Joint FDA / Health Canada Quantitative Assessment of the Risk of Listeriosis from Soft-Ripened Cheese Consumption in the United States and Canada: Replies to Public Comments

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

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Contents

Ninety-six comments were received in docket FDA-2012-N-1182, in regard to Federal Register Notice 78 FR 9701. The risk assessment team ("we") considered the comments that pertained directly to the risk assessment.

The first section of this document provides some replies to general comments. In the second section, each reply answers a group of comments that raised the same issue. The third section provides answers to some individual comments that were unlike any others – i.e., that made unique points and could not be answered collectively.

Replies to general comments

Comments that address issues outside the scope of the risk assessment

The scope of the risk assessment, which was developed by US FDA and Health Canada risk managers, was described in detail in the appendices of the draft report. The risk assessment was focused specifically on the risk of invasive listeriosis linked to consumption of soft-ripened cheese manufactured in the United States and in Canada. In this document, **we do not reply to comments raising issues outside of this scope, such as comments regarding hazards other than *L. monocytogenes*, other categories of cheeses (e.g., hard cheeses, semi-hard cheeses), illegal or unlicensed production of cheese, and cheese manufactured in other countries or territories.**

Clarifications to text and tables

Some comments pointed out specific language, in the main report and appendices, which would benefit from clarification; **we reviewed the text and revised it where necessary**. Other comments pointed out typographical errors, which we corrected. We accommodated some commenters' requests for additional, intermediate results regarding aspects of the risk models. (See the appendices of the draft report.)

Comments on judgments and risk management decisions

The report of the risk assessment does not make value judgments on the estimated risks, consistent with *Codex alimentarius* (1999), the Health Canada Decision-Making Framework (2000), or risk-assessment frameworks developed by the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition (2002). Rather, the risk characterization

component of the risk assessment describes how the risk varies among conditions and circumstances, and, in doing so, invites comparisons among the risks under those different conditions and circumstances.

Nor does the report of the risk assessment make risk management recommendations, and in this response document we do not address comments that referred to hypothetical risk management decisions that would be informed by the risk assessment. The risk assessment follows *Codex alimentarius* and U.S. and Canadian recommendations (*Codex alimentarius* Commission 1999; Health Canada Decision Making Framework 2000; CFSAN Risk Analysis Working Group 2002), pursuant to which evaluations of the availability, feasibility, and cost of mitigations is done not as part of the risk assessment, but externally to the risk assessment, as part of the risk management function that the risk assessment is intended to inform.

Comments on risk-management options

When a comment suggested an evaluation of the risk of invasive listeriosis from consumption of soft-ripened cheese following a risk-mitigation scenario not considered in the draft report, and when we determined that the proposed option was scientifically sound and quantifiable through this risk-assessment model, we analyzed the additional scenario and included it in the final report (see additional scenarios in report for 4, 5, and 6 $\log_{10}$ reductions of *L. monocytogenes* in raw milk and use of surface treatment achieving a 2 $\log_{10}$ reduction). The additional results generated by these additional "what-if" scenarios are also discussed below.

In some cases we evaluated commenters' suggested risk-mitigation scenarios and determined that they were not scientifically sound. We did not include such scenarios in the report. We discuss such comments below and explain our analysis.

The specific strategies that can be examined in a report such as the report of the risk assessment are limited. While the literature on animal husbandry and microbiology describes various strategies for mitigating pathogen contamination of bulk milk to be used as raw material for cheese-making, these studies do not provide information to support any quantitative estimates of the potential reductions in *L. monocytogenes* prevalence, *L. monocytogenes* contamination levels, etc., associated with a particular mitigation. The strength of the process-model structure this risk assessment uses is the capacity to examine how the risk varies, to inform risk managers about uncertainty about the risk results, and to examine the impact of risk-mitigation strategies, whether or not strategies are already considered part of the process. In this way, we avoid endorsing, championing, or appearing to validate any particular system (we do not), while providing risk managers and others with the key points in the farm-to-fork pathway – prevalence, contamination levels, growth rates, consumption amounts – that would be needed to evaluate any food-safety system and strategies involving combinations of preventative controls at those key points.

However, there are mitigation strategies whose effects we cannot incorporate at this time, due to gaps in the current knowledge base. For example, we do not address the potential impact of testing for environmental contamination, because little is known quantitatively about the interrelationship of environmental contamination, its transfer to milk or cheese, and what drives the cross-contamination process. The current model reflects the logical assumption that any decrease in *L. monocytogenes* environmental contamination would decrease the risk of *L. monocytogenes* contamination of pasteurized-milk cheese as well as of non-pasteurized-milk cheese.

