10^{-11}, 1.25\times10^{-11}]$	1.40×10^{-3}
$[4.68\times10^{-13}, 5.04\times10^{-13}]$	2.80×10^{-3}	$[1.25\times10^{-11}, 1.36\times10^{-11}]$	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 5.

Table 5: 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 6). 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 6: Growth models used in this risk assessment.

Unpasteurized-milk cheese	
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 *et al.* (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)
Pasteurized-milk cheese	
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 et al. (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:

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

where[6] $y(t)$ (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.

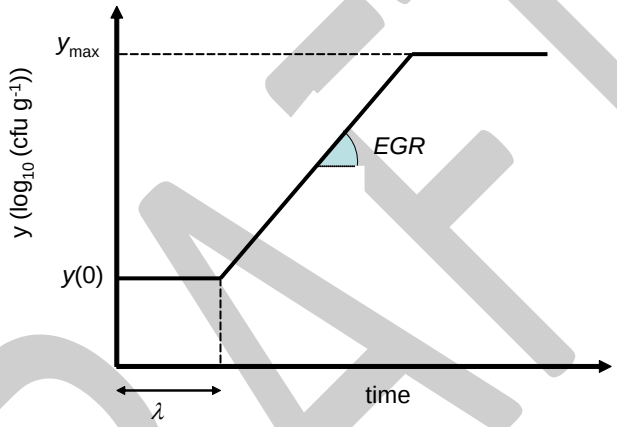

Figure 7: The three-phase linear primary growth model.

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

[6] 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).

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 (°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 $T \le T_{\min}$, where $T_{\min}$ (°C) is the minimum growth temperature. This can be written as:

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

where EGR_{Tref} is the exponential growth rate in a specific food at a chosen T_{ref} (°C) temperature. This model is valid for $T << T_{opt}$. The optimal growth temperature, T_{opt}, for *L. monocytogenes* is ≈37°C (Augustin and Carlier 2000). T_{ref} was arbitrarily set to 20°C. EGR_{Tref} 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°C, a constant T_{min} = -1.18°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

$$\left(w \times \frac{t_j}{\lambda_{T\,j}} \right)$$

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(cfu)/g in a step where $y_{\max,n} = 8$ log(cfu)/g and the bacteria entered another step where $y_{\max,n+1} = 7$ log(cfu)/g, the bacterial population was assumed to decrease to $y_{\max,n+1} = 7$ log(cfu)/g by the end of step *n+1*. If the bacteria then entered a step where $y_{\max,n+2} = 8$ log(cfu)/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(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 (ºC) for 25 studies in liquid microbiological media. Variability in $T_{\min}$ among bacterial strains was described by the equation

$$T_{\min} \sim N\left(\mu_{T_{\min}}, \sigma^2_{T_{\min}}\right).$$

Uncertainty for the unknown parameters μ_{Tmin} and $\sigma^2_{T_{\min}}$ was described using classical statistical theory. The parameters and their uncertainty distributions that were derived are shown in Table 7.

Table 7: 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)
$\sigma^2_{T_{\min}}$	2.55^2	Gamma^{-1}(12.5, 81.28^{-1})

Normal(μ_{min}, σ^2_{Tmin}) distribution.

EGR_{20} in Milk

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

$$\mu_{opt} \sim \text{Normal}\left(\theta_{\mu_{opt}}, \sigma^2_{\mu_{opt}}\right)$$

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 $\sigma^2_{\mu_{opt}}$ shown in Table 8.

Table 8: 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
$\sigma^2_{\mu_{opt}}$	0.18^2	$(\text{LN}(-1.73, 0.160^2) - 9.06\times10^{-4})^2$

(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°C, T_{opt} = 37°C, $T_{\max}$ = 45.5°C (Augustin *et al.* 2005).

EGR_{20} in Camembert

As described above, the baseline product pathway model was developed using parameters appropriate for the manufacture of Camembert. 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 9). Authors' design characteristics are in the rightmost 6 columns in Table 9.

Table 9's EGR_T is a reported *EGR* at study temperature *T*°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 9 indicate the source of the information within the reference article, not to a *Table* or *Figure* in this report.

Table 9: Data for Camembert aging and holding growth rates.

Ident numb.	Source	Cheese part	egr_T	Temp (°C)	*Lm strains* pooled	*Trials* avgd	*Cheese* avgd	Milk	Notes
1	(Ryser and Marth 1987)	Rind	.0298	6	1	1	3	PM	Scott A, Figure 5
2		Rind	.0000	6	1	1	3	PM	V7, Figure 5
3		Rind	.0207	6	1	1	3	PM	CA, Figure 5
4		Rind	.0658	6	1	1	3	PM	OH, Figure 5
5		Rind	.0970	6	1	1	3	PM	Scott A, Figure 1
6		Core	.0450	6	1	1	3	PM	Scott A, Figure 1
7		Rind	.1050	6	1	1	3	PM	CA, Figure 3
8		Core	.0780	6	1	1	3	PM	CA, Figure 3
9		Rind	.1000	6	1	1	3	PM	V7, Figure 2
10		Core	.0538	6	1	1	3	PM	V7, Figure 2
11		Rind	.0750	6	1	1	3	PM	OH, Figure 4
12		Core	.0730	6	1	1	3	PM	OH, Figure 4
13	(Genigeorgis *et al.* 1991)	Core	.8655	30	5	1	1	UNK	Table 1, pg. 664
14		Core	.1456	8	5	1	1	UNK	Table 1, pg. 664
15		Core	.0197	4	5	1	1	UNK	Table 1, pg. 664
16		Rind	.8655	30	5	1	1	UNK	Table 1, pg. 664
17		Rind	.0927	8	5	1	1	UNK	Table 1, pg. 664
18		Rind	.0183	4	5	1	1	UNK	Table 1, pg. 664
19	(Sulzer and Busse 1991)	Rind	.0608	6	1	1	1	PM	*Li*, Figure 1
20		Rind	.0473	6	1	1	1	PM	*Li*, Figure 1
21		Rind	.0583	6	1	1	1	PM	*Lm*, Figure 3
22		Rind	.0288	6	1	1	1	PM	*Lm*, Figure 3
23	(Sulzer and Busse 1993)	Rind	.0909	6	1	1	1	PM	*Li*, Figure 1
24		Core	.0606	6	1	1	1	PM	*Li*, Figure 1
25		Rind	.0500	4	1	1	1	PM	*Li*, Figure 6
26		Rind	.1500	7	1	1	1	PM	*Li*, Figure 6
27		Rind	.475	15	1	1	1	PM	*Li*, Figure 6
28	(Maisnier Patin *et al.* 1992)	Core	.1464	11	1	1	3	PM	Nis-
29		Rind	.2107	11	1	1	3	PM	Nis-

Ident numb.	Source	Cheese part	egr_T	Temp (ºC)	*Lm strains* pooled	*Trials* avgd	*Cheese* avgd	Milk	Notes
30	(Back *et al.* 1993)	Rind	.0600	3	1	2	6	PM	Figure 1a
31		Rind	.0740	6	1	2	6	PM	Figure 1a
32		Rind	.1200	10	1	2	6	PM	Figure 1a
33		Rind	.0467	3	1	1	1	UNK	Table 1
34		Rind	.1467	6	1	1	1	UNK	Table 1
35		Rind	.0867	3	1	1	1	UNK	Table 1
36		Core	-0.028	3	1	2	6	PM	Figure 1b
37		Core	-0.028	6	1	2	6	PM	Figure 1b
38		Core	0	10	1	2	6	PM	Figure 1b
39		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		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		Core	.1100	7	1	3	8	PM	C
46	(Liu *et al.* 2007)	Rind	.0600	7	1	3	8	PM	TS
47		Core	.0467	7	1	3	8	PM	C
48	(Liu *et al.* 2009)	Core	.0333	7	1	3	8	PM	*Lm*, Figure 6c, C
49		Core	.0417	7	1	3	8	PM	*Li*, Figure 6c, C
50		Rind	.0600	7	1	3	8	PM	*Lm*, Figure 6b, TS
51		Rind	.0533	7	1	3	8	PM	*Li*, Figure 6b, TS
52	(D'Amico *et al.* 2008a)	Rind	.0429	4	5	1	3	RM	Figure 4
53		Rind	.0393	4	5	1	3	PM	Figure 4
54		Rind	.0881	4	5	1	3	RM	Figure 4
55		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 9, 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 9) 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(cfu)/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°C, for illustration.

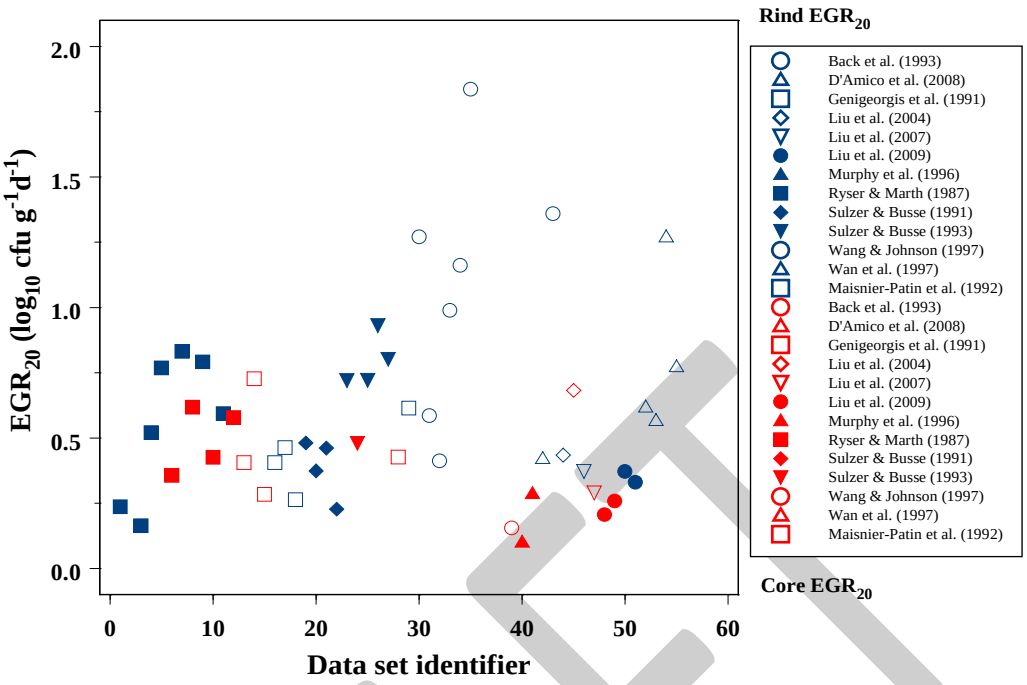

Figure 8: Study EGR_{20} (study-temperature EGR_1-transformed) in Camembert rind (blue symbols) and core (red symbols).

These data were used to estimate parameters for a hierarchical model of EGR_{20} that accounted for variability among (*L. monocytogenes* strains × cheese-making) and among cheeses within (*L. monocytogenes* strains × 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

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

with

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

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 10, Table 11). Figure 9 shows the marginal rind and core EGR_{20} density functions for EGR_{20}>0.

Table 10: Maximum likelihood estimates, for Camembert rind and core EGR_{20}.

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

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

Table 11: 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
$\hat{\lambda}_R$		-0.0033	-0.8505	$\hat{\lambda}_C$		0.0005	-0.6097
$\hat{\theta}_R$			-0.0083	$\hat{\theta}_C$			-0.0044

The EGR_{20}s 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 9). 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.

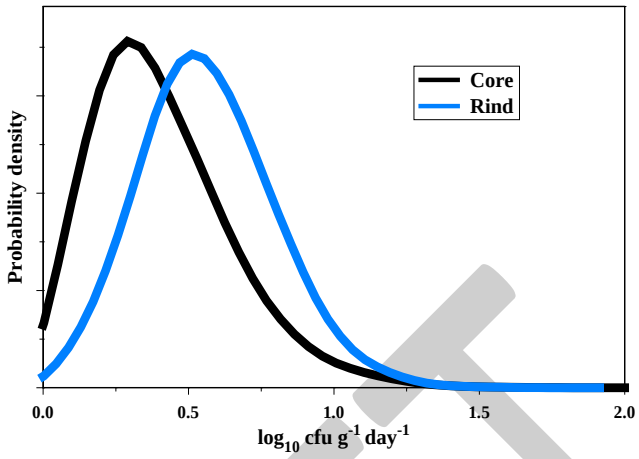

Figure 9: Marginal density functions for single Camembert cheese rind EGR_{20} (blue) and single Camembert cheese core EGR_{20} (black), when EGR_{20} greater than 0.
(Normal Gamma distribution, at Table 10 m.l.e.)

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 12. 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 12: Maximum population density (log(cfu)/g) as a function of temperature and medium.

Medium	Temperature (°C)		
	<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 $y_{\max_T} = f(T) + X$, $X \sim uniform(-0.5, 0.5)$ and $f(T)$ from Table 12.

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^2_ξ parameter estimates was specified as

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

and

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

Note that Sanaa *et al.* (2004) modeled the growth lag in cheese using lag ~ *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; Schvartzman *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 ($1\text{-}10^{-1}$). The number of bacteria in an individual cheese at the end of the initial ripening period X_1 follows:

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

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°C, T_{opt} = 37°C, T_{max} = 45.5°C, a_{wmin} = 0.913, a_{wopt} = 0.997, pH_{min} = 4.26, pH_{opt} = 7.1, pH_{max} = 9.61 in

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

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

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

Sanaa *et al.* (2004) used μ_{opt} = 0.1060 h^{-1} for Camembert, a value that takes into account the a_w impact. Assuming a_w = 0.98 in their process, we used μ_{opt} = 133 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 Schlesser *et al.* (1992, Table 5).

Results

Figure 10 shows the number of generations[7], 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, a lower predicted increase in the core of the cheese. The growth is higher in stabilized cheeses. These curves do *not* include the 1 $\log_{10}$ 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").

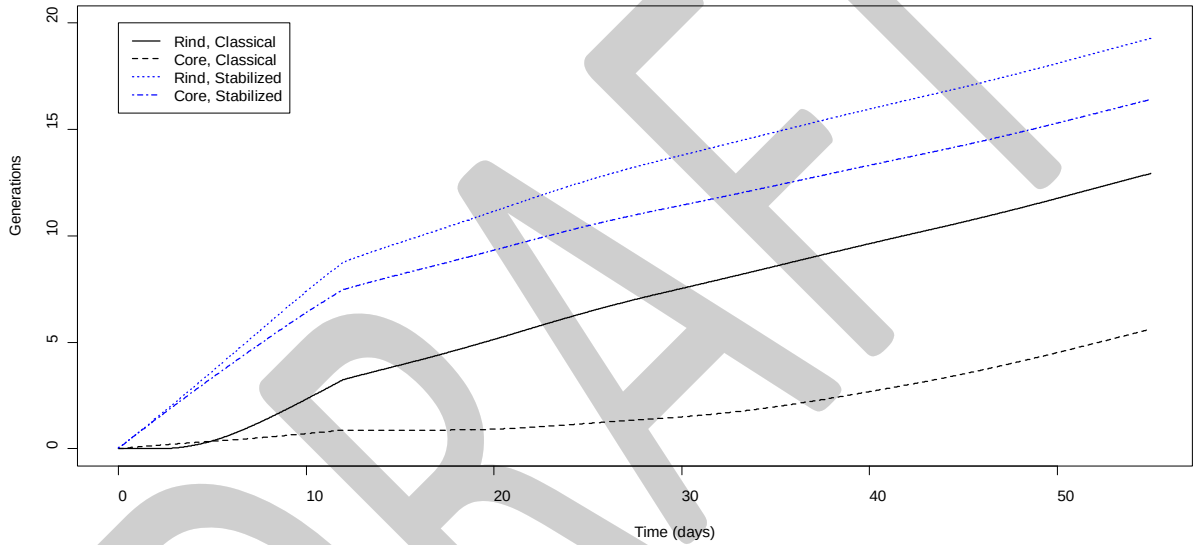

Figure 10: Modeled number of generations during Camembert cheese ripening and manufacturing.

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);

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

- 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 cells[8]. 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):

$$X_1 \sim Binomial(X_0, s).$$

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

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

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

[8] s can, nevertheless, vary from product to product.

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

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, $X_1 = X_0$;
- "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 whey[9];
- During cheese formation, when the bacteria trapped in the bulk curds are distributed among the different individual cheeses;

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

- 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.

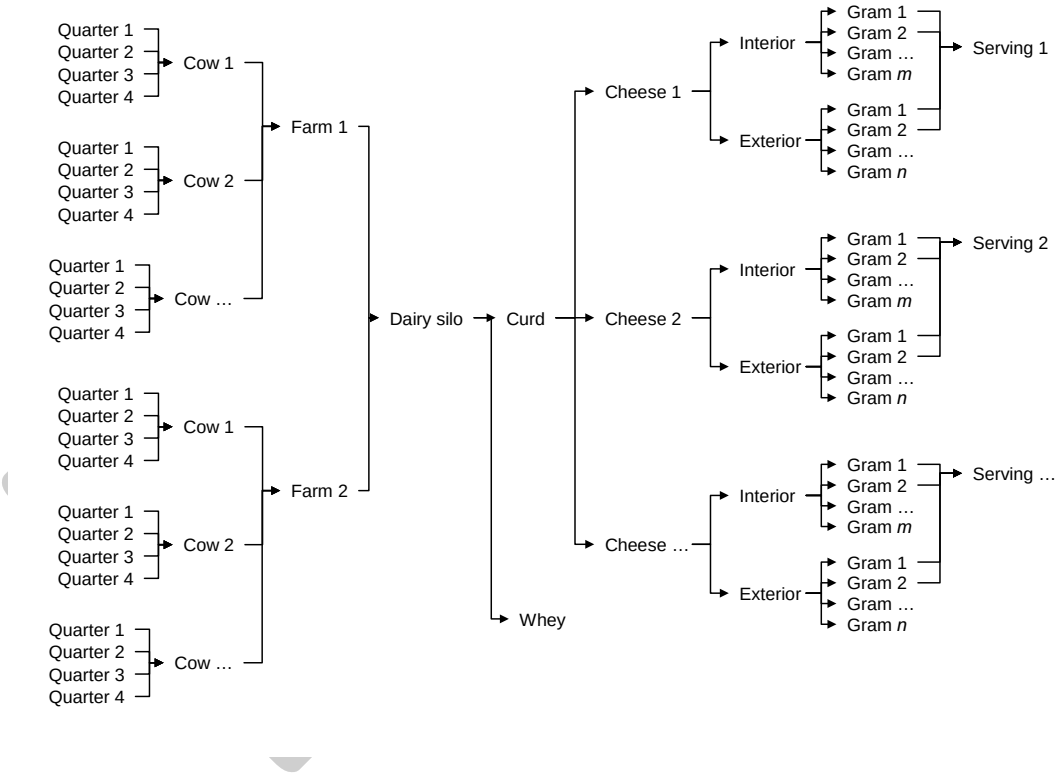

Figure 11: Mixing and partitioning process in the exposure assessment model.