Comments based on references to a single scientific study

The framework of the risk assessment included gathering all the available literature on the subject and selecting all that fell specifically within the scope of the risk assessment (as provided in the appendices of the report). The available datasets deemed appropriate were compiled and used collectively, through statistical and probabilistic methods, to derive a distribution of the variability of the parameter and to estimate the surrounding uncertainty of this estimated distribution. **Whenever possible, we did not base our calculations on just one dataset or on single pieces of data, but rather on the collective datasets and data deemed appropriate.**

Some comments challenged the literature-derived data used in this risk assessment by referring to the results of a single, specific study. **We do not derive additional estimates based on one specific study suggested by a comment, when the single study forms only part of the available knowledge.** Rather, we discuss how the individual studies fall into what one can infer from all available studies about the phenomenon of interest.

Updates to the literature

We also took the opportunity to evaluate updates to the relevant body of knowledge that public comments and our own reviews pointed out, and incorporated them, when appropriate.

Collective replies to specific comments grouped according to point

When multiple comments raised the same issue, we combined them and replied to them collectively. For each such collection of similar comments, we begin by quoting a few representative ones, as examples.

Epidemiologic record

Example comments: “There have been few, if any, outbreaks involving legally made soft cheese in the United States and Canada.” “Perhaps when there are some outbreaks that have actually happened there will be a real cause for concern.” “The literature describes no confirmed outbreaks involving *L. monocytogenes* and unpasteurized milk for over 40 years.”

Answer: Since the release of the draft risk assessment, a listeriosis outbreak linked to an artisanal pasteurized soft-ripened cheese occurred in the United States. It led to six hospitalizations, one death, and one miscarriage (CDC 2013a).

In addition, the majority of listeriosis cases are sporadic cases, not linked to outbreaks [86% of the listeriosis cases reported to CDC (2013b) are not outbreak-associated cases], and there is very little information about the origin of these sporadic cases (Varma *et al.* 2007). **For multiple reasons (small batches, extreme heterogeneity of individual susceptibility), we expect to see primarily sporadic cases of listeriosis linked to small-scale cheese producers. The absence of large outbreak linked to a product from a small-scale cheese producer does not necessarily lead to the conclusion that the risk per serving is low.**

The microbiological literature cites examples of raw materials, handling during manufacture, post-process, repackaging, and consumer storage that have led to sporadic cases of illness from cheeses of various types. These examples are documented in the hazard identification component of the risk assessment.

Use of a single study of *L. monocytogenes*-contaminated cheeses at retail

Example comments: "Unfortunately, the only data employed in the present risk assessment to determine the impact of environmental contamination comes from a single study (Gombas, 2003)." "In an effort to be thorough, several studies should be used to inform this risk assessment. Failure to include multiple studies is a major limitation." "This [(Gombas *et al.* 2003)] data is old (prior to 2003)."

Answer: We agree that little data are available to determine the impact of environmental contamination. **An active literature search did not provide additional data beyond that of the Gombas *et al.* (2003) study at the time the draft risk assessment was developed, nor is additional published data available now.** Little is known quantitatively about the interrelationship of environmental contamination and its transfer to cheeses. We acknowledge this limitation in the "caveat and limitations" section of the risk assessment report.

The published Gombas *et al.* (2003) article and the Gombas *et al.* (2003) dataset available on the FoodRisk.org website enabled us to straightforwardly distinguish the soft-ripened cheese results of interest for this risk assessment from the many other ready-to-eat foods that Gombas *et al.* (2003) reported on, and to use those soft-ripened cheese data to infer the distribution of the levels of post-processing, environment-source *L. monocytogenes* contamination on the surface of cheeses. We have added an explanation to this effect in the report.