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:

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

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 ($i = (1, \ldots, k)$) 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 13. 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 13. Raw results from this study are available on the FoodRisk.org website[10] (Table 14). From this dataset, the distribution of the level of contamination occurring in the plant was estimated by "substracting" 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).

Table 13: Results reported in Gombas *et al.* (2003) for soft-ripened cheeses.

Site	no. of cheeses sampled	no. with no *Lm* 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 14: Raw results, as available on the FoodRisk.org website.

FoodNet site	Freq.	Screening	MPN	Enumeration									
		Vol.	Result	Vol.	Result	Vol.	Result	Vol.	Result	Vol.	Result	Vol.	Result
California	817	25 or 2×25g	-										
California	11		+	3×1	- - -	3×0.1	- - -	3×0.01	- - -	0.1	0	0.002	0
California	1		+	3×1	+ + -	3×0.1	+ - -	3×0.01	- - -	0.1	0	0.002	0
California	1		+	1	+ + +	0.1	- - -	0.01	- - -	0.1	-	0.002	0
Maryland	516		-										
Maryland	1		+	3×1	- - -	3×0.1	- - -	3×0.01	- - -	0.1	0	0.002	0

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

[10] http://foodrisk.org/exclusives/SLMREF/, accessed 12/19/2011.

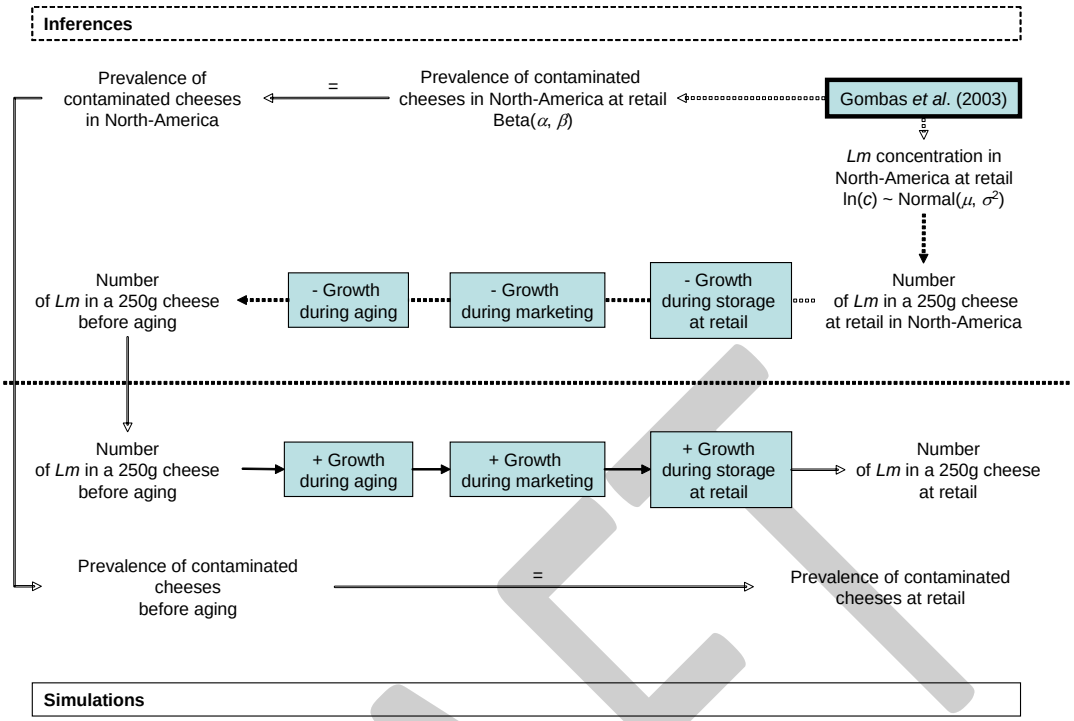

Figure 12: Schematic view of the inference process used to estimate the contamination of cheeses in plant. Top panel: the inference part estimates distributions for the prevalence of contamination and for level of contamination of cheeses in plant from the Gombas *et al.* (2003) data set. Bottom panel: 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 **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 **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 baseline model, as described in the Exposure Assessment section;
- **Y** and **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 **X** = round(**Y**/**G**), with **X** > 0. Values of **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 15.

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

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 16. The overall probability of contamination for a cheese at

random was E[p]|p~Beta(α, β) = 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 16: Parameters α and β used to model the frequency of cheeses with in plant contamination.

Parameter	Point estimate (MLE)	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]; $\rho(\alpha, \beta) = 0.907$

[a] The 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) ~ Normal(μ, σ^2). The parameters μ and σ^2 obtained are given in Table 17. Using this distribution, the estimated distribution of the number of bacteria per contaminated cheese **Y** (**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 17: Maximum likelihood estimates, level of contamination at retail.

Parameter	Point estimate (MLE)	Uncertainty distribution
μ	-1.874	Normal(-1.8737, 0.3386^2)
σ^2	0.5265^2	Gamma^{-1}(7.5, $[3.949]^{-1}$) $\rho(\mu, \sigma) = -0.0127$

ln 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 18).

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

Number of bacteria	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Percentage	10.0	7.35	5.36	4.49	3.55	3.46	3.03	2.69	3.02	2.70	2.74	2.82	2.92	2.81	2.56	2.88

Number of bacteria	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	
Percentage	2.59	2.84	2.64	2.92	2.64	2.66	2.70	2.39	2.39	2.52	2.50	2.32	2.23	2.18	2.07	

(25,000 MC 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 19).

Table 19: 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 MC 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 10.1.2.).

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 v 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 v ml follows a Poisson distribution with mean μv 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:

$$\Pr(reject \mid \mu) = \varphi \times (1 - \exp(-\eta v \mu)),$$

i.e. the probability that the milk is tested multiplied by the probability that at least one bacterial cell is detected in the v 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 a composite of v g, *i.e.* 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 composite 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:

$$\Pr(reject \mid \mu) = \varphi \times \left(1 - (1-\eta)^m\right),$$

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 Camembert 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.

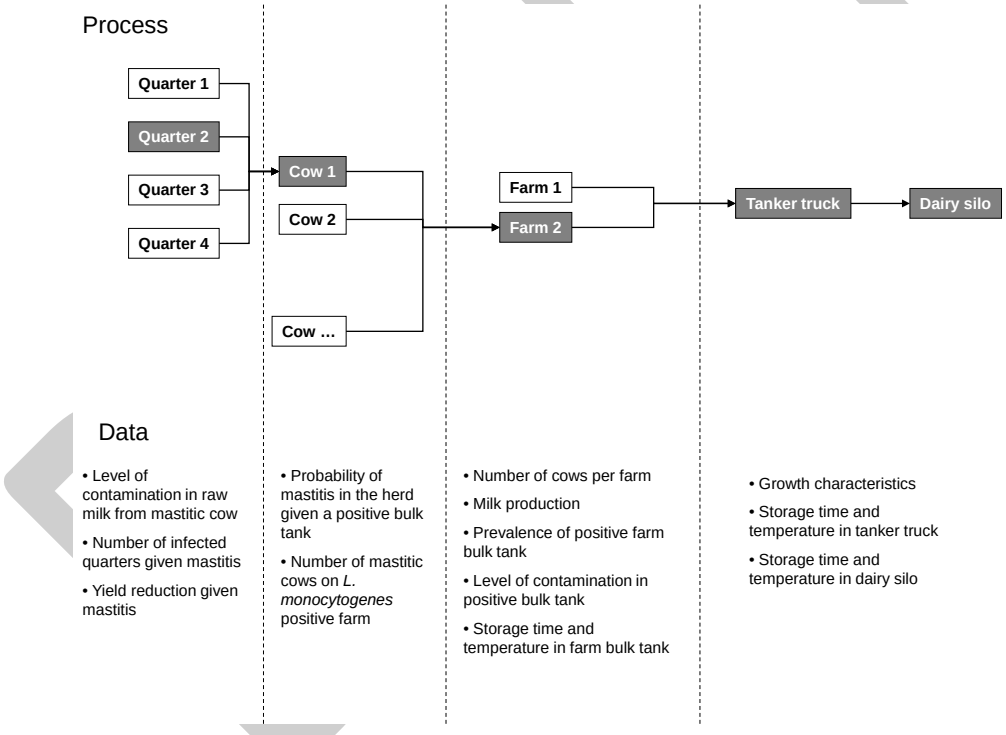

Figure 13: On farm process and data used.
Grayed boxes stand for contaminated units. In this example, the dairy silo is contaminated from the tanker truck. This tanker truck is contaminated from Farm #2. Farm #2 is contaminated from a mastitic cow (Cow #1) with one infected quarter (Quarter #2).

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

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

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 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 baseline 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 20 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 21 shows the same outputs for the baseline artisanal-scale case. Data uncertainty propagated to these on farm outputs are reported separately (Table 22, Table 23).

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.

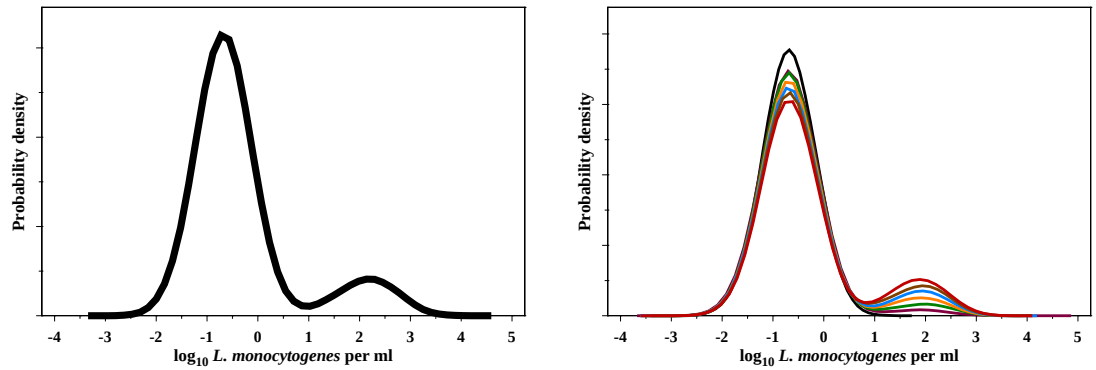

Figure 14: Distribution of the concentration (log cfu/ml) of *L. monocytogenes* in positive milk collection and change in concentration distribution as Pr(*Lm* mastitis | *Lm*+ environment) increases over range 0 (0.025) 0.15.

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, under an assumption like the one on page **Error! Bookmark not defined.** that relates the prevalence of a contaminated collection to the prevalence in its parts. 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 22 and Table 23 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 20: Point estimates of the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Baseline model, farmstead-scale operations.

	Canada					United States			
	Summary statistics		**Prevalence**	**Concentration (cfu / ml, log_{10} statistics)**		**Summary statistics**		**Prevalence**	**Concentration (cfu / ml, log_{10} statistics)**
←Variability→	Median		0.225	-0.289	**←Variability→**	Median		0.371	-0.289
	Mean		0.236	1.60		Mean		0.424	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
		2.5%	0.0957	-1.30			2.5%	0.00696	-1.30
		5%	0.0112	-1.14			5%	0.00965	-1.14
		10%	0.0132	-0.957			10%	0.0136	-0.957
		25%	0.0172	-0.649			25%	0.0228	-0.649
		50%	0.0225	-0.289			50%	0.0371	-0.289
		75%	0.0288	0.140			75%	0.0564	0.140
		90%	0.0353	1.68			90%	0.0780	1.68
		95%	0.0396	2.34			95%	0.0930	2.34
		97.5%	0.0436	2.66			97.5%	0.107	2.66
		99%	0.0485	2.93			99%	0.125	2.93

Table 21: Point estimates of the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Baseline model, artisanal-scale operations.

Canada					United States				
	Summary statistics		Prevalence	Concentration (cfu / ml, $\log_{10}$ statistics)		Summary statistics		Prevalence	Concentration (cfu / ml, $\log_{10}$ statistics)
←Variability→	Median		0.0445	-0.584	←Variability→	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
		2.5%	0.0191	-1.67			2.5%	0.0139	-1.67
		5%	0.0222	-1.49			5%	0.0192	-1.48
		10%	0.0263	-1.30			10%	0.0271	-1.29
		25%	0.0341	-0.968			25%	0.0451	-0.959
		50%	0.0445	-0.584			50%	0.0729	-0.582
		75%	0.0567	-0.127			75%	0.110	-0.131
		90%	0.0693	1.38			90%	0.150	1.39
		95%	0.0776	2.02			95%	0.177	2.04
		97.5%	0.0852	2.34			97.5%	0.203	2.36
		99%	0.0946	2.62			99%	0.234	2.62

Table 22: Prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Baseline farmstead-scale case, uncertainty considered.

Canada								United States							
Summary statistics			Prevalence			Concentration (cfu/ml, $\log_{10}$ statistics)			Summary statistics		Prevalence			Concentration (cfu/ml, $\log_{10}$ statistics)	
			←Uncertainty→			←Uncertainty→					←Uncertainty→			←Uncertainty→	
			Median	CI95%		Median	CI95%				Median	CI95%		Median	CI95%	
←Variability→	Median		0.0234	6.25×10^{-4}	0.208	-0.282	-0.390	-0.121	←Variability→	Median	0.0410	0.0102	0.224	-0.282	-0.390	-0.121
	Mean		0.0252	6.77×10^{-4}	0.209	1.60	1.21	1.98		Mean	0.0474	0.0118	0.227	1.60	1.21	1.98
	Std. Dev.		0.00917	3.43×10^{-4}	0.0320	2.34	2.04	2.61		Std. Dev.	0.0258	0.00791	.0633	2.34	2.04	2.61
	Quantile	.01	0.00640	9.48×10^{-5}	0.168	-1.49	-1.64	-1.33		.01	0.0056	4.14×10^{-4}	0.117	-1.49	-1.64	-1.33
		.025	0.00765	1.37×10^{-4}	0.174	-1.30	-1.44	-1.16		.025	0.00821	9.00×10^{-4}	0.131	-1.30	-1.44	-1.16
		.05	0.00944	1.94×10^{-4}	0.180	-1.15	-1.27	-1.00		.05	0.0117	0.00164	0.144	-1.15	-1.27	-1.00
		.1	0.0112	3.06×10^{-4}	0.186	-0.965	-1.082	-0.826		.1	0.0168	0.00302	0.160	-0.965	-1.08	-0.826
		.25	0.0154	4.38×10^{-4}	0.196	-0.652	-0.762	-0.519		.25	0.0264	0.00574	0.189	-0.652	-0.762	-0.519
		.5	0.0234	6.25×10^{-4}	0.208	-0.282	-0.390	-0.121		.5	0.0410	0.0102	0.224	-0.282	-0.390	-0.121
		.75	0.0328	8.60×10^{-4}	0.222	0.142	-0.0146	1.36		.75	0.0626	0.0157	0.262	.142	-0.015	1.36
		.9	0.0385	0.00112	0.240	1.71	0.395	2.39		.9	0.0845	0.0223	0.299	1.71	.395	2.39
		.95	0.0438	0.00129	0.251	2.34	1.04	2.69		.95	0.0996	0.0270	0.332	2.34	1.04	2.69
		.975	0.0487	0.00146	0.261	2.63	2.22	2.92		.975	0.112	0.0315	0.367	2.63	2.22	2.92
		.99	0.0551	0.00169	0.272	2.91	2.62	3.21		.99	0.127	0.0372	0.409	2.91	2.62	3.21

Table 23: Estimates for the prevalence of positive collections and the *L. monocytogenes* concentration in positive collections. Baseline artisanal-scale case, uncertainty considered.

Canada								United States							
Summary statistics			Prevalence			Concentration (cfu/ml, $\log_{10}$ statistics)			Summary statistics			Prevalence			Concentration (cfu/ml, $\log_{10}$ statistics)	
			←Uncertainty→			←Uncertainty→						←Uncertainty→			←Uncertainty→	
			Median	CI95%		Median	CI95%					Median	CI95%		Median	CI95%	
←Variability→	Median		0.0463	0.00125	0.372	-0.582	-0.694	-0.369	←Variability→	Median		0.0803	0.020	0.397	-0.569	-0.686	-0.363
	Mean		0.0496	0.00135	0.373	1.29	0.923	1.64		Mean		0.0917	0.0234	0.399	1.28	0.916	1.64
	Std. Dev.		0.0175	6.85×10^{-4}	.0515	2.01	1.73	2.26		Std. Dev.		0.0482	0.0155	0.103	2.00	1.74	2.27
	Quantile	.01	0.0128	1.90×10^{-4}	0.308	-1.86	-2.01	-1.72		Quantile	.01	0.0111	8.28×10^{-4}	0.219	-1.85	-2.00	-1.70
		.025	0.0152	2.74×10^{-4}	0.318	-1.66	-1.81	-1.53			.025	0.0163	0.00180	0.243	-1.66	-1.79	-1.51
		.05	0.0188	3.88×10^{-4}	0.326	-1.50	-1.63	-1.36			.05	0.0233	0.00327	0.266	-1.49	-1.62	-1.35
		.1	0.0223	6.12×10^{-4}	0.336	-1.30	-1.42	-1.16			.1	0.0333	0.00604	0.293	-1.29	-1.42	-1.15
		.25	0.0305	8.75×10^{-4}	0.353	-0.968	-1.08	-0.822			.25	0.0522	0.0115	0.341	-0.959	-1.08	-0.812
		.5	0.0463	0.00125	0.372	-0.582	-0.694	-0.369			.5	0.0803	0.0203	0.397	-0.569	-0.686	-0.363
		.75	0.0646	0.00172	0.394	-0.116	-0.294	1.30			.75	0.121	0.0311	0.455	-0.114	-0.293	1.31
		.9	0.0755	0.00223	0.422	1.47	0.137	2.08			.9	0.162	0.0441	0.508	1.47	0.143	2.11
		.95	0.0856	0.00258	0.438	2.03	0.818	2.37			.95	0.189	0.0532	0.554	2.04	0.854	2.37
		.975	0.0950	0.00292	0.453	2.32	1.96	2.58			.975	0.211	0.0619	0.599	2.32	1.94	2.60
		.99	0.107	0.00338	0.470	2.58	2.33	2.84			.99	0.237	0.0730	0.651	2.59	2.33	2.86

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.

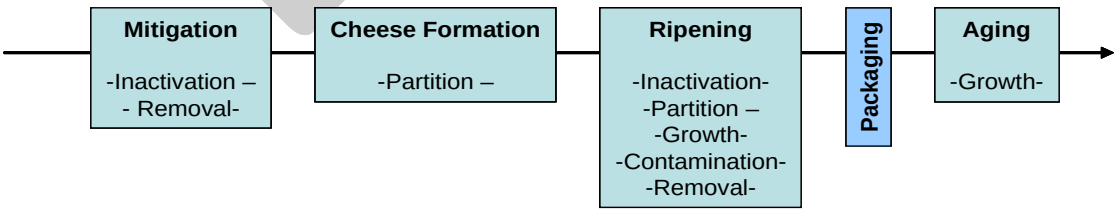

Figure 15: Schematic view of cheese processing and associated basic processes.

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 model 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 $\boldsymbol{T}|\boldsymbol{Y}=y \sim Uniform(1, y)$ days before packaging for ripening period length $\boldsymbol{Y}$.

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 ≈10°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 Camembert is a 10.8 cm diameter × 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 55) 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 24, 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 24: 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 Camembert" 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 24. 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 24, 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.

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 model. However, an option to add this mitigation was included to facilitate testing alternative intervention scenarios (see section 6.5). This option assumed that $\nu = 5\times5$ 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 η = 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:

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

Cheeses made from non-pasteurized Milk

The 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:

$$T_{aging} = \left(\pi X + (1-\pi)Y - 32\right) \times 5/9$$
$$\pi \sim Bernoulli(0.5)$$
$$X \sim Triangular(35, 37, 40)$$
$$Y \sim Triangular(37, 38, 40)$$

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 model are described here.

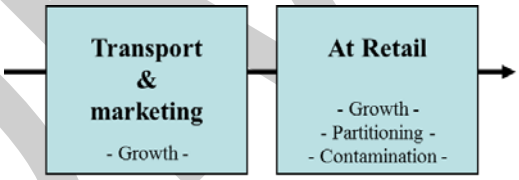

Figure 17: Schematic view of the Transport, Marketing and Retail steps and associated basic processes.

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:

$$t_m \sim Triangular(1, 5, 10)$$

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:

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

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 Camembert is displayed in the retail display cabinet were estimated as <1 day, 5 days and 14 days, respectively. This was modeled as:

$$\begin{aligned} m_{tr} &\sim Uniform(0,1) \\ t_r &\sim Triangular(m_{tr}, 5, 14) \end{aligned}.$$

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.[11] 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 baseline 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 25.

[11] http://foodrisk.org/exclusives/EcoSure assessed 4/11/2012.

Table 25 Summary statistics for storage temperature (°F) for retail semi-solid cottage cheese dairy product, supermarket.

N	Mean	Var.	Min.	Percentile									Max
				1%	5%	10%	25%	50%	75%	90%	95%	99%	
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:

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

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

where $TruncatedNormal(\mu, \sigma, 28.5, 60.8)$ is the normal distribution with a mean μ, a standard deviation σ, truncated at [28.5; 60.8]°F (*i.e.* [-1.94; 16]°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 × location (within state) selection with equal probabilities, and a random store type and participant (within state × city × 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 26.

Table 26: Specification of the temperature T_{rF} (ºF) at retail.

DEFF	Maximum likelihood estimate	Uncertainty distribution (ºF)
420	$\mu = 3.6647$ $\sigma^2 = 0.01143$	μ~Normal(3.665, 0.005203^2) σ^2~[Gamma(210, $[2.394]^{-1}$)]$^{-1}$ $\rho(\mu, \sigma^2)$=0.000022

T_{rF} is specified using $\ln(T_{rF}) \sim$ TruncatedNormal(μ, σ^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 Camembert 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).

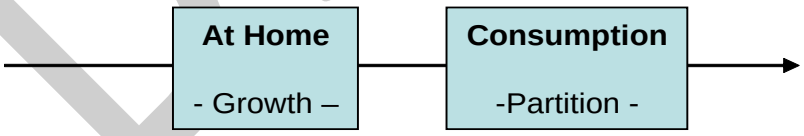

Figure 18: Schematic view of the Home and Consumption steps and associated basic processes.

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 27. 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 27: Brie and Camembert serving size distributions for Canadian population.

Population group	DRI age-sex groups	Resp.	Fraction eaters Est. (±s.e.) [95% c.i.] (bootstrap)	logNormal distribution, eaters	
				$\hat{\mu}$ ln(g)	$\hat{\sigma}^2$ ln(g)
Elderly	M >70, F >70	4,130	0.0057 (±0.0015) [0.0032, 0.0089]	3.04 (±0.293)	1.00 (±0.280)
Pregnant women	F 19-30, F 31-50	4,772	0.012 (±0.0032) [0.0032, 0.0064]	3.05 (±0.283)	0.823 (±0.278)
General & Immunocompromised	Children ≥ 1, Adults ≤ 70	29,278	0.0093 (±0.0013) [0.0070, 0.012]	3.24 (±0.140)	0.845 (±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 28. 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 28: 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 29 shows summary statistics for the serving size distributions for Canada and U.S. used in the model.

Table 29: Serving size (g) distribution summary statistics, soft-ripened cheese, Canada and U.S.

Country	Group		Mean	Percentiles		
				5th	50th	95th
Canada	Elderly	Point estimate	40	11	28	115
		Uncertainty	38 [28; 50]	11 [11; 14]	28 [20; 37]	100 [69; 150]
	Pregnant women	Point estimate	37	11	27	100
		Uncertainty	38 [22; 53]	11 [11; 13]	27 [18; 37]	100 [44; 150]
	General and Immunocompromised	Point estimate	42	12	30	117
		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.[12] Participants were asked to complete a questionnaire on storage times for

[12] http://www.foodrisk.org/exclusives/index.cfm assessed 4/11/2012.

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 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, $\boldsymbol{X}$, has distribution represented by a Binomial distribution, that is, $\boldsymbol{X} \mid \boldsymbol{N}$ servings, $\boldsymbol{M}$ occasions ~ Binomial(N-M+1, $[1+M]^{-1}$).

Table 30 shows the derived consumer storage practices, Table 31 shows the parameter estimates for the distribution of storage times until a package is first opened, and Table 32 shows the

parameter estimates for the distributions for the number of consumption occasions and the distribution for the time between successive consumption occasions.

Table 30: Soft cheese storage attributes, fraction of cheeses consumed with listed characteristic.

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

Table 31: Weibull distribution for time the product is unopened until the 1st consumption (d).

Population group	m.l.e. (±se) [95% ci] (Wald-type)		$\rho(\hat{\lambda}, \hat{\theta})$
	α (shape)	λ (scale)	
Elderly	0.95 ±0.084 [0.83, 1.2]	5.6 ±0.60 [4.5, 6.9]	0.36
Pregnant women	0.80 ±0.079 [0.64, 0.95]	5.9 ±0.87 [4.2, 7.6]	0.21
General & Immunocompromised	0.83 ±0.067 [0.70, 0.97]	5.0 ±0.60 [3.9, 6.2]	0.31

Table 32: Consumption occasions (Poisson λ) and time (d) between successive occasions from opened package (Exponential θ).

Population group	m.l.e. (±se) [95% ci] (Wald-type)		$\rho(\hat{\lambda}, \hat{\theta})$
	λ	θ	
Elderly	1.2 ±0.85 [-0.51, 2.8]	8.2 ±3.1 [2.1, 14.3]	-0.95
Pregnant women	2.5 ±0.95 [0.62, 4.4]	6.7 ±2.3 [2.2, 11.3]	-0.93
General & Immunocompromised	2.7 ±1.0 [0.72, 4.7]	3.3 ±1.1 [1.1, 5.5]	-0.96

The simulation outputs (storage time to serving at random, in days) are shown in Table 33. 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 29). 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 30-Table 32) and because of different serving size distributions (Table 29, Canada).

Table 33: 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		Mean	Percentile		
				5th %ile	Median	95th %ile
Canada	Elderly	Point estimate	13	.47	9.4	37
		Uncertainty	13 [10; 15]	.44 [0.21; 0.92]	9.8 [7.6; 12]	36 [28; 42]
	Pregnant women	Point estimate	15	.40	12	42
		Uncertainty	16 [10; 19]	.33 [0.13; 1.4]	8.6 [13; 17]	39 [27; 48]
	General and	Point estimate	10	.43	8.5	27
	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
		Uncertainty	12 [10; 14]	.43 [0.22; 0.87]	7.4 [9.3; 11]	34 [28; 40]
	Pregnant women	Point estimate	14	.35	11	39
		Uncertainty	13 [9.8; 17]	.31 [0.13; 0.91]	10 [7.9; 14]	34 [25; 45]
	General and	Point estimate	10	.41	8.2	26
	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 34. 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 34: Parameter estimates for fitted Laplace distributions for refrigerator storage temperature (ºC).

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

Simulated Temperature

Table 35 summarizes the properties of the temperature distributions (in °C) used in the exposure assessment.

Table 35: Temperature (ºC), serving at random.
Entries [2.5%, Mean, 97.5%] uncertainty replicates.

Country	Population		Mean	5th %ile	Median	95th %ile
Canada	Elderly	Point estimate	4.5	0.3	4.5	9.1
		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]
	Pregnant women	Point estimate	4.8	-0.22	4.3	11
		Uncertainty	4.6 [4.4; 4.8]	-0.44 [-0.20; 0.18]	4.6 [4.4; 4.8]	12 [10; 17]
	General and Immunocompromised	Point estimate	4.5	-0.13	4.1	10
		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
		Uncertainty	4.7 [4.4; 5.4]	0.35 [0.094; 0.61]	4.3 [4.2; 4.4]	9.6 [8.7; 16]
	Pregnant women	Point estimate	4.8	-0.21	4.4	11
		Uncertainty	5.1 [4.7; 5.8]	-0.20 [-0.46; 0.18]	4.6 [4.4; 4.8]	12 [10; 17]
	General and Immunocompromised	Point estimate	4.5	-0.15	4.1	9.9
		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 $C_{c,p} = N_{c,p} \times \overline{R}_{s_{c,p}}$, where $N_{c,p}$ is the number of servings consumed by

population *p* in country *c* during this period and $\overline{R}_{s_{c,p}}$ is the mean risk per serving for this population *p* during this period of time[13]. 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 (indexed 0) is then equal to:

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

Other risk characterization outputs of interest are:

- the **risk per contaminated serving**, $R_{cs_{s,p}}$, 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.

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

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 Camembert. 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, 0.025^{th} and 0.975^{th} 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 95^{th} 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.

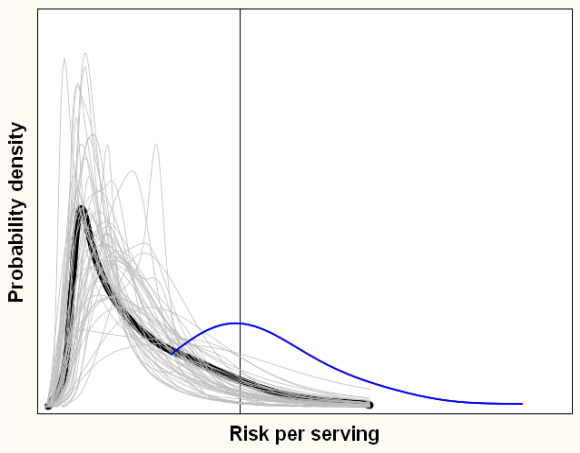

Figure 19: Illustration of second-order Monte-Carlo results.

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

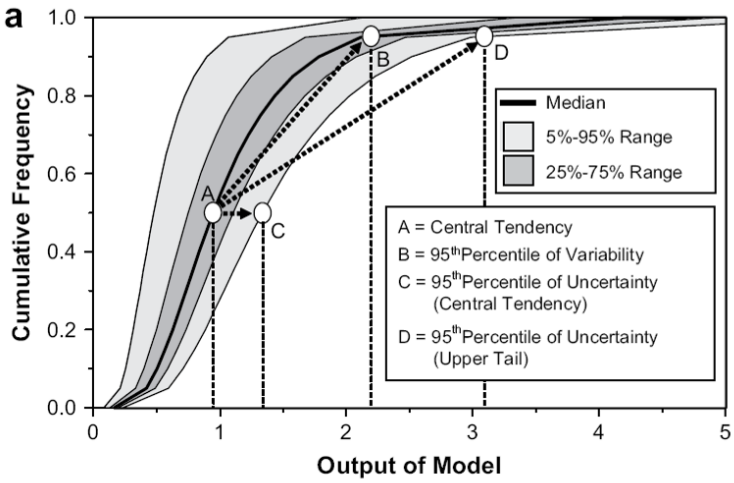

Figure 20: Illustration of the measure of Variability and Uncertainty (Ozkaynak *et al.* 2009).

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 baseline (section 6.4, Table 18-Table 19) to exactly 1, 10, 100, 1,000, 10,000, 100,000 cfu/cheese;
- Growth characteristics: from baseline (section 6.1, Table 10-Table 11) to EGR_{20}s equal to 0, ½ baseline, 2 × baseline; from baseline (section 6.1, Table 12) to maximum population densities equal to the baseline -1 $\log_{10}$ or to the baseline +1 $\log_{10}$;
- Transport and Marketing temperature: baseline -1°C, baseline +1°C;
- Temperature at Retail: baseline -1°C, baseline +1°C;
- Home storage temperature: baseline -1°C, baseline +1°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 0.025^{th} quantile, and the 0.975^{th} 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 Baseline Model

The baseline model 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 36 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 × 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 18, p. 66) and *L. monocytogenes* per serving distribution at the time of consumption (Table 36) 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 37 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 37'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 37 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 36: Number of *L. monocytogenes* cells per contaminated Camembert serving, pasteurized-milk cheeses, no uncertainty considered.

Summary statistics from distributions describe variability among contaminated servings.

	Canada					United States			
	Elderly	**Pregnant**	**IC***	**General**		**Elderly**	**Pregnant**	**IC**	**General**
Median	17	21	16	16		18	21	16	16
Mean	1,061,159	2,642,105	886,087	886,087		1,043,170	2,584,927	736,435	736,435
Std. dev	16,170,615	25,442,612	15,322,622	15,322,622		17,375,826	28,545,263	14,442,700	14,442,700
Percentile									
1%	1	1	1	1		1	1	1	1
2.5%	1	1	1	1		1	1	1	1
5%	1	1	1	1		1	1	1	1
10%	1	1	1	1		1	1	1	1
25%	3	3	3	3		3	3	3	3
50%	17	21	16	16		18	21	16	16
75%	254	390	204	204		252	357	197	197
90%	5,135	14,773	3,252	3,252		4,812	10,379	3,027	3,027
95%	56,025	200,000	26,679	26,679		49,358	124,014	24,187	24,187
97.5%	286,470	3,162,278	157,764	157,764		241,020	2,354,498	135,692	135,692
99%	6,324,555	100,000,000	2,900,146	2,900,146		3,850,756	100,000,000	1,949,514	1,949,514

* IC: Immunocompromised.

Table 37: *L. monocytogenes* cells per g at process pathway steps, pasteurized-milk contaminated cheeses, 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*).

	Contaminated cheese				Contaminated serving
	Initial Contamination	**After Aging**	**After Transport and Marketing**	**After Retail**	**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
Percentile					
1%	0.004	0.004	0.004	0.004	0.04
2.5%	0.004	0.004	0.004	0.004	0.07
5%	0.004	0.004	0.007	0.009	0.12
10%	0.004	0.010	0.013	0.013	0.25
25%	0.018	0.034	0.040	0.049	0.89
50%	0.053	0.088	0.106	0.128	4.49
75%	0.093	0.218	0.485	1.013	72.53
90%	0.119	0.959	2.492	6.813	1,525.79
95%	0.128	2.126	6.596	24.377	14,450.68
97.5%	0.133	4.193	16.225	77.817	94,720.30
99%	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 soft-ripened 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 36.

Table 38 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 × 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 baseline result is about 6-7 per 1,000 servings in Canada and in the U.S. (Table 38). 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 38, 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 38, between columns, same summary statistic (row)).

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

Table 38: Prevalence of contaminated Camembert servings, pasteurized-milk cheeses, no uncertainty considered. Summary statistics from distributions describe variability among contaminated servings prevalence.