We did not otherwise revise the report in response to this comment because we were not able to identify additional data that should be added to our analysis. We acknowledge the suggestion that the Gombas *et al.* (2003) study is old and that improvement in cheese-manufacturing practices might have occurred since then. We did not modify the report on this basis because we could identify no data or information supporting this conclusion. As such, we consider it to be

speculative. However, solely for purposes of discussion in this document, we estimate the risk using, as a working hypothesis, a lower probability of environmental contamination, specifically the prevalence distribution defined by the lowest 20% of the baseline prevalence (Table 1, this document, Figure 1, this document). This specification of the prevalence mimics an hypothetical situation in which the probabilities of environmental contamination of cheeses during manufacture would be equal to the probabilities of the fifth best manufacturing practices observed in 2000-2001, as inferred from the Gombas *et al.* (2003) study. In this alternative, the average probability of environmentally contaminated cheeses would be 0.22% (vs. 0.94% in the baseline reported in the draft report) and, in the pasteurized-milk cheese baseline, the mean prevalence of contaminated cheeses would then be 0.22% (vs. 0.94% in the baseline reported in the draft report). The mean prevalence of contaminated servings would be 0.15% in Canada and 0.16% in the United States for the elderly population (as an example) (vs. 0.64% in Canada and 0.66% in the United States, for the baseline in the draft report).

The corresponding results, in terms of risk of invasive listeriosis per serving, are provided in Table 1 (this document) for the elderly populations in the United States and in Canada. The median risk of invasive listeriosis following the consumption of a serving of pasteurized-milk soft-ripened cheese would be about a third that of the draft report's baseline for pasteurized milk cheese, and the mean risk would be about a quarter that of the baseline.

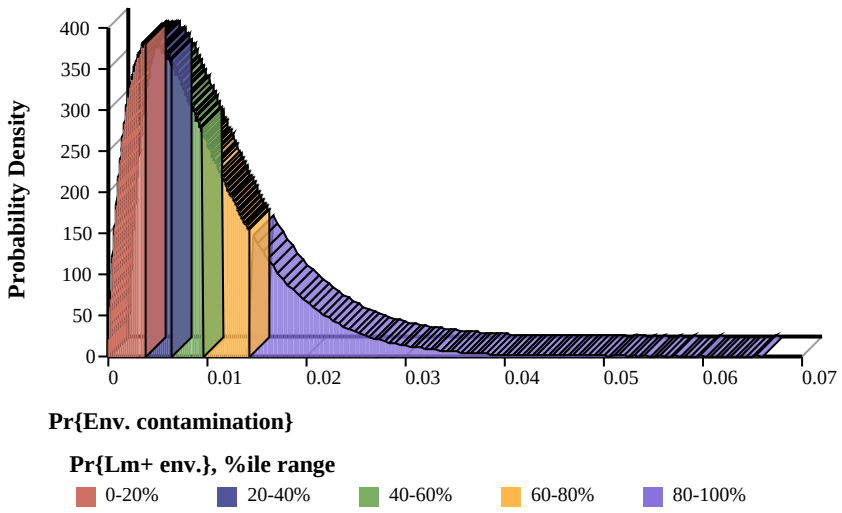

Figure 1: Baseline distribution of prevalence of environmental contamination, as inferred from Gombas *et al.* (2003) data, divided into fifths.

Table 1: Risk of invasive listeriosis per pasteurized-milk cheese serving. Environment contamination frequencies as in the baseline (draft report) vs. environment contamination frequencies as in the lowest fifth of Figure 1's baseline distribution.

Pasteurized-milk cheese	Summary statistics	Canada	United States
Baseline (Recall*)	**Median**	1.16×10^{-13}	1.27×10^{-13}
Baseline (Recall*)	**Mean**	7.23×10^{-9}	8.04×10^{-9}
Lower environmental contamination -0-20% percentiles of the baseline-	**Median (*dMedian PMC***)**	4.19×10^{-14} (0.36)	4.32×10^{-14} (0.34)
Lower environmental contamination -0-20% percentiles of the baseline-	**Mean (*dMean PMC*)**	1.83×10^{-9} (0.25)	2.03×10^{-9} (0.25)

* Results might be slightly different than in the draft report, because they were obtained from an updated version of the Analytica™ model.
** Recall: *dMean* (respectively (resp.). *dMedian*) is the change in the mean (resp. median) risk output with reference to a change in a particular model: PMC: pasteurized-milk cheese, RMC: raw-milk cheese. Example: *dMean PMC* = 0.36 means that the mean in this alternative is 0.36 times higher than the mean for the pasteurized-milk cheese alternative.

The current risk assessment does not assess the potential impact of testing the environment to prevent contamination, because little is known about the interrelationship of environmental contamination, its transfer to cheeses, and the drivers of that cross-contamination process. Promising studies have recently been published on the subject (Tenenhaus-Aziza *et al.* 2013).