Canada					United States				
	Elderly	**Pregnant**	**IC***	**General**		**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%
Percentile					Percentile				
1%	0.02%	0.02%	0.02%	0.02%	1%	0.02%	0.02%	0.02%	0.02%
2.5%	0.04%	0.04%	0.04%	0.04%	2.5%	0.04%	0.04%	0.04%	0.04%
5%	0.06%	0.06%	0.06%	0.06%	5%	0.06%	0.06%	0.06%	0.06%
10%	0.10%	0.10%	0.10%	0.10%	10%	0.10%	0.10%	0.10%	0.10%
25%	0.22%	0.21%	0.23%	0.23%	25%	0.23%	0.23%	0.23%	0.23%
50%	0.47%	0.47%	0.49%	0.49%	50%	0.49%	0.49%	0.49%	0.49%
75%	0.89%	0.88%	0.91%	0.91%	75%	0.92%	0.92%	0.92%	0.92%
90%	1.41%	1.39%	1.43%	1.43%	90%	1.45%	1.45%	1.45%	1.45%
95%	1.80%	1.77%	1.81%	1.81%	95%	1.84%	1.84%	1.84%	1.84%
97.5%	2.19%	2.16%	2.20%	2.20%	97.5%	2.22%	2.22%	2.22%	2.22%
99%	2.66%	2.65%	2.69%	2.69%	99%	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 36). 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 39) among populations (country × subpopulation) accrue

- Between countries, from differences in distributions of the number of *L. monocytogenes* in a contaminated serving (Table 36);
- 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 36); 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 36'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 $\log_{10}$ 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 $\log_{10}$ offset. Differences in the distribution of *L. monocytogenes* in contaminated servings among subpopulations (Table 36) have much less influence on Table 39'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 39: Risk of invasive listeriosis per contaminated Camembert cheese serving, pasteurized-milk cheeses, no uncertainty considered.
Summary statistics from distributions describe variability among the risk per contaminated serving.

Canada					United States				
	Elderly	**Pregnant**	**IC***	**General**		**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}
Percentile					Percentile				
1%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	1%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
2.5%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	2.5%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
5%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	5%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
10%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}	10%	1.06×10^{-12}	1.06×10^{-12}	1.06×10^{-12}	2.36×10^{-14}
25%	3.18×10^{-12}	3.18×10^{-12}	3.18×10^{-12}	7.11×10^{-14}	25%	3.18×10^{-12}	3.18×10^{-12}	3.18×10^{-12}	7.11×10^{-14}
50%	1.80×10^{-11}	2.23×10^{-11}	1.70×10^{-11}	3.79×10^{-13}	50%	1.91×10^{-11}	2.23×10^{-11}	1.70×10^{-11}	3.79×10^{-13}
75%	2.69×10^{-10}	4.14×10^{-10}	2.16×10^{-10}	4.83×10^{-12}	75%	2.67×10^{-10}	3.78×10^{-10}	2.09×10^{-10}	4.67×10^{-12}
90%	5.44×10^{-09}	1.57×10^{-08}	3.45×10^{-09}	7.71×10^{-11}	90%	5.10×10^{-09}	1.10×10^{-08}	3.21×10^{-09}	7.17×10^{-11}
95%	5.94×10^{-08}	2.12×10^{-07}	2.83×10^{-08}	6.32×10^{-10}	95%	5.23×10^{-08}	1.31×10^{-07}	2.56×10^{-08}	5.73×10^{-10}
97.5%	3.04×10^{-07}	3.35×10^{-06}	1.67×10^{-07}	3.74×10^{-09}	97.5%	2.55×10^{-07}	2.50×10^{-06}	1.44×10^{-07}	3.22×10^{-09}
99%	6.70×10^{-06}	1.06×10^{-04}	3.07×10^{-06}	6.87×10^{-08}	99%	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 40) 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 39.

The mean per serving risk is >150 times higher for Pregnant women than for the U.S. General population, as a baseline 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 41).

Table 40: Risk of invasive listeriosis per Camembert serving, pasteurized-milk cheeses, no uncertainty considered. Summary statistics from distributions describe variability among the risk per serving at random.

Canada					United States				
	Elderly	**Pregnant**	**IC***	**General**		**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}
Percentile					Percentile				
1%	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	1%	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
2.5%	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	2.5%	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
5%	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	5%	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
10%	6.81×10^{-15}	6.72×10^{-15}	6.92×10^{-15}	1.54×10^{-16}	10%	7.03×10^{-15}	7.03×10^{-15}	7.03×10^{-15}	1.57×10^{-16}
25%	2.04×10^{-14}	2.01×10^{-14}	2.08×10^{-14}	4.64×10^{-16}	25%	2.11×10^{-14}	2.11×10^{-14}	2.11×10^{-14}	4.71×10^{-16}
50%	1.16×10^{-13}	1.41×10^{-13}	1.11×10^{-13}	2.48×10^{-15}	50%	1.26×10^{-13}	1.48×10^{-13}	1.12×10^{-13}	2.51×10^{-15}
75%	1.73×10^{-12}	2.62×10^{-12}	1.41×10^{-12}	3.16×10^{-14}	75%	1.77×10^{-12}	2.51×10^{-12}	1.38×10^{-12}	3.09×10^{-14}
90%	3.50×10^{-11}	9.92×10^{-11}	2.25×10^{-11}	5.03×10^{-13}	90%	3.38×10^{-11}	7.29×10^{-11}	2.13×10^{-11}	4.75×10^{-13}
95%	3.81×10^{-10}	1.34×10^{-09}	1.85×10^{-10}	4.13×10^{-12}	95%	3.47×10^{-10}	8.71×10^{-10}	1.70×10^{-10}	3.80×10^{-12}
97.5%	1.95×10^{-09}	2.12×10^{-08}	1.09×10^{-09}	2.44×10^{-11}	97.5%	1.69×10^{-09}	1.65×10^{-08}	9.53×10^{-10}	2.13×10^{-11}
99%	4.31×10^{-08}	6.72×10^{-07}	2.01×10^{-08}	4.49×10^{-10}	99%	2.71×10^{-08}	7.03×10^{-07}	1.37×10^{-08}	3.06×10^{-10}

* IC: Immunocompromised.

Table 41: Relative mean risk of invasive listeriosis per serving at random, no uncertainty considered.

	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 baseline model that applies to section 9.1.2. 's results also applies to this section's results.

Risk per Serving

Table 42 and the Table 43 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 42 and the Table 43 is positively skewed. (Recall Figure 19, page 100). 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 42: Risk of invasive listeriosis per Camembert serving at random, pasteurized-milk cheeses, 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 (variability)		Elderly			Pregnant			Immunocompromised			General		
		← Uncertainty →			← Uncertainty →			← Uncertainty →			← Uncertainty →		
		Median	CI95		Median	CI95		Median	CI95		Median	CI95	
↓ Variability →	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}
	Percentile												
	1%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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}

Table 43: Risk of invasive listeriosis per Camembert serving at random, pasteurized-milk cheeses, 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 (variability)		Elderly			Pregnant			Immunocompromised			General		
		← Uncertainty →			← Uncertainty →			← Uncertainty →			← Uncertainty →		
		Median	CI95		Median	CI95		Median	CI95		Median	CI95	
↓ Variability →	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}
	Percentile												
	1%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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}

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

Figure 21 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.

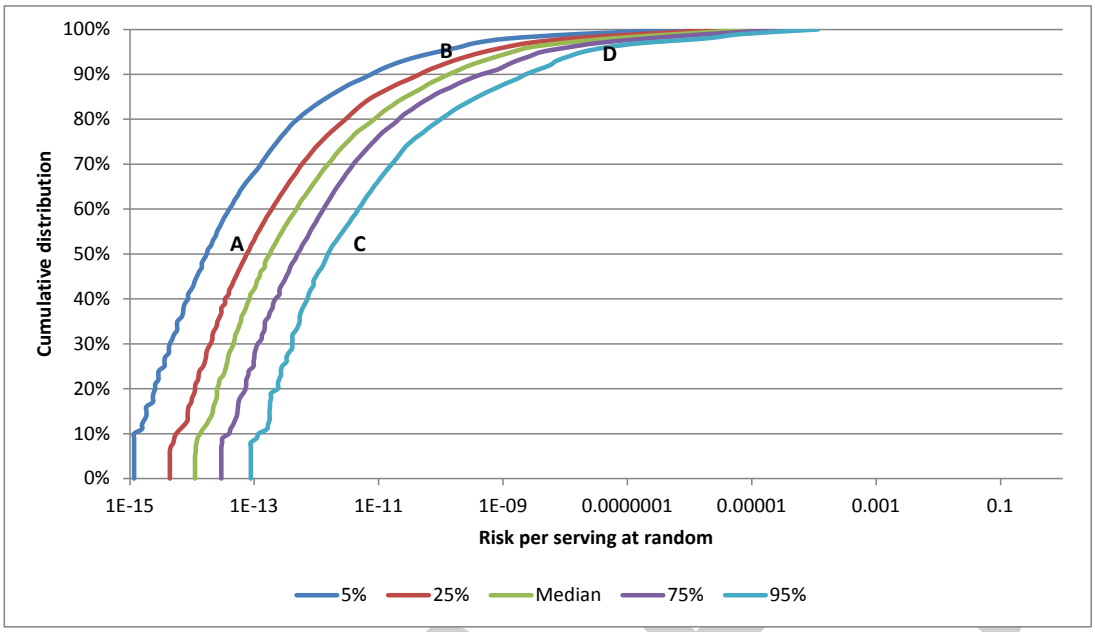

Figure 21: Distribution for risk of invasive listeriosis per soft-ripened cheese serving at random, Elderly population, Canada.

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 its 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 its baseline model setting, as the ratios:

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

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

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 the 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 the 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. Other outputs' or other subpopulations' results are also reported if the characteristics of the measures of change are different.

9.2.1. Prevalence

The risk per serving is directly linked to the contaminated cheese prevalence and the effect of changing the prevalence is straightforward: multiplying the baseline prevalence by 10 multiplies the risk per serving by 10; dividing the baseline prevalence by 10 divides the risk per serving also by 10.

9.2.2. Environmental Contamination Levels

The baseline model uses 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 18, p. 66).

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 44).

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 44). This result suggests that other factors than high initial environmental contamination are needed to affect the mean risk per contaminated serving.

Table 44: Sensitivity of the risk per contaminated serving, Canadian Elderly population, to the level of environmental contamination.

	Baseline	Number of cfu per cheese					
		1	10	100	1,000	10,000	100,000
dMedian	1.00	0.47	1.0	7.3	74	740	7,141
dMean	1.00	0.51	0.9	2.6	4.4	6.5	8.8

9.2.3. 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 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 baseline growth, dividing the baseline EGR_{20} by a factor of 2, when growth occurs;
- higher than baseline growth, multiplying the baseline EGR_{20} by a factor of 2, when growth occurs.

When growth occurs as in the baseline model, the mean risk per serving is >53,000 times larger than the mean risk per contaminated serving when no growth occurs (Table 45, *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 baseline model's median risk.

In the gamma concept predictive microbiology framework (Zwietering *et al.* 1996), environmental factors act independently ($0 \le \gamma_i(x_i) \le 1$) or with a positive synergy ($0 \le \gamma_{int}(x_{1, \ldots, x_n}) \le 1$) on the *EGR* according to $EGR = EGR_{20}\left(\prod_i \gamma_i(x_i)\right)\gamma_{int}(x_i,...,x_n)$. So, to halve the *EGR*, modify any one or more factors such that $\gamma_{int}(x_i,...,x_n)\prod_i \gamma_i(x_i) = ½$; modifying one or more factors such that $\gamma_{int}(x_i,...,x_n)\prod_i \gamma_i(x_i) = 2$, 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 $\log_{10}$ higher and a 1 $\log_{10}$ lower maximum population density for *L. monocytogenes* in soft-ripened cheese has a large impact on the mean risk per contaminated serving (Table 45, *dMean*) and no impact on the median risk per contaminated serving (Table 45, *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 45: Sensitivity of the risk per contaminated serving to growth characteristics.

	Baseline	EGR_{20}			Maximum population density	
		0	½ ×baseline	2 × baseline	- 1 $\log_{10}$	+1 $\log_{10}$
dMedian	1.00	0.35	0.47	9.5	1.0	1.0
dMean	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 baseline
- a general increase of 1°C during transport and marketing, at retail and during storage in the home refrigerator, compared to the baseline;
- a maximum duration of home storage of 28 days (*vs.* 56 days in the 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 46, 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 46, 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 46: Sensitivity of the risk per contaminated serving to the storage time and temperature.

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

Max. home storage duration	Baseline	Max. home storage: 28 days *vs.* baseline (56 days)			
		Elderly	Pregnant women	Immunocompromised	General
dMedian	1.00	0.88	0.81	0.94	0.94
dMean	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 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 of this baseline case.

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 47 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 47, "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 47'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 47: Spearman's rank correlations between various inputs and the risk per serving of soft-ripened cheese at random, pasteurized-milk cheese, for the Elderly population, Canada.

Input		Estimate
Specified (Parent) parameters	Derived (Child) parameters	
	Number of *Lm* in contaminated servings	1.00
	Number of *Lm* in contaminated cheese after home storage	0.95
	Number of *Lm* in contaminated cheese after retail storage	0.83
	Number of *Lm* in contaminated cheese after transport and marketing storage	0.75
	Number of *Lm* in contaminated cheese after aging	0.64
Parameter K_{ξ} for *Lm* growth lag time		**-0.54**
	Number of *Lm* 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 *Lm*, Environmental contamination		**0.21**
	Number of grams of products without *Lm*	-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 48 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 48: Spearman's rank correlation between the Mean or the 97.5th percentile of the risk per serving at random and some uncertain parameters.

Uncertain parameter	For Mean risk	For 97.5th 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 1st 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
$Var(T_{min})$	-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
$Var(\ln Kx_i)$	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 and Alternatives for Raw-milk cheese

In the 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.

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 20 and Table 21 in section 7.1).

Raw-milk and unpasteurized-milk cheeses are processed using a "traditional" (*i.e.* "non stabilized") process while pasteurized-milk cheeses 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 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.

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 the baseline model setting using the *dMean* and *dMedian* statistics as in section 9. *dMean* and *dMedian* are evaluated as the ratios:

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

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

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.

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 Riskoutputs").

10.1.1. Baseline for Raw-Milk Cheese

Farmstead-scale Operation

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 49). 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 fully pasteurized-milk cheese (section 9).

Table 49: Risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, cheeses from farmstead-scale operations under the current 60-day aging regulation.
Summary statistics from distributions describe variability among the risk per serving.

Canada					United States				
	Elderly	**Pregnant**	**IC***	**General**		**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}
Percentile					Percentile				
1%	3.14×10^{-14}	3.13×10^{-14}	3.15×10^{-14}	7.03×10^{-16}	1%	5.12×10^{-14}	5.12×10^{-14}	5.12×10^{-14}	1.14×10^{-15}
2.5%	3.14×10^{-14}	3.13×10^{-14}	3.15×10^{-14}	7.03×10^{-16}	2.5%	1.02×10^{-13}	1.02×10^{-13}	1.02×10^{-13}	2.29×10^{-15}
5%	6.28×10^{-14}	6.26×10^{-14}	6.30×10^{-14}	1.41×10^{-15}	5%	2.05×10^{-13}	2.05×10^{-13}	1.54×10^{-13}	3.43×10^{-15}
10%	2.20×10^{-13}	2.19×10^{-13}	2.21×10^{-13}	4.93×10^{-15}	10%	5.64×10^{-13}	6.15×10^{-13}	5.64×10^{-13}	1.26×10^{-14}
25%	2.17×10^{-12}	2.41×10^{-12}	2.14×10^{-12}	4.79×10^{-14}	25%	5.28×10^{-12}	5.84×10^{-12}	4.87×10^{-12}	1.09×10^{-13}
50%	4.40×10^{-11}	5.63×10^{-11}	3.96×10^{-11}	8.85×10^{-13}	50%	9.97×10^{-11}	1.25×10^{-10}	8.39×10^{-11}	1.88×10^{-12}
75%	2.55×10^{-09}	3.28×10^{-09}	2.06×10^{-09}	4.61×10^{-11}	75%	5.12×10^{-09}	6.75×10^{-09}	4.10×10^{-09}	9.17×10^{-11}
90%	3.45×10^{-08}	6.26×10^{-08}	2.90×10^{-08}	6.49×10^{-10}	90%	6.88×10^{-08}	1.18×10^{-07}	5.64×10^{-08}	1.26×10^{-09}
95%	1.63×10^{-07}	5.08×10^{-07}	1.35×10^{-07}	3.03×10^{-09}	95%	3.12×10^{-07}	8.34×10^{-07}	2.49×10^{-07}	5.57×10^{-09}
97.5%	8.21×10^{-07}	6.08×10^{-06}	6.82×10^{-07}	1.52×10^{-08}	97.5%	1.62×10^{-06}	8.42×10^{-06}	1.14×10^{-06}	2.54×10^{-08}
99%	6.28×10^{-06}	2.19×10^{-05}	6.31×10^{-06}	1.41×10^{-07}	99%	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 50) 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 fully 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 baseline.

Table 50: Risk of invasive listeriosis per serving: raw-milk cheese *vs.* pasteurized-milk cheese, farmstead-scale operations under the current 60-day aging regulation.

Canada						United States			
	Elderly	**Pregnant**	**IC***	**General**		**Elderly**	**Pregnant**	**IC***	**General**
dMedian	381	399	358	357	*dMedian*	788	844	746	746
dMean	53	52	69	69	*dMean*	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 20);
        - *L. monocytogenes* in those contaminated cheeses are predicted to grow to high enough levels to evoke the risk results in Table 39;
- 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 baseline case — *L. monocytogenes* from the raw milk— and because the number of *L. monocytogenes* in a contaminated cheese is larger than in the 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 risk 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 20, section 7.1, estimated mean: 2.4% in Canada *vs*. 4.2% in the United States).

Uncertainty considered

Table 51 and Table 52 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 51),

- 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 $[2.4 \times 10^{-8}, 2.7 \times 10^{-5}]$ (2.5^{th} and 97.5^{th} 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 51: Risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, cheeses from 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 (variability)		Elderly			Pregnant			Immunocompromised			General		
		← Uncertainty →			← Uncertainty →			← Uncertainty →			← Uncertainty →		
		Median	CI95		Median	CI95		Median	CI95		Median	CI95	
← Variability →	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}
	Percentile												
	1%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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}

Table 52: Risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, cheeses from 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 (variability)		Elderly			Pregnant			Immunocompromised			General		
		← Uncertainty →			← Uncertainty →			← Uncertainty →			← Uncertainty →		
		Median	CI95		Median	CI95		Median	CI95		Median	CI95	
← Variability →	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}
	Percentile												
	1%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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}

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 53) 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 53: Risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, cheeses from 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.