Prevalence in bulk-tank milk

Example comments: "My primary concern relates to the use of farm bulk tank *L. monocytogenes* prevalence and concentration data to model risk scenarios. I would argue that bulk tank milk prevalence/concentration models are irrelevant because stringent microbiological criteria are required to produce a raw milk Camembert which will be of saleable quality following 60 days of aging." "…the incidence of *L. monocytogenes* [for raw-milk cheese is] lower than that typically seen in commodity fluid milk bulk tank surveys." "The data set used to determine contamination rates and levels is obtained from surveys of bulk tanks of milk from producers harvesting commodity fluid milk for pasteurization, and not necessarily that intended for the manufacture of cheese or from the bulk milk of cheese producers, large or small."

Answer: Two scientific articles (D'Amico *et al.* 2008b; D'Amico and Donnelly 2010) reported *L. monocytogenes* prevalence and concentration in bulk-tank milk at small-scale cheese producers in the United States; data from those articles were included in the meta-analysis of bulk-tank milk surveys used in this risk assessment. A third article (Latorre *et al.* 2011) reported prevalence of *L. monocytogenes* bulk milk produced by farms licensed to sell raw milk.[1]

D'Amico *et al.* (2008b) found *L. monocytogenes* concentrations of <1 cfu ml^{-1} in each of 3 milk samples positive for *L. monocytogenes*, with limits of detection in the order of 10 *L. monocytogenes* per ml. This result would not be a very unusual result if their *L. monocytogenes* concentrations were drawn from the baseline distribution this risk assessment

[1] "In New York State, raw milk can be purchased at licensed farms, where consumers either bring their own containers and have them filled directly from the bulk tank in their presence, or purchase bottled raw milk from on-farm stores" (Latorre *et al*., 2011)

used: it is a result that one would find approximately 29% [14%, 42%] (95% conf. int.) of the time when milk samples detected positive for *L. monocytogenes* came from the same *L. monocytogenes*-concentration distribution that the risk assessment inferred from the microbiological literature for *L. monocytogenes*-contaminated farm bulk milk. Indeed, one might have evidence that the *L. monocytogenes* concentrations in a sampling population have different (higher) concentrations, in distribution, than the one we derived from the microbiological literature for this risk assessment, but only if one observed 3 of 3 milk samples with >1 cfu ml^{-1} [which would occur 3.9% (1.6%, 11%) of the time, if the sampling population for milk's concentrations were the same as the one this risk assessment derived.]. **In summary, the concentration reported by D'Amico *et al.* (2008b) from bulk-tank milk at small-scale cheese producers is not incompatible with the baseline distribution of concentration used in this study.**

D'Amico and Donnelly (2010) and D'Amico *et al.* (2008b) reported how often their studies detected *L. monocytogenes* in bulk-tank milk at small-scale cheese producers, [3/62 samples (95%CI: 1.0-13.5%] and [0/101 samples (95%CI: 0-3.6%)]. We carefully reviewed the scientific article by Latorre *et al.* (2011), but were not able to find any significant difference between *Listeria* prevalence in dairy farms vs. licensed farms, particularly since the data they reported do not include any sample size (number of samples) or any relevant information on the sampling plan for the collection of the samples of milk. Those three-point prevalences ((D'Amico *et al.* 2008b; D'Amico and Donnelly 2010; Latorre *et al.* 2011)) are all below the median of the farm bulk-tank prevalence distribution used in this risk assessment, as fully one-half of the farm bulk-tank prevalence distribution would fall below the median; their large confidence intervals cover substantial portions of the whole distribution (Figure 2, this document). **As a conclusion, we identified no statistical evidence that the prevalence of *Listeria monocytogenes*-contaminated milk used by small-scale cheese producers is lower than the estimated prevalence used in this risk assessment.** Therefore, we did not modify the report in response to these comments.