Canada					United States				
	Elderly	**Pregnant**	**IC***	**General**		**Elderly**	**Pregnant**	**IC**	**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}
Percentile					Percentile				
1%	5.39×10^{-14}	5.38×10^{-14}	5.42×10^{-14}	1.21×10^{-15}	1%	9.10×10^{-14}	9.10×10^{-14}	9.10×10^{-14}	2.03×10^{-15}
2.5%	5.39×10^{-14}	5.38×10^{-14}	1.08×10^{-13}	2.42×10^{-15}	2.5%	1.82×10^{-13}	1.82×10^{-13}	1.82×10^{-13}	4.07×10^{-15}
5%	1.62×10^{-13}	1.61×10^{-13}	1.63×10^{-13}	3.63×10^{-15}	5%	2.73×10^{-13}	2.73×10^{-13}	2.73×10^{-13}	6.10×10^{-15}
10%	3.78×10^{-13}	3.77×10^{-13}	3.79×10^{-13}	8.48×10^{-15}	10%	8.19×10^{-13}	9.10×10^{-13}	8.19×10^{-13}	1.83×10^{-14}
25%	2.86×10^{-12}	3.07×10^{-12}	2.76×10^{-12}	6.18×10^{-14}	25%	6.19×10^{-12}	6.82×10^{-12}	5.64×10^{-12}	1.26×10^{-13}
50%	5.70×10^{-11}	7.03×10^{-11}	5.12×10^{-11}	1.15×10^{-12}	50%	1.18×10^{-10}	1.46×10^{-10}	1.00×10^{-10}	2.24×10^{-12}
75%	3.20×10^{-09}	5.36×10^{-09}	2.61×10^{-09}	5.84×10^{-11}	75%	6.52×10^{-09}	9.10×10^{-09}	4.94×10^{-09}	1.10×10^{-10}
90%	4.52×10^{-08}	8.61×10^{-08}	4.02×10^{-08}	8.99×10^{-10}	90%	9.52×10^{-08}	1.62×10^{-07}	7.84×10^{-08}	1.75×10^{-09}
95%	2.21×10^{-07}	7.04×10^{-07}	1.92×10^{-07}	4.30×10^{-09}	95%	4.46×10^{-07}	1.15×10^{-06}	3.55×10^{-07}	7.93×10^{-09}
97.5%	1.18×10^{-06}	6.63×10^{-06}	1.01×10^{-06}	2.26×10^{-08}	97.5%	2.27×10^{-06}	1.06×10^{-05}	1.72×10^{-06}	3.85×10^{-08}
99%	1.08×10^{-05}	3.10×10^{-05}	1.08×10^{-05}	2.42×10^{-07}	99%	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 fully pasteurized-milk cheese (Table 54), 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 risk 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 54).

Table 54: Relative risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, artisanal-scale operations, under current 60 day aging regulation *vs*. pasteurized-milk cheese and *vs*. raw-milk cheeses from farmstead-scale operations, under current 60 day aging regulation.

		Relative to pasteurized-milk cheese					Relative to farmstead-scale operations			
		Elderly	**Pregnant women**	**IC***	**General**		**Elderly**	**Pregnant women**	**IC***	**General**
Canada	*dMedian*	493	499	462	462	*dMedian*	1.29	1.25	1.29	1.29
	dMean	90	77	100	100	*dMean*	1.71	1.48	1.45	1.45
United States	*dMedian*	934	992	891	891	*dMedian*	1.18	1.18	1.19	1.19
	dMean	176	146	227	227	*dMean*	1.57	1.52	1.45	1.45

* IC: Immunocompromised.

These slightly higher mean and median risks for artisanal-scale operations compared to 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 21 (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 21, Section 7.1) does not compensate for this higher level of prevalence.

Uncertainty considered

Table 55 and Table 56 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 55),

- 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 [1.7×10^{-8}, 4.6×10^{-5}] (2.5^{th} and 97.5^{th} 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 99^{th} 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 95^{th} 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 95^{th} 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 55: Risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, cheeses from 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 (variability)		Elderly			Pregnant			Immunocompromised			General		
		← Uncertainty →			← Uncertainty →			← Uncertainty →			← Uncertainty →		
		Median	CI95		Median	CI95		Median	CI95		Median	CI95	
↓ Variability →	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}
	Percentile												
	1%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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}

Table 56: Risk of invasive listeriosis per raw-milk soft-ripened cheese serving at random, cheeses from 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 (variability)		Elderly			Pregnant			Immunocompromised			General		
		← Uncertainty →			← Uncertainty →			← Uncertainty →			← Uncertainty →		
		Median	CI95		Median	CI95		Median	CI95		Median	CI95	
↓ Variability →	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}
	Percentile												
	1%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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%	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}

10.1.2. 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 $\log_{10}$ *L. monocytogenes* reduction in raw milk before cheese-making; 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 "Fully Pasteurized-milk cheese Baseline" (section 9); and,
- a "Raw-milk cheese Baseline" (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 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 $\log_{10}$ reduction in *L. monocytogenes* concentration in bulk milk, 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 57, 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 fully pasteurized-milk soft-ripened cheese (Table 57, 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 57: Relative size of mean and median from distribution for risk per raw-milk soft-ripened cheese serving at random when there is no restriction on the aging duration.

Baseline case		No aging time restriction			
		Canada		United States	
		Farmstead	Artisanal	Farmstead	Artisanal
Fully pasteurized-milk cheese	*dMedian*	56	70	113	135
	dMean	36	52	62	97
Raw-milk cheese	*dMedian*	0.15	0.14	0.14	0.14
	dMean	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 Camembert cheese, 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 log_{10} 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 58) 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 58). 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 fully 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 58: Relative size of mean and median from distribution for risk per raw-milk soft-ripened cheese serving at random under 3 log_{10} reduction mitigation applied to bulk raw milk.

Baseline cases		3 log_{10} reduction applied to bulk raw milk			
		Canada		United States	
		Farmstead	Artisanal	Farmstead	Artisanal
Fully pasteurized-milk cheese	*dMedian*	14	19	20	29
	dMean	7.4	11	11	17
Raw-milk cheese	*dMedian*	0.036	0.038	0.025	0.031
	dMean	0.14	0.12	0.10	0.10

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 composite made of 5 g from each of 5 cheeses at random from the lot; 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 model.

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 59, for the Elderly population in Canada, and Table 60, 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 fully 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 risk 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 risk per serving than any of the bulk milk testing alternatives, and is the only alternative that reduces the mean and median risk per serving for raw-milk cheese below the pasteurized-milk cheese baseline scenario.

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 59),

- 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 fully 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 fully pasteurized-milk soft-ripened cheese serving.

Table 59: Impact of testing bulk milk or cheese lots on the risk per serving, relative to the risk per serving of baseline cases for Elderly population in Canada.

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

Table 60: Impact of testing bulk milk or cheese lots on the risk per serving, relative to the risk per serving of baseline cases for Elderly population in the U.S.

		Relative to Baseline, Pasteurized-milk cheese			Relative to Baseline, Raw-milk cheese	
		Farmstead	Artisanal		Farmstead	Artisanal
Farm level, test every milk collection.	*dMedian*	5.3	5.2	*dMedian*	0.007	0.006
	dMean	3.0	2.9	*dMean*	0.027	0.016
Farm level, test at every farm	*dMedian*	-	15.4	*dMedian*	-	0.016
	dMean	-	8.9	*dMean*	-	0.051
Dairy silo	*dMedian*	4.7	8.6	*dMedian*	0.006	0.009
	dMean	2.3	4.3	*dMean*	0.021	0.025
Cheese lots	*dMedian*	0.242	1.036	*dMedian*	0.000	0.001
	dMean	0.134	0.672	*dMean*	0.001	0.004

Similarly, for farmstead-scale operations in the U.S. (Table 60),

- 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 fully 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 fully pasteurized-milk soft-ripened cheese serving.

The relative effect of testing farms (once) rather than testing every milk collection (Table 59, Table 60, 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).

Cheese Lot Testing

The mean and median risk 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 risk 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 risk per serving at random according to the various alternatives is proposed Figure 22.

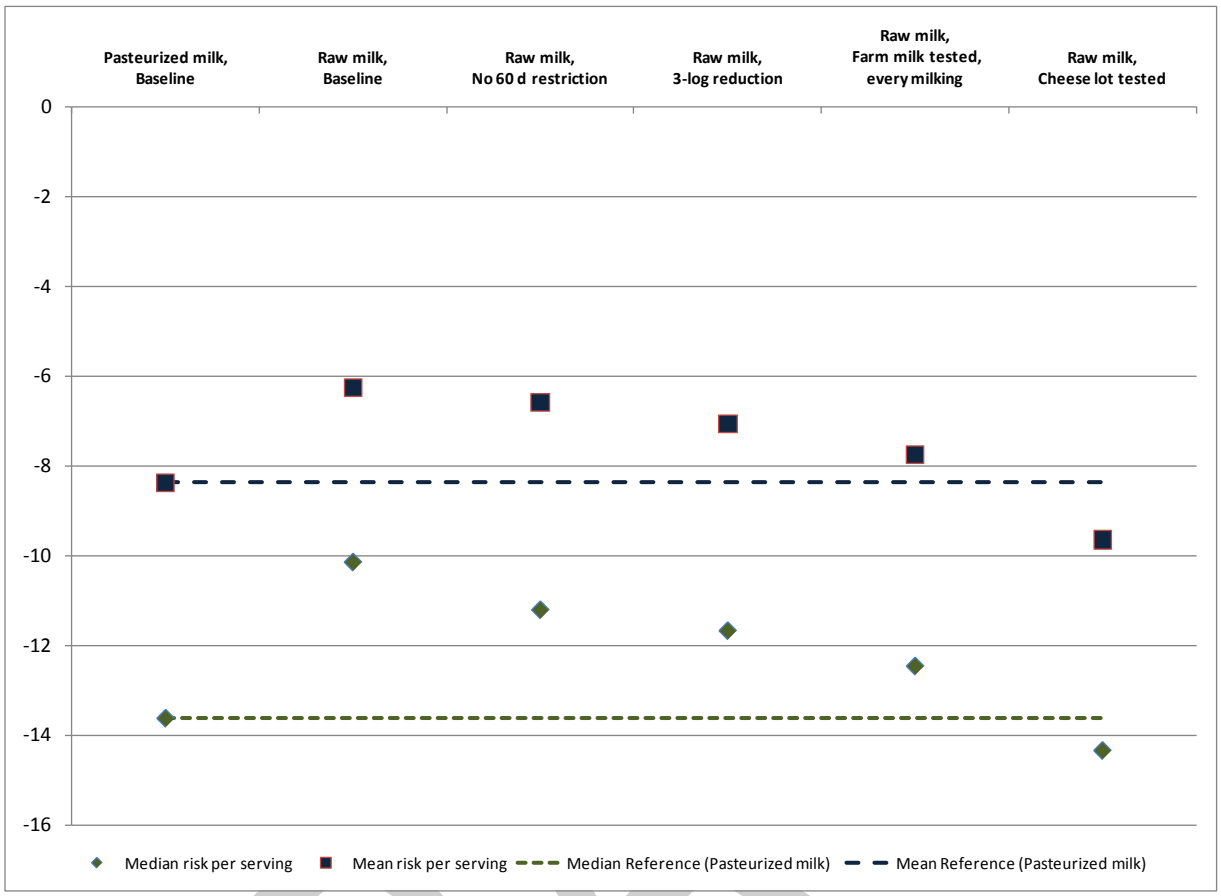

Figure 22: Log_{10}(median) (♦) and log_{10}(mean) (■) risk per serving at random for the Elderly population, Canada, comparing Pasteurized-milk cheese baseline, Farmstead raw-milk cheese baseline, Farmstead raw-milk cheese without 60-day aging regulation, Farmstead raw-milk cheese with a 3-log reduction 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.

Testing Considerations

The bulk milk testing alternatives above 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 composite 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:
    - the volume tested was 125 ml, compared to 25 ml in the baseline;

    - the single *L. monocytogenes* detection probability was 0.50 or 0.90, compared to 0.75 in the baseline; and,
    - 95% of bulk milk is tested, rather than 100%.
- For cheese lot testing:
    - the mass tested was a composite of 125 g, including 25 g from 5 cheeses, compared to 25 g (5 g × 5 cheeses) in the baseline;
    - the tested 25 g originated from 1 single cheese, compared to 5 cheeses;
    - the single *L. monocytogenes* detection probability was 0.50 or 0.90, compared to 0.75 in the baseline;
    - 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,
    - 95% of cheese lots are tested, rather than 100%.

The results (Table 61, 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 a composite 5 × 25g = 125 g is much more efficient than testing 5 × 5g = 25g. Whatever the change made in these parameters, the testing of dairy silo milk leads to a higher mean and median risk 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.

As well, Table 61 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*)

composites of 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.

Table 61: Impact of parameters of testing bulk milk or 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			Relative to Baseline, Baseline Test			Relative to Baseline, Pasteurized-milk cheese, no testing		
				Canada	U.S.		Canada	U.S.
Farm bulk milk testing	Baseline farm bulk testing	*dMedian*	1 (ref)	1 (ref)	*dMedian*	4.63	5.31
		dMean	1 (ref)	1 (ref)	*dMean*	2.22	2.99
	125 ml *vs.* 25 ml	*dMedian*	0.74	0.63	*dMedian*	3.42	3.36
		dMean	0.80	0.62	*dMean*	1.79	1.86
	Single *Lm* detection probability: 0.50 *vs.* 0.75	*dMedian*	1.30	1.43	*dMedian*	6.01	7.59
		dMean	1.26	1.24	*dMean*	2.79	3.71
	Single *Lm* detection probability: 0.90 *vs.* 0.75	*dMedian*	0.92	0.88	*dMedian*	4.24	4.66
		dMean	0.95	0.83	*dMean*	2.11	2.49
	95% bulk milk tested *vs.* 100% bulk milk tested	*dMedian*	2.06	2.72	*dMedian*	9.56	14.42
		dMean	2.60	2.48	*dMean*	5.77	7.41
	90% bulk milk tested *vs.* 100% bulk milk tested	*dMedian*	3.50	5.29	*dMedian*	16.20	28.09
		dMean	3.65	4.84	*dMean*	8.09	14.47
Cheese lots testing	Baseline cheese lots testing	*dMedian*	1 (ref)	1 (ref)	*dMedian*	0.16	0.24
		dMean	1 (ref)	1 (ref)	*dMean*	0.08	0.13
	125 g *vs.* 25 g	*dMedian*	0.0005	0.0026	*dMedian*	0.0001	0.0006
		dMean	0.0038	0.0047	*dMean*	0.0003	0.0006
	1 Cheese *vs.* 5 Cheeses	*dMedian*	0.58	0.69	*dMedian*	0.09	0.17
		dMean	0.77	0.72	*dMean*	0.06	0.10
	Single *Lm* detection probability: 0.50 *vs.* 0.75	*dMedian*	1.28	1.38	*dMedian*	0.21	0.33
		dMean	1.51	1.37	*dMean*	0.12	0.18
	Single *Lm* detection probability: 0.90 *vs.* 0.75	*dMedian*	0.82	0.98	*dMedian*	0.13	0.24
		dMean	0.86	0.96	*dMean*	0.07	0.13
	In-plant env. *Lm* contaminates: .5% *vs.* 2.5%	*dMedian*	0.94	1.18	*dMedian*	0.15	0.28
		dMean	1.11	1.08	*dMean*	0.09	0.14
	In-plant env. *Lm* contaminates: 1% *vs.* 2.5%	*dMedian*	0.82	1.15	*dMedian*	0.13	0.28
		dMean	1.10	1.22	*dMean*	0.09	0.16
	95% cheese lots tested *vs.* 100% cheese lots tested	*dMedian*	59	79.49	*dMedian*	9.68	19.2
		dMean	24	23.83	*dMean*	1.94	3.20
	90% cheese lots tested *vs.* 100% cheese lots tested	*dMedian*	160	218	*dMedian*	26.1	52.7
		dMean	44.4	46.9	*dMean*	3.57	6.31

(ref): reference case.

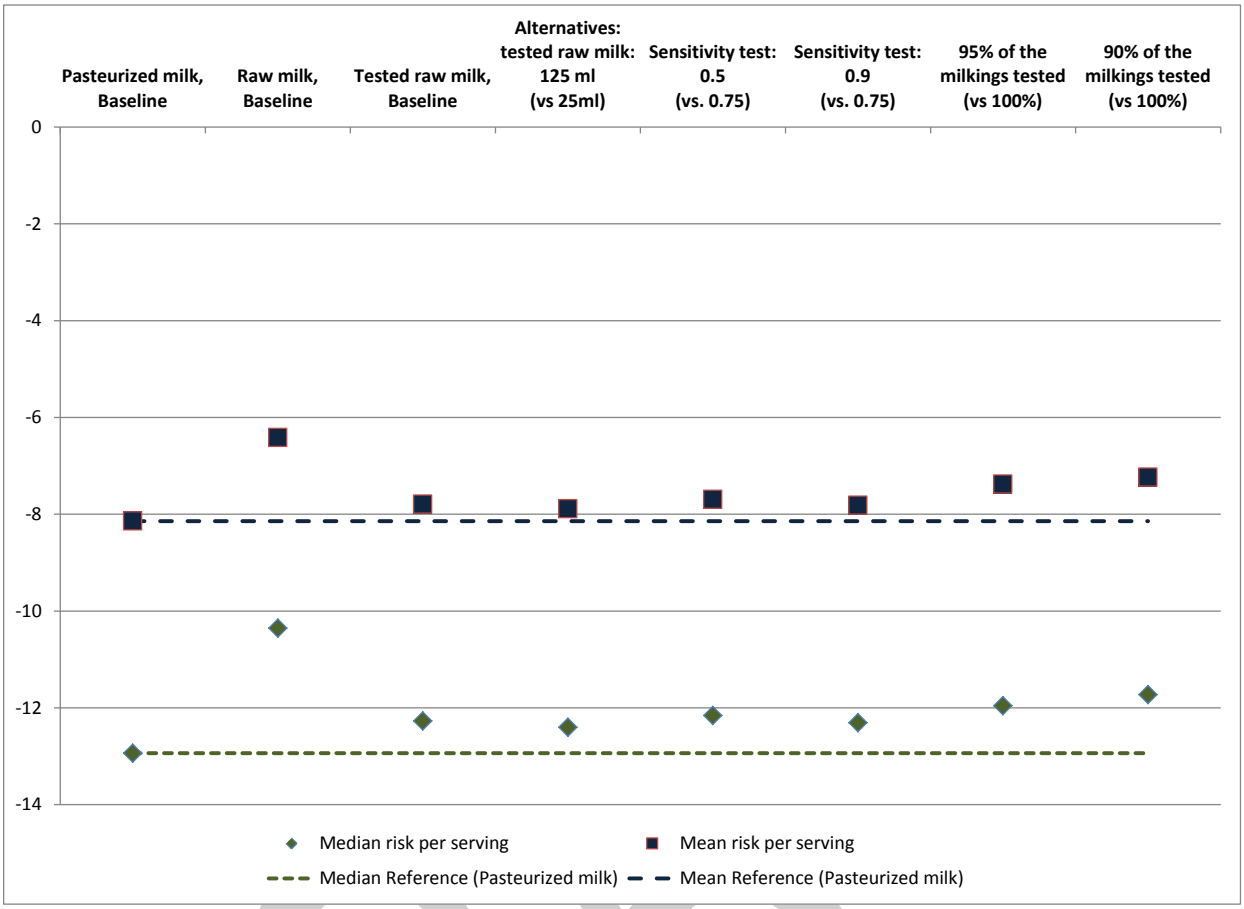

Figure 23: Log_{10}(median) (♦) and log_{10}(mean) (■) risk per serving at random for the Elderly population, Canada, comparing Pasteurized-milk cheese baseline, Farmstead raw-milk cheese with farm bulk milk tested (every milk collection) and alternatives. See text for details.