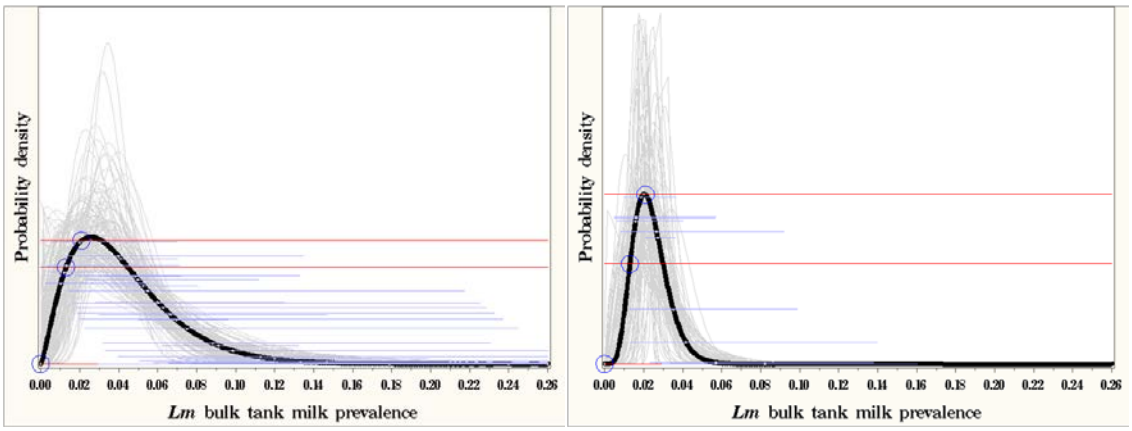

Figure 2: United States (left), Canada (right) farm bulk-tank milk prevalence distribution as inferred in the report (beta mixture of binomial samples); individual studies with 95% Confidence Interval (small white dots along density function). Specific observations from (D'Amico and Donnelly 2010) (1 point) and (Latorre *et al.* 2011) (2 points) are reported.

We acknowledge that some comments suggested that the prevalence of *Listeria monocytogenes* in milk used specifically for raw-milk cheese making may be lower than in milk used for pasteurization or for cheese making generally. We could identify no data or information supporting this conclusion. As such, we consider it to be speculative. However, solely for purposes of discussion in this document, we modeled the results if it could be shown that there was a lower prevalence of contaminated farm bulk milk used for raw-milk cheese making than we estimated. Risk estimates were calculated (Table 2, this document) using a lower prevalence of contaminated farm bulk milk than in the baseline; specifically, the prevalence distribution defined by the lowest 20% of the baseline prevalence distribution (Figure 3, this document). That mimics a situation in which, in Canada and the United States, milk for raw-milk cheeses would originate from farm bulk milk drawn from farm bulk milk with *L. monocytogenes* contamination prevalence within the lowest 20% of contamination prevalence, as inferred by our meta-analysis. In this analysis, the mean (resp. median) prevalence of contaminated bulk-tank milk would be 1.3% (resp. 1.3%) in Canada vs. 2.4% (resp. 2.3%) in the baseline and 1.3% (resp. 1.4%) in the United States, vs. 4.2% (resp. 3.7%) in the baseline. Note that, at a level of prevalence of 1.3% and under the assumption of 100% test sensitivity (probability to detect the contamination if the milk is contaminated), the probability to observe 0 positive samples out of 101 samples [as in D'Amico and Donnelly (2010)] is 27%, and the probability to observe 3 or less positive samples out of 62 [as in D'Amico *et al.* (2008b)] is 99%.

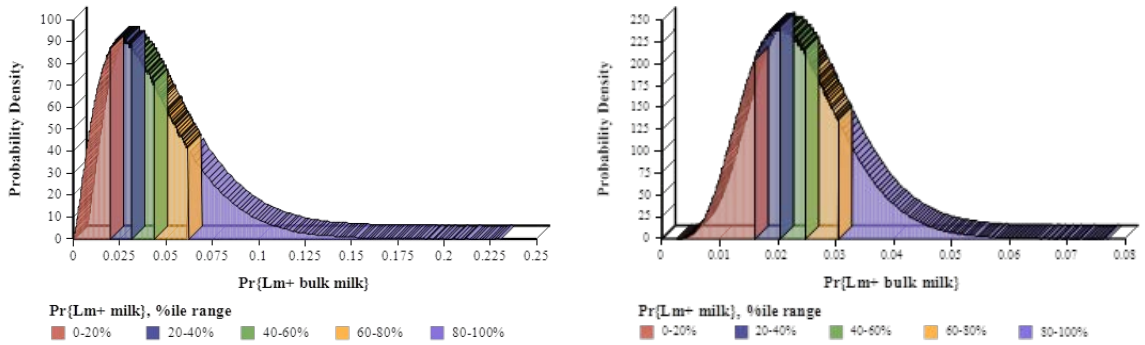

Figure 3: United States (left), Canada (right) farm bulk-tank milk prevalence distributions divided into fifths.

For contaminated farm bulk-milk prevalence limited to the first fifth of the baseline prevalence distribution, the mean risk per raw-milk soft-ripened Camembert cheese serving is less by a factor of 2 in Canada and 3 in the United States, compared with the baseline raw-milk cheese case for the Elderly population.[2] The mean risk of listeriosis per serving of raw-milk soft-ripened Camembert cheese would be 32 times higher in Canada and 32 times higher in the United States, compared with the mean risk of listeriosis per serving of a pasteurized-milk soft-ripened cheese.

Table 2: Risk of invasive listeriosis per raw-milk cheese serving, Elderly population. Bulk-tank milk prevalence as in the baseline for raw-milk cheese (report) vs. bulk-tank milk prevalence as in the lowest fifth of the baseline distribution.

Raw-milk cheese	Summary statistics	Canada	United States
Baseline (Recall*)	Median	4.36×10^{-11}	9.94×10^{-11}
Baseline (Recall*)	Mean	4.37×10^{-07}	8.42×10^{-7}
Lower bulk milk tank prevalence -0-20% percentiles of the baseline-	Median (*dMedian RMC***) (*dMedian PMC*)	1.77×10^{-11} (0.41) (153)	1.93×10^{-11} (0.19) (153)
Lower bulk milk tank prevalence -0-20% percentiles of the baseline-	Mean (*dMean RMC*) (*dMean PMC*)	2.31×10^{-7} (0.53) (32)	2.54×10^{-7} (0.30) (32)

* Results might be slightly different than in the draft report, because they were obtained from an updated version of the Analytica™ model.
** Recall: *dMean* (resp. *dMedian*) is the change in the mean (resp. median) risk output with reference to a change in a particular model: PMC: pasteurized-milk cheese, RMC: raw-milk cheese. Example: *dMean PMC* = 32 means that the mean in this alternative is 32 times higher than the mean for the pasteurized-milk cheese alternative.

Risk-management options other than pasteurization

Example comments: "The analysis does not consider a wide range of preventative controls and strategies incorporating combinations of preventative controls." "A wide range of food safety approaches exist for the production of raw milk products."

[2] Results for the Elderly subpopulation are presented as examples, but comparable results would be obtained for other subpopulations of interest (Pregnant, Immuno-compromised, General).

Answer: In the United States, the requirements for milk pasteurization are set out in the Code of Federal Regulations (21 CFR 1240.61).

The animal husbandry and microbiological literature identify various strategies for mitigating pathogen contamination of bulk milk to be used as raw material for cheese-making, but the studies do not provide information to support any quantitative estimates of the potential reductions in *L. monocytogenes* prevalence/concentration associated with a particular mitigation. Thus, the risk assessment compares risk results under changes to its baseline *L. monocytogenes* prevalence and concentration distributions without attributing those changes to specific mitigations. We considered that the risk assessment could analyze hypothetical, unspecified mitigations that could be applied to bulk milk as raw material for cheese making that achieve different levels of reduction of *L. monocytogenes* (in addition to the 3 $\log_{10}$ reduction mitigation we analyzed in the draft report).

We expanded the risk assessment report to include raw-milk cheese scenarios that apply a 4 $\log_{10}$ reduction, a 5 $\log_{10}$ reduction, and a 6 $\log_{10}$ reduction to the level of *L. monocytogenes* contamination in contaminated bulk milk destined for raw-milk cheese manufacture, as if by applying (unspecified) processes to all bulk milk destined for raw-milk cheese manufacture. Results (Table 3 of this document) suggest that the mean predicted level of risk per serving for the elderly populations in Canada and in the United States would then be slightly higher than for pasteurized-milk cheese if a 5 $\log_{10}$ reduction of the level of *L. monocytogenes* in the bulk-tank milk destined for raw-milk cheese was obtained. A 6 $\log_{10}$ reduction in concentration in the bulk-tank milk destined for raw-milk cheese would lead to a mean predicted risk lower than the one predicted for the pasteurized-milk cheeses.

How to achieve such levels of $\log_{10}$ reduction as systematically as a pasteurization process does and how to control this reduction are questions outside the scope of the risk assessment.

Table 3: Risk of invasive listeriosis per raw-milk cheese serving. Bulk-tank milk concentration as in the baseline for raw-milk cheese (draft report) *vs*. bulk-tank milk concentration following a 3, 4 5, or 6 $\log_{10}$ safety-performance criterion. Elderly population.