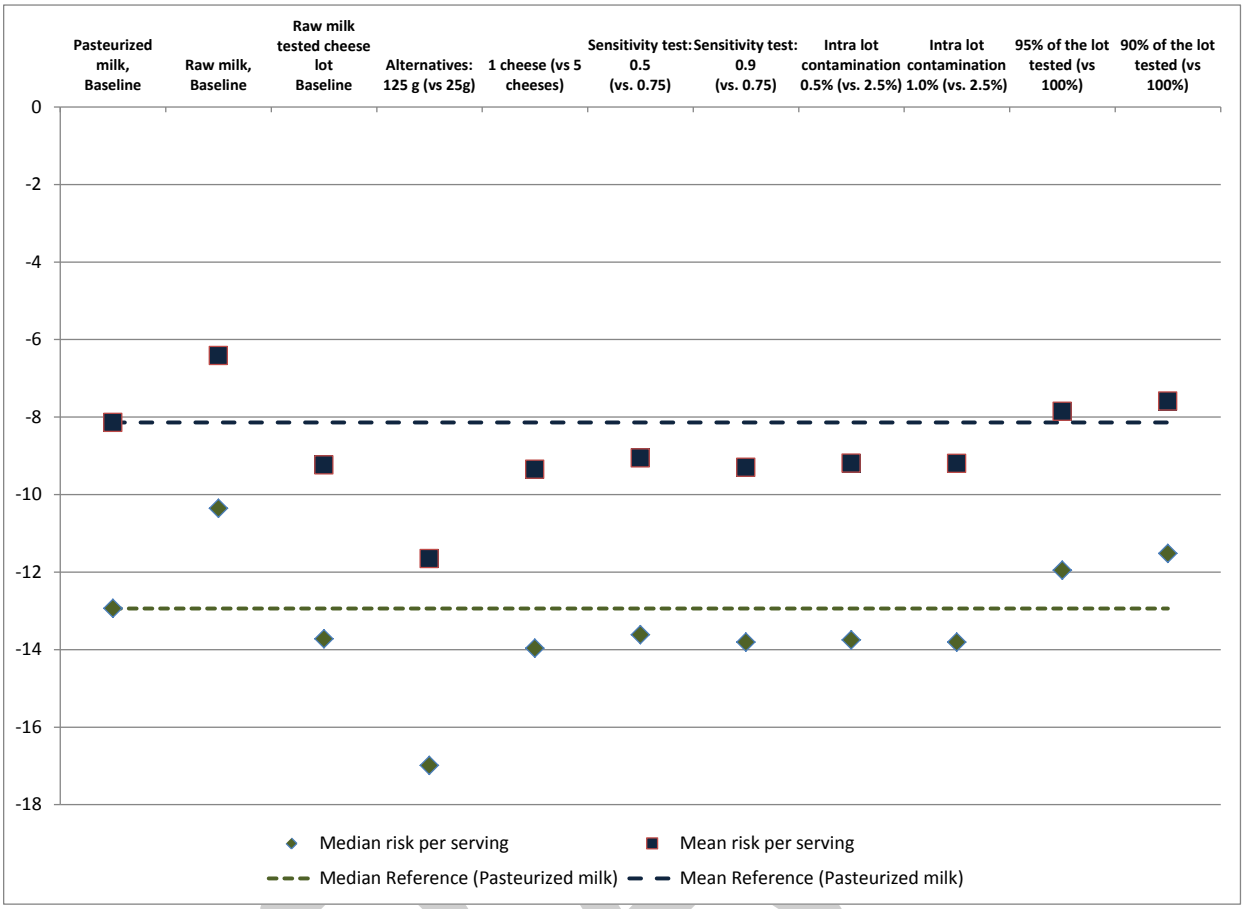

Figure 24: Log_{10}(median) (♦) and log_{10}(mean) (■) risk per serving at random for the Elderly population, Canada, comparing Pasteurized-milk cheese baseline, Farmstead raw-milk cheese with cheese lots tested and alternatives. See text for details.

10.2. Pasteurized-milk cheese

10.2.1. Testing Cheese Lots

The impact of testing lots of cheeses made from pasteurized milk on the mean and median risk per serving (Table 62) 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 62: Risk of invasive listeriosis per serving of pasteurized-milk Camembert: relative risk when cheese lot testing is implemented.

Canada						United States			
	Elderly	**Pregnant**	**IC***	**General**		**Elderly**	**Pregnant**	**IC***	**General**
dMedian	1.1	1.1	1.1	1.1	*dMedian*	1.1	1.1	1.1	1.1
dMean	0.8	0.8	0.8	0.8	*dMean*	0.7	0.8	0.8	0.8

IC*: Immunocompromised.

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 and the cheese (Camembert-like cheese or cheeses with similar characteristics) considered here. Notably, the growth function parameterization relies on the more extensive growth information available for Camembert cheese. Without further discussion, the results should not be extrapolated to other soft-ripened cheeses such as "Brie-type" and "Vacherin-type", other soft cheeses such as Mexican-style cheese or cheeses with other textures such as Gouda and Cheddar. 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 63 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 63: 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**				**United States**			
	Elderly	**Pregnant women**	**IC***	**General**	**Elderly**	**Pregnant women**	**IC***	**General**
L. monocytogenes contamination in farm	A	A	A	A	B	B	B	B
L. monocytogenes 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
L. monocytogenes 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 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 "traditionnal" process for farmstead and artisanal cheese. Nevertheless, there is a lack of information about

- 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 cheeses from large commercial cheese manufacturing operations and 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.

There is a lack of information about *L. monocytogenes* growth in naturally contaminated cheese and a lack of information about the growth of *L. monocytogenes* in the presence of natural cheese flora, such as in raw-milk cheese rather than in pasteurized-milk cheese. This risk assessment uses the assumption that *L. monocytogenes* growth is similar in soft-ripened cheese made from pasteurized milk and that made from raw milk. That is consistent with some published literature (for example, for fresh soft cheese, (FDA/FSIS 2003)) but is not consistent with other published literature (for example Schvartzman *et al.* 2011). Further studies are needed on that subject.

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 model. 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 appendixes, 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.

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. Keeping in mind the limitations, 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 to other data 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.

References

1) AAFC (2006). Statistics of the Canadian Dairy Industry, 2006. Agriculture & Agri-Foods Canada-AID Dairy Section**:** 174.
2) AFSSA (2000). Rapport de la Commission d'étude des risques liés à *Listeria monocytogenes*. Maisons-Alfort, France, Agence Française de Sécurité Sanitaire des Aliments**:** 143.
3) Albert, I., E. Grenier, J. B. Denis and J. Rousseau (2008). "Quantitative risk assessment from farm to fork and beyond: a global Bayesian approach concerning food-borne diseases." Risk Anal **28**(2): 557-571.
4) Albert, I., R. Pouillot and J. B. Denis (2005). "Stochastically modeling *Listeria monocytogenes* growth in farm tank milk." Risk Anal **25**(5): 1171-1185.
5) Antognoli, M., J. Lombard, B. Wagner, B. McCluskey, J. Van Kessel and J. Karns (2008). "Risk factors associated with the presence of viable *Listeria monocytogenes* in bulk tank milk from US dairies." Zoon Publ Health **56** (2): 77-83.
6) Arqués, J. L., E. Rodríguez, P. Gaya, M. Medina and M. Nuñez (2005). "Effect of combinations of high pressure treatment and bacteriocin producing lactic acid bacteria on the survival of *Listeria monocytogenes* in raw milk cheese." Intl Dairy J **15**(16-19): 893-900.
7) Augustin, J.-C. and V. Carlier (2000). "Mathematical modelling of the growth rate and lag time for *Listeria monocytogenes*." Int J Food Microbiol **56**(1): 29-51.
8) Augustin, J. C., V. Zuliani, M. Cornu and L. Guillier (2005). "Growth rate and growth probability of *Listeria monocytogenes* in dairy, meat and seafood products in suboptimal conditions." J Appl Microbiol **99**(5): 1019-1042.
9) Back, J. P., S. A. Langford and R. G. Kroll (1993). "Growth of *Listeria monocytogenes* in Camembert and Other Soft Cheeses at Refrigeration Temperatures." J Dairy Res **60**(3): 421-429.
10) Baranyi, J. and T. A. Roberts (1994). "A dynamic approach to predicting bacterial growth in food." Int J Food Microbiol **23**(3-4): 277-294.
11) Beckers, H. J., P. S. S. Soentoro and E. H. M. Delgou-van Asch (1987). "The occurrence of *Listeria monocytogenes* in soft cheeses and raw milk and its resistance to heat." Int J Food Microbiol **4**(3): 249-256.

12) Bemrah, N., M. Sanaa, M. H. Cassin, M. W. Griffiths and O. Cerf (1998). "Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk." Prev Vet Med **37**(1-4): 129-145.

13) Bille, J., D. S. Blanc, H. Schmid, K. Boubaker, A. Baumgartner, H. H. Siegrist, M. L. Tritten, R. Lienhard, D. Berner, R. Anderau, M. Treboux, J. M. Ducommun, R. Malinverni, D. Genne, P. H. Erard and U. Waespi (2006). "Outbreak of human listeriosis associated with tomme cheese in northwest Switzerland, 2005." Euro Surveill **11**(6): 91-93.

14) Borgonovo, E. (2006). "Measuring uncertainty importance: investigation and comparison of alternative approaches." Risk Anal **26**(5): 1349-1361.

15) Bougle, D. L. and V. Stahl (1994). "Survival of *Listeria monocytogenes* after irradiation treatment of Camembert cheeses made from raw milk." J Food Prot **57**(9): 811-813.

16) Bourry, A. and B. Poutrel (1996). "Bovine mastitis caused by *Listeria monocytogenes*: kinetics of antibody responses in serum and milk after experimental infection." J Dairy Sci **79**(12): 2189-2195.

17) Bourry, A., B. Poutrel and J. Rocourt (1995). "Bovine mastitis caused by *Listeria monocytogenes*: characteristics of natural and experimental infections." J Med Microbiol **43**(2): 125-132.

18) Breand, S., G. Fardel, J. P. Flandrois, L. Rosso and R. Tomassone (1997). "A model describing the relationship between lag time and mild temperature increase duration." Int J Food Microbiol **38**(2-3): 157-167.

19) Breand, S., G. Fardel, J. P. Flandrois, L. Rosso and R. Tomassone (1999). "A model describing the relationship between regrowth lag time and mild temperature increase for *Listeria monocytogenes*." Int J Food Microbiol **46**(3): 251-261.

20) Buchanan, R. L., R. C. Whiting and W. C. Damert (1997). "When is simple good enough: a comparison of the Gompertz, Baranyi, and three-phase linear models for fitting bacterial growth curves." Food Microbiol **14**(4): 313-326.

21) Bula, C. J., J. Bille and M. P. Glauser (1995). "An epidemic of food-borne listeriosis in western Switzerland: description of 57 cases involving adults." Clin Infect Dis **20**(1): 66-72.

22) Cates, S. C., K. M. Kosa, S. A. Karns, S. L. Godwin and D. Chambers (2007). "Consumer Storage Practices for Refrigerated Ready-to-Eat Foods: Results of a Web-enabled Survey." Food Prot Trends **27**(7): 530-543.

23) CDC (1985). "Listeriosis outbreak associated with Mexican-style cheese--California." MMWR Morb Mortal Wkly Rep **34**(24): 357-359.

24) CDC (2006). Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet surveillance final report for 2004 (with Tables). Atlanta, Georgia, U.S. Department of health and Human Services, CDC.

25) CDC (2008). Foodborne Diseases Active Surveillance Network (FoodNet): FoodNet surveillance final report for 2005. Atlanta, Georgia, U.S. Department of health and Human Services, CDC.

26) CDC (2011). "Vital Signs: Incidence and Trends of Infection with Pathogens Transmitted Commonly Through Food --- Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996--2010." MMWR Morb Mortal Wkly Rep **60**(22): 749-755.

27) CDC. (2012). "FoodBorne Outbreak Online Database." Retrieved March 15th,, 2012, from http://wwwn.cdc.gov/foodborneoutbreaks/Default.aspx#.

28) CFSAN (2008). Data on storage times and temperatures for soft-ripened cheese from expert solicitation prepared by International Dairy Foods Association - Expert elicitation - July 30, 2008.

29) CFSAN Risk Analysis Working Group (2002). Initiation and conduct of all 'major' risk assessments within a risk analysis framework, CFSAN/FDA**:** 69.

30) Chen, Y., W. H. Ross, M. J. Gray, M. Wiedmann, R. C. Whiting and V. N. Scott (2006). "Attributing risk to *Listeria monocytogenes* subgroups: dose response in relation to genetic lineages." J Food Prot **69**(2): 335-344.

31) Chen, Y., W. H. Ross, R. C. Whiting, A. Van Stelten, K. K. Nightingale, M. Wiedmann and V. N. Scott (2011). "Variation in *Listeria monocytogenes* dose response in relation to subtypes encoding a full-length or truncated internalin A." Appl Environ Microbiol **77**(4): 1171-1180.

32) Clark, C. G., J. Farber, F. Pagotto, N. Ciampa, K. Dore, C. Nadon, K. Bernard and L. K. Ng (2010). "Surveillance for *Listeria monocytogenes* and listeriosis, 1995-2004." Epidemiol Infect **138**(4): 559-572.

33) *Codex alimentarius* Commission (1999). Principles and guidelines for the conduct of microbiological risk assessment. Rome, FAO edition**:** 6.

34) D'Amico, D. J. and C. W. Donnelly (2009). "Detection, isolation, and incidence of *Listeria* spp. in small-scale artisan cheese processing facilities: a methods comparison." J Food Prot **72**(12): 2499-2507.

35) D'Amico, D. J. and C. W. Donnelly (2010). "Microbiological quality of raw milk used for small-scale artisan cheese production in Vermont: effect of farm characteristics and practices." J Dairy Sci **93**(1): 134-147.

36) D'Amico, D. J., M. J. Druart and C. W. Donnelly (2008a). "60-day aging requirement does not ensure safety of surface-mold-ripened soft cheeses manufactured from raw or pasteurized milk when *Listeria monocytogenes* is introduced as a postprocessing contaminant." J Food Prot **71**(8): 1563-1571.

37) D'Amico, D. J., E. Groves and C. W. Donnelly (2008b). "Low incidence of foodborne pathogens of concern in raw milk utilized for farmstead cheese production." J Food Prot **71**(8): 1580-1589.

38) Danielsson-Tham, M. L., E. Eriksson, S. Helmersson, M. Leffler, L. Ludtke, M. Steen, S. Sorgjerd and W. Tham (2004). "Causes behind a human cheese-borne outbreak of gastrointestinal listeriosis." Foodborne Pathog Dis **1**(3): 153-159.

39) Davidson, R. J., D. W. Sprung, C. E. Park and M. K. Rayman (1989). "Occurrence of *Listeria monocytogenes*, *Campylobacter* spp and *Yersinia enterocolitica* in Manitoba raw milk " Canadian Institute of Food Science and Technology Journal-Journal de l'Institut Canadien de Science et Technologie Alimentaires **22**(1): 70-74.

40) De Graaf, T. and R. H. Dwinger (1996). "Estimation of milk production losses due to sub-clinical mastitis in dairy cattle in Costa Rica." Prev Vet Med **26**(3-4): 215-222.

41) De Vries, J. and R. Strikwerda (1956). "[A case of bovine udder listeriosis.]." Zentralbl Bakteriol Orig **167**(3): 229-232.

42) Delignette-Muller, M. L. (1998). "Relation between the generation time and the lag time of bacterial growth kinetics." Int J Food Microbiol **43**(1-2): 97-104.

43) Desmasures, N. and M. Gueguen (1997). "Monitoring the microbiology of high quality milk by monthly sampling over 2 years." J Dairy Res **64**(2): 271-280.

44) Deuffic, S., L. Buffat, T. Poynard and A. J. Valleron (1999). "Modeling the hepatitis C virus epidemic in France." Hepatology **29**(5): 1596-1601.

45) Djoenne, B. C. (1982). "[A case of *Listeria* abortion in [dairy] cattle]." Norsk Veterinaertidsskrift **94**(12): 803-805.

46) Doyle, M. E., A. S. Mazzotta, T. Wang, D. W. Wiseman and V. N. Scott (2001). "Heat resistance of *Listeria monocytogenes*." J Food Prot **64**(3): 410-429.

47) Doyle, M. P., K. A. Glass, J. T. Beery, G. A. Garcia, D. J. Pollard and R. D. Schultz (1987). "Survival of *Listeria monocytogenes* in milk during high-temperature, short-time pasteurization." Appl Environ Microbiol **53**(7): 1433-1438.

48) Dutta, P. K. and B. S. Malik (1981). "Isolation and characterization of *Listeria monocytogenes* from animals and human beings." Ind J Anim Sci **51**: 1045-1052.

49) EcoSure. (2008). "2007 U.S. Cold Temperature Evaluation: Design and Summary Pages." Retrieved June, 4, 2008, from http://foodrisk.org/exclusives/EcoSure/.

50) Ellouze, M., J. P. Gauchi and J. C. Augustin (2010). "Global sensitivity analysis applied to a contamination assessment model of *Listeria monocytogenes* in cold smoked salmon at consumption." Risk Anal **30**(5): 841-852.

51) Erdogan, H. M., B. Cetinkaya, L. E. Green, P. J. Cripps and K. L. Morgan (2001). "Prevalence, incidence, signs and treatment of clinical listeriosis in dairy cattle in England." Vet Rec **149**(10): 289-293.

52) Everson, T. C. (1988). "Industry response to the problems of pathogenic bacteria." J Dairy Sci **71**(10): 2820-2829.

53) Faleiro, M. L., P. W. Andrew and D. Power (2003). "Stress response of *Listeria monocytogenes* isolated from cheese and other foods." Int J Food Microbiol **84**(2): 207-216.

54) FAO/WHO (2004). Risk assessment of *Listeria monocytogenes* in ready to eat foods - Technical report. Microbiological Risk Assessment Series, no 5. Rome, Food and Agriculture Organization of the United Nations and World Health Organization**:** 269.

55) Farber, J. M., W. H. Ross and J. Harwig (1996). "Health risk assessment of *Listeria monocytogenes* in Canada." Int J Food Microbiol **30**(1-2): 145-156.

56) Farber, J. M., G. W. Sanders and S. A. Malcolm (1988). "The presence of *Listeria* spp in raw milk in Ontario." Can J Microbiol **34**(2): 95-100.

57) FDA (2009). Grade 'A' Pasteurized milk ordinance, U.S. Department of Health and Human Services, Public Health Service, Food and Drug Administration**:** 398.

58) FDA/FSIS (2001). Draft assessment of the relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods, Food and Drug Administration, United States Department of Agriculture, Centers for Disease Control and Prevention**:** 381.

59) FDA/FSIS (2003). Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods, Food and Drug Administration, United States Department of Agriculture, Centers for Disease Control and Prevention**:** 541.

60) Fedio, W. M. and H. Jackson (1990). "Incidence of *Listeria monocytogenes* in raw bulk milk in Alberta." Canadian Institute of Food Science and Technology Journal-Journal de l'Institut Canadien de Science et Technologie Alimentaires **23**(4-5): 236-238.

61) Fedio, W. M. and H. Jackson (1992). "On the origin of *Listeria monocytogenes* in raw bulk-tank milk." Intl Dairy **2**: 197-208.

62) Fedio, W. M., M. Schoonderwoerd, R. H. Shute and H. Jackson (1990). "A case of bovine mastitis caused by *Listeria monocytogenes*." Can Vet J **31**(11): 773-775.

63) Fenlon, D. R., T. Stewart and W. Donachie (1995). "The incidence, numbers and types of *Listeria monocytogenes* isolated from farm bulk tank milks." Lett Appl Microbiol **20**(1): 57-60.

64) Fenlon, D. R. and J. Wilson (1989). "The incidence of *Listeria monocytogenes* in raw milk from farm bulk tanks in north-east Scotland." J Appl Bacteriol **66**(3): 191-196.

65) Frey, H. C. (1992). Quantitative Analysis of Uncertainty and Variability in Environmental Policy Making, American Association for the Advancement of Science / U.S. Environmental Protection Agency.

66) Frey, H. C. and S. R. Patil (2002). "Identification and review of sensitivity analysis methods." Risk Anal **22**(3): 553-578.

67) FSIS (2003). FSIS Risk Assessment for *Listeria monocytogenes* in deli meats, FSIS.

68) Garcia-Graells, C., C. Valckx and C. W. Michiels (2000). "Inactivation of *Escherichia coli* and *Listeria innocua* in milk by combined treatment with high hydrostatic pressure and the lactoperoxidase system." Appl Environ Microbiol **66**(10): 4173-4179.

69) Gaulin, C., D. Ramsay, L. Ringuette and J. Ismail (2003). "First documented outbreak of *Listeria monocytogenes* in Quebec, 2002." Can Commun Dis Rep **29**(21): 181-186.

70) Gay, M. and A. Amgar (2005). "Factors moderating *Listeria monocytogenes* growth in raw milk and in soft cheese made from raw milk." Lait **85**(3): 153-170.

71) Gaya, P., J. Sanchez, M. Medina and M. Nunez (1998). "Incidence of *Listeria monocytogenes* and other *Listeria* species in raw milk produced in Spain." Food Microbiol **15**(5): 551-555.

72) Genigeorgis, C., M. Carniciu, D. Dutulescu and T. B. Farver (1991). "Growth and survival of *Listeria monocytogenes* in market cheeses stored at 4 degrees C to 30 degrees C." J Food Prot **54**(9): 662-668.

73) Ghani, A. C., N. M. Ferguson, C. A. Donnelly, T. J. Hagenaars and R. M. Anderson (1998). "Epidemiological determinants of the pattern and magnitude of the vCJD epidemic in Great Britain." Proc Biol Sci **265**(1413): 2443-2452.

74) Gianfranceschi, M., M. C. D'Ottavio, A. Gattuso, M. Pourshaban, I. Bertoletti, R. Bignazzi, P. Manzoni, M. Marchetti and P. Aureli (2006). "Listeriosis associated with gorgonzola (Italian blue-veined cheese)." Foodborne Pathog Dis **3**(2): 190-195.

75) Gilleberg, B. A. and M. L. Nordhaug (1989). "[Bovine mastites caused by *Listeria monocytogenes*. Zoonoses dangers]." Norsk Veterinaertidsskrift **100**: 131-132.

76) Gilot, P., C. Hermans, M. Yde, J. Gigi, M. Janssens, A. Genicot, P. Andre and G. Wauters (1997). "Sporadic case of listeriosis associated with the consumption of a *Listeria monocytogenes*-contaminated 'Camembert' cheese." J Infect **35**(2): 195-197.

77) Gitter, M., R. Bradley and P. H. Blampied (1980). "*Listeria monocytogenes* infection in bovine mastitis." Vet Rec **107**(17): 390-393.

78) Gombas, D. E., Y. Chen, R. S. Clavero and V. N. Scott (2003). "Survey of *Listeria monocytogenes* in ready-to-eat foods." J Food Prot **66**(4): 559-569.

79) Goulet, V., M. Hebert, C. Hedberg, E. Laurent, V. Vaillant, H. De Valk and J. C. Desenclos (2012). "Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis." Clin Infect Dis **54**(5): 652-660.

80) Goulet, V., C. Jacquet, V. Vaillant, I. Rebiere, E. Mouret, C. Lorente, E. Maillot, F. Stainer and J. Rocourt (1995). "Listeriosis from consumption of raw-milk cheese." Lancet **345**(8964): 1581-1582.

81) Greenwood, M. H., D. Roberts and P. Burden (1991). "The occurrence of *Listeria* species in milk and dairy products: a national survey in England and Wales." Int J Food Microbiol **12**(2-3): 197-206.

82) Gröhn, Y. T., D. J. Wilson, R. N. Gonzalez, J. A. Hertl, H. Schulte, G. Bennett and Y. H. Schukken (2004). "Effect of pathogen-specific clinical mastitis on milk yield in dairy cows." J Dairy Sci **87**(10): 3358-3374.

83) Guerzoni, M. E., R. Lanciotti, S. Torriani and F. Dellaglio (1994). "Growth modeling of *Listeria monocytogenes* and *Yersinia enterocolitica* in food model systems and dairy-products." Int J Food Microbiol **24**(1-2): 83-92.

84) Haas, C. N., J. B. Rose and C. P. Gerba (1999). Quantitative microbial risk assessment. New York, Wiley.

85) Harvey, J. and A. Gilmour (1992). "Occurrence of *Listeria* species in raw milk and dairy products produced in Northern Ireland." J Appl Bacteriol **72**(2): 119-125.

86) Hassan, L., H. O. Mohammed and P. L. McDonough (2001). "Farm-management and milking practices associated with the presence of *Listeria monocytogenes* in New York state dairy herds." Prev Vet Med **51**(1-2): 63-73.

87) Hassan, L., H. O. Mohammed, P. L. McDonough and R. N. Gonzalez (2000). "A cross-sectional study on the prevalence of *Listeria monocytogenes* and *Salmonella* in New York dairy herds." J Dairy Sci **83**(11): 2441-2447.

88) Health Canada Decision Making Framework (2000). Health Canada decision-making framework for identifying, assessing, and managing health risks, Health Canada - Santé Canada**:** 80.

89) Helloin, E., A. Bouttefroy, M. Gay and L. P. Thanh (2003). "Impact of preheating on the behavior of *Listeria monocytogenes* in a broth that mimics Camembert cheese composition." J Food Prot **66**(2): 265-271.

90) Ho, A. J., V. R. Lappi and M. Wiedmann (2007). "Longitudinal monitoring of *Listeria monocytogenes* contamination patterns in a farmstead dairy processing facility." J. Dairy Sci. **90**: 2517-2524.

91) IDFA (2008). Expert elicitation.

92) Jacquet, C., C. Saint-Cloment, F. Brouille, B. Catimel and J. Rocourt (1998). "La listériose humaine en France en 1997 – Données du Centre National de référence des *Listeria*." Bulletin Epidémiologique Hebdomadaire **33**: 142-143.

93) Jayarao, B. M., S. C. Donaldson, B. A. Straley, A. A. Sawant, N. V. Hegde and J. L. Brown (2006). "A survey of foodborne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania." J Dairy Sci **89**(7): 2451-2458.

94) Jayarao, B. M. and D. R. Henning (2001). "Prevalence of foodborne pathogens in bulk tank milk." J Dairy Sci **84**(10): 2157-2162.

95) Jensen, A., W. Frederiksen and P. Gerner-Smidt (1994). "Risk factors for listeriosis in Denmark, 1989-1990." Scand J Infect Dis **26**(2): 171-178.

96) Jensen, N. E., F. M. Aarestrup, J. Jensen and H. C. Wegener (1996). "*Listeria monocytogenes* in bovine mastitis. Possible implication for human health." Int J Food Microbiol **32**(1-2): 209-216.

97) Johnsen, B. O., E. Lingaas, D. Torfoss, E. H. Strom and I. Nordoy (2010). "A large outbreak of *Listeria monocytogenes* infection with short incubation period in a tertiary care hospital." J Infect **61**(6): 465-470.

98) Koch, J., R. Dworak, R. Prager, B. Becker, S. Brockmann, A. Wicke, H. Wichmann-Schauer, H. Hof, D. Werber and K. Stark (2010). "Large listeriosis outbreak linked to cheese made from pasteurized milk, Germany, 2006-2007." Foodborne Pathog Dis **7**(12): 1581-1584.

99) Kongo, J. M., F. X. Malcata, A. J. Ho and M. Wiedmann (2006). "Detection and characterization of *Listeria monocytogenes* in Sao Jorge (Portugal) cheese production." J Dairy Sci **89**(11): 4456-4461.

100) Kosa, K. M., S. C. Cates, S. Karns, S. L. Godwin and D. Chambers (2007a). "Consumer home refrigeration practices: results of a web-based survey." J Food Prot **70**(7): 1640-1649.

101) Kosa, K. M., S. C. Cates, S. Karns, S. L. Godwin and D. Chambers (2007b). "Consumer knowledge and use of open dates: results of a WEB-based survey." J Food Prot **70**(5): 1213-1219.

102) Kosikowski, F. V. and V. V. Mistry (1987). Cheese and Fermented Milk Foods. Vol I: Origins and Principles. Westport (CT), Kosikowski, F.V. .

103) Latorre, A. A., J. A. Van Kessel, J. S. Karns, M. J. Zurakowski, A. K. Pradhan, R. N. Zadoks, K. J. Boor and Y. H. Schukken (2009). "Molecular ecology of *Listeria*

monocytogenes: evidence for a reservoir in milking equipment on a dairy farm." Appl Environ Microbiol **75**(5): 1315-1323.

104) Lawrence, R. C., L. K. Creamer and J. Gilles (1987). "Texture development during cheese ripening." J Dairy Sci **70**(8): 1748-1760.

105) Leclercq-Perlat, M. N., D. Picque, H. Riahi and G. Corrieu (2006). "Microbiological and biochemical aspects of Camembert-type cheeses depend on atmospheric composition in the ripening chamber." J Dairy Sci **89**(8): 3260-3273.

106) Lecuit, M., S. Dramsi, C. Gottardi, M. Fedor-Chaiken, B. Gumbiner and P. Cossart (1999). "A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*." EMBO J **18**(14): 3956-3963.

107) Lecuit, M., S. Vandormael-Pournin, J. Lefort, M. Huerre, P. Gounon, C. Dupuy, C. Babinet and P. Cossart (2001). "A transgenic model for listeriosis: role of internalin in crossing the intestinal barrier." Science **292**(5522): 1722-1725.

108) Leuschner, R. G. K. and M. P. Boughtflower (2002). "Laboratory-scale preparation of soft cheese artificially contaminated with low levels of *Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella enterica* serovars Typhimurium, Enteritidis, and Dublin." J Food Prot **65**(3): 508-514.

109) Liewen, M. B. and M. W. Plautz (1988). "Occurrence of *Listeria monocytogenes* in raw milk in Nebraska." J Food Prot **51**(11): 840-841.

110) Linton, M., A. B. Mackle, V. K. Upadhyay, A. L. Kelly and M. F. Patterson (2008). "The fate of *Listeria monocytogenes* during the manufacture of Camembert-type cheese: A comparison between raw milk and milk treated with high hydrostatic pressure." Innovative Food Science & Emerging Technologies **9**(4): 423-428.

111) Liu, S. and V. M. Puri (2004). Measurement of pH and water activity values during ripening of Camembert cheese. Northeast Agricultural & Biological Engineering Conference. University Park, Pennsylvania, United States of America.

112) Liu, S. and V. M. Puri (2008). "Dynamic growth models for *L. monocytogenes* during ripening in Camembert cheese." Lwt-Food Science and Technology **41**(3): 511-520.

113) Liu, S., V. M. Puri and A. Demirci (2004). Fate of *Listeria innocua* during manufacturing and ripening of Camembert cheese. Northeast Agricultural & Biological Engineering Conference. University Park, Pennsylvania, United States of America.

114) Liu, S., V. M. Puri and A. Demirci (2007). "Spatial distribution of population of *Listeria monocytogenes* during manufacturing and ripening of Camembert cheese." J Food Safety **27**(1): 43-55.

115) Liu, S. W., V. M. Puri and A. Demirci (2009). "Evaluation of *Listeria innocua* as a suitable indicator for replacing *Listeria monocytogenes* during ripening of Camembert cheese." International Journal of Food Science and Technology **44**(1): 29-35.

116) Loessner, M., S. Guenther, S. Steffan and S. Scherer (2003). "A pediocin-producing *Lactobacillus plantarum* strain inhibits *Listeria monocytogenes* in a multispecies cheese surface microbial ripening consortium." Appl Environ Microbiol **69**(3): 1854-1857.

117) Lovett, J., D. W. Francis and J. M. Hunt (1987). "*Listeria monocytogenes* in raw milk - detection, incidence, and pathogenicity." J Food Prot **50**(3): 188-192.

118) Lumina Decision Systems. (2010). "Analytica." Retrieved March 20, 2012, from www.lumina.com.

119) Lund, A. M., E. A. Zottola and D. J. Pusch (1991). "Comparison of methods for isolation of *Listeria* from raw milk." J Food Prot **54**(8): 602-606.

120) MacDonald, P. D., R. E. Whitwam, J. D. Boggs, J. N. MacCormack, K. L. Anderson, J. W. Reardon, J. R. Saah, L. M. Graves, S. B. Hunter and J. Sobel (2005). "Outbreak of listeriosis among Mexican immigrants as a result of consumption of illicitly produced Mexican-style cheese." Clin Infect Dis **40**(5): 677-682.

121) Maisnier Patin, S., N. Deschamps, S. R. Tatini and J. Richard (1992). "Inhibition of *Listeria monocytogenes* in Camembert cheese made with a nisin-producing starter." Lait **72**: 249-263.

122) Makino, S. I., K. Kawamoto, K. Takeshi, Y. Okada, M. Yamasaki, S. Yamamoto and S. Igimi (2005). "An outbreak of food-borne listeriosis due to cheese in Japan, during 2001." Int J Food Microbiol **104**(2): 189-196.

123) MAPAQ (2010). Éclosion d'infections à *Listeria monocytogenes* pulsovar 93 liée à la consommation de fromages Québécois, 2008. Province de Québec. Rapport d'investigation et d'intervention., Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec**:** 78.

124) Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin and R. V. Tauxe (1999). "Food-related illness and death in the United States." Emerg Infect Dis **5**(5): 607-625.

125) Mellefont, L. A., T. A. McMeekin and T. Ross (2003). "The effect of abrupt osmotic shifts on the lag phase duration of foodborne bacteria." Int J Food Microbiol **83**(3): 281-293.

126) Mellefont, L. A., T. A. McMeekin and T. Ross (2004). "The effect of abrupt osmotic shifts on the lag phase duration of physiologically distinct populations of *Salmonella* typhimurium." Int J Food Microbiol **92**(2): 111-120.

127) Meyer-Broseta, S., A. Diot, S. Bastian, J. Riviere and O. Cerf (2003). "Estimation of low bacterial concentration: *Listeria monocytogenes* in raw milk." Int J Food Microbiol **80**(1): 1-15.

128) Modzelewska-Kapitula, M. and F. Marin-Iniesta (2005). "The possibility of using *Lactobacillus Fermentum* strains of human origin as protective cultures in soft cheese." Elec J Polish Agri Univ **8**(4): article 14.

129) Mohammed, H. O., K. Stipetic, P. L. McDonough, R. N. Gonzalez, D. V. Nydam and E. R. Atwill (2009). "Identification of potential on-farm sources of *Listeria monocytogenes* in herds of dairy cattle." Am J Vet Res **70**(3): 383-388.