Raw-milk cheese	Summary statistics	Canada	United States
Baseline (Recall*)	Median	4.36×10^{-11}	9.94×10^{-11}
Baseline (Recall*)	Mean	4.37×10^{-07}	8.42×10^{-7}
3 $\log_{10}$ reduction (Recall*)	Median (*dMedian RMC***) (*dMedian PMC*)	1.57×10^{-12} (0.036) (14)	2.52×10^{-12} (0.025) (20)
3 $\log_{10}$ reduction (Recall*)	Mean (*dMean RMC*) (*dMean PMC*)	5.34×10^{-8} (0.14) (7.4)	8.05×10^{-8} (0.10) (11)
4 $\log_{10}$ reduction	Median (*dMedian RMC*) (*dMedian PMC*)	1.30×10^{-13} (0.003) (1.1)	1.87×10^{-13} (0.002) (1.5)
4 $\log_{10}$ reduction	Mean (*dMean RMC*) (*dMean PMC*)	1.23×10^{-8} (0.028) (1.7)	1.65×10^{-8} (0.020) (2.0)
5 $\log_{10}$ reduction	Median (*dMedian RMC*) (*dMedian PMC*)	7.52×10^{-14} (0.002) (0.65)	1.00×10^{-13} (0.001) (0.79)
5 $\log_{10}$ reduction	Mean (*dMean RMC*) (*dMean PMC*)	7.68×10^{-9} (0.018) (1.1)	9.64×10^{-9} (0.011) (1.2)
6 $\log_{10}$ reduction	Median (*dMedian RMC*) (*dMedian PMC*)	6.37×10^{-14} (0.001) (0.55)	7.53×10^{-14} (0.001) (0.60)
6 $\log_{10}$ reduction	Mean (*dMean RMC*) (*dMean PMC*)	6.09×10^{-9} (0.014) (0.84)	6.41×10^{-9} (0.008) (0.80)

* Results might be slightly different than in the draft report, because they were obtained from an updated version of the Analytica™ model.
** Recall: *dMean* (resp. *dMedian*) is the change in the mean (resp. median) risk output with reference to a change in a particular model: PMC: pasteurized-milk cheese, RMC: raw-milk cheese. Example: *dMean RMC* = 7.4 means that the mean in this alternative is 7.4 times higher than the mean for the pasteurized-milk cheese alternative.

L. monocytogenes growth in raw-milk cheeses and pasteurized-milk cheeses

Example comments: "It is clear from the literature that (a) the resulting microbial growth profile of soft-ripened cheese is distinct from that in similar cheeses from pasteurized milk; and (b) that the indigenous bacteria characterizing raw camembert production have antimicrobial characteristics." "Now a pasteurized cheese is a "dead" cheese and has absolutely no bacteria that can fight intruders." "Raw milk cheese is self-protected against major pathogens and is less exposed to recontamination by major pathogens."

Answer: There is no such thing as a "dead" soft-ripened cheese; all soft-ripened cheese productions use a starter culture (e.g. *Lactococci*, *Lactobacilli*, *Streptococci*) that is added to milk (Kosikowski and Mistry 1987).

The microbiological literature attributes the growth profile of *L. monocytogenes* in soft-ripened cheese to the cheese environment, as a function of that environment's pH, a_w, and temperature properties. The applicable literature does not demonstrate *L. monocytogenes* growth rate differences at the same pH, a_w and temperature among raw-milk and pasteurized-milk

Camembert cheeses during aging [(Genigeorgis *et al.* 1991; D'Amico *et al.* 2008a); appendix of the risk assessment].

The collected body of work that describes *L. monocytogenes* growth in soft-ripened Camembert cheese allows inferences about how growth rates during cheese ripening and aging vary – among strains of *L. monocytogenes*, among cheeses, within cheese among cheese parts, and in response to environmental conditions –with uncertainty that the risk assessment captures. **The risk assessment addresses growth in cheeses made with pasteurized milk and in cheeses made with non-pasteurized milk in a manner consistent with the available data**; i.e.:

- During cheese ripening, different cheese-making processes for pasteurized-milk cheese and raw-milk cheese lead to different conditions of pH in pasteurized-milk cheeses and raw-milk cheeses, and then to different growth.
    - the pasteurized-milk cheese baseline model considers the manufacture of soft-ripened cheese using the "stabilized cheese process" (Kosikowski and Mistry 1987; Lawrence et al. 1987); the raw-milk cheese baseline model considers the manufacture of soft-ripened cheese using the "traditional process" (Kosikowski and Mistry 1987; Sanaa et al. 2004). This reflects our understanding of the processes typically used by cheese makers for the relevant types of soft-ripened cheeses (raw or pasteurized). During ripening, the stabilized cheese process is characterized by a higher pH than the traditional process (Kosikowski and Mistry 1987; Lawrence et al. 1987). Higher pH environments are more favorable to *L. monocytogenes* growth than lower pH environments (Ryser 2007);
- During cheese aging, available data do not demonstrate differences in *L. monocytogenes* growth rates in pasteurized-milk cheese and raw-milk cheese, but do demonstrate differences in *L. monocytogenes* growth rates
    - among different *L. monocytogenes* strains and among *L. monocytogenes* within the same strain;
    - among batches from the same cheese-making process;
    - among cheeses within the same batch; and,
    - between cheeses' interiors and exteriors or among cheeses' parts with different physico-chemical properties.