130) Mokhtari, A. and H. C. Frey (2005). "Sensitivity analysis of a two-dimensional probabilistic risk assessment model using analysis of variance." Risk Anal **25**(6): 1511-1529.

131) Moustafa, S. I. and E. H. Marth (1993). "Occurrence of *Escherichia coli*, *Pseudomonas aeruginosa* and *Listeria monocytogenes* in abnormal milk." Dairy Food Environ Sanit **13**(2): 70-73.

132) Muraoka, W., C. Gay, D. Knowles and M. Borucki (2003). "Prevalence of *Listeria monocytogenes* subtypes in bulk milk of the Pacific Northwest." J Food Prot **66**(8): 1413-1419.

133) Murinda, S. E., L. T. Nguyen, H. M. Nam, R. A. Almeida, S. J. Headrick and S. P. Oliver (2004). "Detection of sorbitol-negative and sorbitol-positive Shiga toxin-producing *Escherichia coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Salmonella* spp. in dairy farm environmental samples." Foodborne Pathog Dis **1**(2): 97-104.

134) Murphy, P. M., M. C. Rea and D. Harrington (1996). "Development of a predictive model for growth of *Listeria monocytogenes* in a skim milk medium and validation studies in a range of dairy products." J Appl Bacteriol **80**(5): 557-564.

135) National Center for Health Statistics. (2003-2004). "National Health and Nutrition Examination Survey (NHANES III),." from http://www.cdc.gov/nchs/nhanes.htm

136) National Research Council (1994). Science and Judgment in Risk Assessment. Washington, DC.

137) Nauta, M. (2008). The Modular Process Risk Model (MPRM): a structured approach to food chain exposure assessment. Microbial Risk Analysis of Foods. D. W. Schaffner. Washington, D.C., ASM Press**:** 99-136.

138) Nightingale, K. K., E. D. Fortes, A. J. Ho, Y. H. Schukken, Y. T. Grohn and M. Wiedmann (2005). "Evaluation of farm management practices as risk factors for clinical listeriosis and fecal shedding of *Listeria monocytogenes* in ruminants." JAVMA **227**(11): 1808-1814.

139) Nightingale, K. K., Y. H. Schukken, C. R. Nightingale, E. D. Fortes, A. J. Ho, Z. Her, Y. T. Grohn, P. L. McDonough and M. Wiedmann (2004). "Ecology and transmission of *Listeria monocytogenes* infecting ruminants and in the farm environment." Appl Environ Microbiol **70**(8): 4458-4467.

140) Norton, D. M. and C. R. Braden (2007). Foodborne Listeriosis. *Listeria*, Listeriosis and Food Safety. E. T. Ryser and E. H. Marth. Boca Raton, Florida, CRC Press.

141) O'Donnell, E. T. (1995). "The incidence of *Salmonella* and *Listeria* in raw milk from farm bulk tanks in England and Wales." International Journal of Dairy Technology **48**(1): 25-29.

142) OzFoodNet (2007). "Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet network." Communicable Diseases Intelligence **32**(4): 400.

143) Ozkaynak, H., H. C. Frey, J. Burke and R. W. Pinder (2009). "Analysis of coupled model uncertainties in source-to-dose modeling of human exposures to ambient air pollution: a PM2.5 case study." Atmos Envir **43**(9): 1641-1649.

144) Patterson, R. L., D. J. Pusch and E. A. Zottola (1989). "The isolation and identification of *Listeria* species from raw milk." J Food Prot **52**: 745.

145) Picque, D., M. N. Leclercq-Perlat and G. Corrieu (2006). "Effects of atmospheric composition on respiratory behavior, weight loss, and appearance of Camembert-type cheeses during chamber ripening." J Dairy Sci **89**(8): 3250-3259.

146) Potel, J. (1953). "Atiologie der Granuulomatosis Infantiseptica." Wiss Z Marttin Luther Univ-Ha Wittenberg **3**: 341.

147) Pouillot, R., I. Albert, M. Cornu and J.-B. Denis (2003). "Estimation of uncertainty and variability in bacterial growth using Bayesian inference. Application to *Listeria monocytogenes*." Int J Food Microbiol **81**(2): 87-104.

148) Pouillot, R., M. B. Lubran, S. C. Cates and S. Dennis (2010). "Estimating parametric distributions of storage time and temperature of ready-to-eat foods for U.S. households." J Food Prot **73**(2): 312-321.

149) Pritchard, T. J., C. M. Beliveau, K. J. Flanders and C. W. Donnelly (1994). "Increased incidence of *Listeria* Species in dairy processing plants having adjacent farm facilities." J Food Prot **57**(9): 770-775.

150) Pritchard, T. J., K. J. Flanders and C. W. Donnelly (1995). "Comparison of the incidence of *Listeria* on equipment versus environmental sites within dairy processing plants." Int J Food Microbiol **26**(3): 375-384.

151) Promed. (2008). "Listeriosis, Fatal - Chile: (Santiago), Cheese Suspected, Request for Information. Archive Number 20081128.3754 ", from http://www.promedmail.org/pls/apex/f?p=2400:1202:2080716593049925::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,74952.

152) R Development Core Team. (2008). "R: a language and environment for statistical computing." Retrieved March 20, 2012, from http://www.R-project.org.

153) Rajala-Schultz, P. J., Y. T. Grohn, C. E. McCulloch and C. L. Guard (1999). "Effects of clinical mastitis on milk yield in dairy cows." J Dairy Sci **82**(6): 1213-1220.

154) Ramsaran, H., J. Chen, B. Brunke, A. Hill and M. W. Griffiths (1998). "Survival of bioluminescent *Listeria monocytogenes* and *Escherichia coli* O157:H7 in soft cheese." J. Dairy. Sci. **81**: 1810-1817.

155) Ratkowsky, D. A., J. Olley, T. A. McMeekin and T. A. Ball (1982). "Relationship between temperature and growth rate of bacterial cultures." J Bacteriol **149**: 1-5.

156) Rawool, D. B., S. V. Malik, I. Shakuntala, A. M. Sahare and S. B. Barbuddhe (2007). "Detection of multiple virulence-associated genes in *Listeria monocytogenes* isolated from bovine mastitis cases." Int J Food Microbiol **113**(2): 201-207.

157) Rocourt, J. (1996). "Risk factors for listeriosis." Food Control **7**(4-5): 195-202.

158) Rohrbach, B. W., F. A. Draughon, P. M. Davidson and S. P. Oliver (1992). "Prevalence of *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Salmonella* in Bulk Tank Milk - Risk-Factors and Risk of Human Exposure." J Food Prot **55**(2): 93-97.

159) Ross, T. and T. A. McMeekin (2003). "Modeling microbial growth within food safety risk assessments." Risk Anal **23**(1): 179-197.

160) Ross, T., S. Rasmussen, A. Fazil, G. Paoli and J. Summer (2009). "Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat meats in Australia." Int J Food Microbiol **131**(2-3): 128-137.

161) Rosso, L., J. R. Lobry and J. P. Flandrois (1993). "An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model." J Theor Biol **162**(4): 447-463.

162) RTI International, Tennessee State University and Kansas State University. (2005). "Consumer storage practices for refrigerated ready-to-eat (RTE) foods: study design." from http://foodrisk.org/.

163) Ryser, E. T. (2007). Incidence and Behavior of *Listeria monocytogenes* in cheese and other fermented dairy products. *Listeria*, listeriosis and Food Safety. E. T. Ryser and E. H. Marth. Boca Raton, U.S.A., CRC Press.

164) Ryser, E. T. and E. H. Marth (1987). "Fate of *Listeria monocytogenes* during the manufacture and ripening of Camembert cheese." J Food Prot **50**(5): 372-378.

165) Sanaa, M., A. Audurier, B. Poutrel, J. L. Menard and F. Serieys (1996). "Origin of bovine milk contamination by *Listeria monocytogenes*." Int Dairy Fed **25**: 163-179.

166) Sanaa, M., L. Coroller and O. Cerf (2004). "Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux." Risk Anal **24**(2): 389-399.

167) Sanaa, M. and J. L. Menard (1994). "Raw milk contamination by *Listeria monocytogenes* in dairy farms - origins, risk-factors, prevention." Rec Med Vet **170**(6-7): 437-442.

168) Sanaa, M., B. Poutrel, J. L. Menard and F. Serieys (1993). "Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms." J Dairy Sci **76**(10): 2891-2898.

169) Sauders, B. D., M. Z. Durak, E. Fortes, K. Windham, Y. Schukken, A. J. Lembo, B. Akey, K. K. Nightingale and M. Wiedmann (2006). "Molecular characterization of *Listeria monocytogenes* from natural and urban environments." J Food Prot **69**(1): 93-105.

170) Sauders, B. D., K. Mangione, C. Vincent, J. Schermerhorn, C. M. Farchione, N. B. Dumas, D. Bopp, L. Kornstein, E. D. Fortes, K. Windham and M. Wiedmann (2004). "Distribution of *Listeria monocytogenes* molecular subtypes among human and food isolates from New York State shows persistence of human disease--associated *Listeria monocytogenes* strains in retail environments." J Food Prot **67**(7): 1417-1428.

171) Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones and P. M. Griffin (2011). "Foodborne illness acquired in the United States—major pathogens." Emerg Infect Dis **17**(1): 7-12.

172) Schlesser, J. E., S. J. Schmidt and R. Speckman (1992). "Characterization of chemical and physical changes in Camembert cheese during ripening." J Dairy Sci **75**(7): 1753-1760.

173) Schoder, D., P. Winter, A. Kareem, W. Baumgartner and M. Wagner (2003). "A case of sporadic ovine mastitis caused by *Listeria monocytogenes* and its effect on contamination of raw milk and raw-milk cheeses produced in the on-farm dairy." J Dairy Res **70**(4): 395-401.

174) Schulz, G. (1967). "Untersuchungen über das Vorkommen von Listerien in Rohmilch." Mh Vet Med **22**: 766-768.

175) Schvartzman, M. S., A. Maffre, F. Tenenhaus-Aziza, M. Sanaa, F. Butler and K. Jordan (2011). "Modelling the fate of *Listeria monocytogenes* during manufacture and ripening of smeared cheese made with pasteurised or raw milk." Int J Food Microbiol **145 Suppl 1**: S31-38.

176) Servello, V., A. R. Hill and R. W. Lencki (2004). "Towards an optimum mixing protocol for on-farm bulk milk sampling." J Dairy Sci **87**(9): 2846-2853.

177) Sharp, M. W. (1989). "Bovine mastitis and *Listeria monocytogenes*." Vet Rec **125**(20): 512-513.

178) Skandamis, P. N., J. D. Stopforth, Y. Yoon, P. A. Kendall and J. N. Sofos (2009). "Heat and acid tolerance responses of *Listeria monocytogenes* as affected by sequential exposure to hurdles during growth." J Food Prot **72**(7): 1412-1418.

179) Slade, P. J. and D. L. Collins-Thompson (1988a). "Comparison of 2-stage and direct selective enrichment techniques for isolating *Listeria* spp from raw milk." J Food Sci **53**(6): 1694-&.

180) Slade, P. J. and D. L. Collins-Thompson (1988b). "Enumeration of *Listeria monocytogenes* in raw milk." Lett Appl Microbiol **6**(5): 121-123.

181) Slade, P. J., D. L. Collins-Thompson and F. Fletcher (1988). "Incidence of *Listeria* species in Ontario raw milk." Canadian Institute of Food Science and Technology Journal-Journal De L Institut Canadien De Science Et Technologie Alimentaires **21**(4): 425-429.

182) Slade, P. J., E. C. Fistrovici and D. L. Collins-Thompson (1989). "Persistence at source of *Listeria* spp in raw milk." Int J Food Microbiol **9**(3): 197-203.

183) Statistics Canada. (2004). "Canadian Community Health Survey 2.2, 24-hour dietary recall component." from http://www.statcan.gc.ca/cgi-bin/imdb/p2SV.pl?Function=getSurvey&SDDS=5049&lang=en&db=imdb&adm=8&dis=2.

184) Steele, M. L., W. B. McNab, C. Poppe, M. W. Griffiths, S. Chen, S. A. Degrandis, L. C. Fruhner, C. A. Larkin, J. A. Lynch and J. A. Odumeru (1997). "Survey of Ontario bulk tank raw milk for food-borne pathogens." J Food Prot **60**(11): 1341-1346.

185) Stephan, R., D. Senczek, C. Muller and C. Feusi (2000). "[Isolation of *Listeria* spp. and *Aspergillus fumigatus*--two case reports from mastitis diagnosis]." Schweiz Arch Tierheilkd **142**(7): 387-390.

186) Sulzer, G. and M. Busse (1991). "Growth inhibition of *Listeria s*pp on Camembert cheese by bacteria producing inhibitory substances." Int J Food Microbiol **14**(3-4): 287-296.

187) Sulzer, G. and M. Busse (1993). "Behavior of *Listeria s*pp during the production of Camembert cheese under various conditions of inoculation and ripening." Milchwissenschaft-Milk Science International **48**(4): 196-200.

188) Swaminathan, B. and P. Gerner-Smidt (2007). "The epidemiology of human listeriosis." Microbes Infect **9**(10): 1236-1243.

189) Tan, A., S. Beaton, K. Dimovski, G. Hogg, V. di Paola and H. Haines (2008). "Pathogen survival in portioned retail soft cheeses." Australian Journal of Dairy Technology **63**(2): 39-44.

190) Thompson, K. M. (2002). "Variability and uncertainty meet risk management and risk communication." Risk Anal **22**(3): 647-654.

191) Tiwari, N. P. and S. G. Aldenrath (1990). "Occurrence of *Listeria* species in food and environmental samples in Alberta." Canadian Institute of Food Science and Technology Journal-Journal De L Institut Canadien De Science Et Technologie Alimentaires **23**(2-3): 109-113.

192) US EPA (1997). Guiding Principles for Monte Carlo Analysis. Washington, DC, U.S. Environmental Protection Agency,**:** 39.

193) USDA. (2011). "USDA Summary of 2010 Herd Averages DHI Report K-3." from http://aipl.arsusda.gov/publish/dhi/dhi11/haall.html.

194) van Daelen, A. M. and F. H. Jaartsveld (1988). "[*Listeria* mastitis in cattle]." Tijdschr Diergeneeskd **113**(7): 380-383.

195) van Gerwen, S. J. C. and M. H. Zwietering (1998). "Growth and inactivation models to be used in quantitative risk assessments." J Food Prot **61**(11): 1541-1549.

196) Van Kessel, J. S., J. S. Karns, L. Gorski, B. J. McCluskey and M. L. Perdue (2004). "Prevalence of *Salmonellae*, *Listeria monocytogenes*, and fecal coliforms in bulk tank milk on US dairies." J Dairy Sci **87**(9): 2822-2830.

197) Vilar, M. J., E. Yus, M. L. Sanjuan, F. J. Dieguez and J. L. Rodriguez-Otero (2007). "Prevalence of and risk factors for *listeria* species on dairy farms." J Dairy Sci **90**(11): 5083-5088.

198) Vishinsky, Y., A. Grinberg and R. Ozery (1993). "*Listeria monocytogenes* udder infection and carcase contamination." Vet Rec **133**(19): 484.

199) Von Hartwigk, H. (1958). "Zum nachweis von Listerien in der Kuhmilch." Berl. Munch. Tierarztl. Wochenschr. **71**: 82-85.

200) Waak, E., W. Tham and M. L. Danielsson-Tham (2002). "Prevalence and fingerprinting of *Listeria monocytogenes* strains isolated from raw whole milk in farm bulk tanks and in dairy plant receiving tanks." Appl Environ Microbiol **68**(7): 3366-3370.

201) Wagner, M., L. Podstatzky-Lichtenstein, A. Lehner, H. Asperger, W. Baumgartner and E. Brandl (2000). "Prolonged excretion of *Listeria monocytogenes* in a subclinical case of mastitis." Milchwissenschaft-Milk Science International **55**(1): 3-6.

202) Wan, J., K. Harmark, B. E. Davidson, A. J. Hillier, J. B. Gordon, A. Wilcock and M. J. Coventry (1997). "Inhibition of *Listeria monocytogenes* by piscicolin 126 in milk and Camembert cheese manufactured with a thermophilic starter." J Appl Microbiol **82**(3): 273-280.

203) Wang, L. L. and E. A. Johnson (1997). "Control of *Listeria monocytogenes* by monoglycerides in foods." J Food Prot **60**(2): 131-138.

204) Wesley, I. V. (2007). Listeriosis in animals. Listeria, listeriosis and food safety. E. T. Ryser and E. H. Marth. Boca Raton, Florida, U.S.A., CRC Press, **:** 55-84.

205) Whitley, E., D. Muir and W. M. Waites (2000). "The growth of *Listeria monocytogenes* in cheese packed under a modified atmosphere." J Appl Microbiol **88**(1): 52-57.

206) Williams, D., E. A. Irvin, R. A. Chmielewski, J. F. Frank and M. A. Smith (2007). "Dose-response of *Listeria monocytogenes* after oral exposure in pregnant guinea pigs." J Food Prot **70**(5): 1122-1128.

207) Wilson, D. J., R. N. Gonzalez, J. Hertl, H. F. Schulte, G. J. Bennett, Y. H. Schukken and Y. T. Grohn (2004). "Effect of clinical mastitis on the lactation curve: a mixed model estimation using daily milk weights." J Dairy Sci **87**(7): 2073-2084.

208) Winter, P., F. Schilcher, Z. Bago, D. Schoder, M. Egerbacher, W. Baumgartner and M. Wagner (2004). "Clinical and histopathological aspects of naturally occurring mastitis caused by *Listeria monocytogenes* in cattle and ewes." J Vet Med B Infect Dis Vet Public Health **51**(4): 176-179.

209) Yoshida, T., Y. Kato, M. Sato and K. Hirai (1998). "Sources and routes of contamination of raw milk with *Listeria monocytogenes* and its control." J Vet Med Sci **60**(10): 1165-1168.

210) Zwietering, M. H., J. C. de Wit and S. Notermans (1996). "Application of predictive microbiology to estimate the number of *Bacillus cereus* in pasteurised milk at the point of consumption." Int J Food Microbiol **30**(1-2): 55-70.