Ripening

Changes in pH dominate the important effects during ripening Camembert cheese at 12-14°C, so it is the production process, not the type of milk, that permits shorter lag times and faster *L. monocytogenes* growth in pasteurized-milk cheese (stabilized process), compared with raw-milk cheese (traditional process), during the cheese-ripening stage.

Aging

After ripening (during aging), pH and a_w in raw-milk cheese and pasteurized-milk cheese are

alike (Kosikowski and Mistry 1987; Lawrence et al. 1987; Sanaa et al. 2004). Camembert cheese aging temperatures are temperatures at which growth by *Lactobacillus* spp., *Lactococcus* spp., or *Pseudomonas* spp. that dominate the species identified in raw-milk cheeses would have negligible effect on the growth of other bacteria in the cheese, such as *L. monocytogenes* (Claeys *et al.* 2013). Claeys *et al.* (2013) documented no instances in which *Pseudomonas* spp. or *Lactobacillus* spp. dominated to the total detriment of other microorganisms or to levels that those authors described as having a protective effect; those authors did comment on the emergence, in their sampling, of psychrotrophic bacteria (bacteria that are capable of surviving in a cold environment) within 24 hours and on how bacterial dynamics vary among milk samples.

Conclusion

While some studies investigated the potential antimicrobial characteristics of some raw milk components, all those studies reported a great variability in terms of raw-milk ecology. To our knowledge, no study showed a systematic absence of growth or a systematic reduction in the growth rate of *Listeria monocytogenes* in raw-milk soft-ripened Camembert cheese, compared with *Listeria monocytogenes* in pasteurized-milk soft-ripened Camembert cheese, when processed similarly. In the absence of data supporting this hypothesis, there is no evidence that the natural environment of raw-milk cheese is able to systematically provide a hurdle to reduce *L. monocytogenes* growth. Because the presence of beneficial antimicrobial bacteria in raw-milk cheeses is random (and is not found in all such cheeses), we do not consider reliance on the raw-milk microbial environment to be a scientifically sound mitigation strategy. Therefore, we did not modify the report in response to these comments.

However, solely for purposes of discussion in this document, we considered what would happen if the raw-milk microbial environment could be demonstrated to provide a scientifically sound mitigation strategy. We examined hypothetical scenarios for raw-milk cheeses, in which the exponential growth rate, EGR_{20}, would be systematically halved, compared with the baseline growth-rate distribution (Table 4, this document). The mean risk of invasive listeriosis per raw-milk cheese serving at random under such a scenario would be 7.1 times higher (Canada, elderly population) and 11.4 times higher (United States, elderly population) than the risk per pasteurized-milk cheese serving at random, as estimated in the baseline.

Table 4: Risk of invasive listeriosis per raw-milk cheese serving. Exponential growth rate as estimated in the report *vs.* exponential growth rate divided by a factor of 2. Eledrly population.

Raw-milk cheese	Summary statistics	Canada	United States
Baseline (Recall*)	Median	4.36×10^{-11}	9.94×10^{-11}
Baseline (Recall*)	Mean	4.37×10^{-07}	8.42×10^{-7}
EGR_{20} systematically divided by 2	Median (*dMedian RMC***) (*dMedian PMC*)	5.28×10^{-12} (0.12) (45)	1.14×10^{-11} (0.12) (90)
EGR_{20} systematically divided by 2	Mean (*dMean RMC*) (*dMean PMC*)	5.17×10^{-8} (0.12) (7.1)	9.20×10^{-8} (0.11) (11.4)

* Results might be slightly different than in the draft report, because they were obtained from an updated version of the Analytica™ model.
** Recall: *dMean* (resp. *dMedian*) is the change in the mean (resp. median) risk output with reference to a change in a particular model: PMC: pasteurized-milk cheese, RMC: raw-milk cheese. Example: *dMean PMC* = 45 means that the mean in this alternative is 45 times higher than the mean for the pasteurized-milk cheese alternative.

Use of antimicrobial substance

Example comment: "A beneficial revision critical to ensuring the safety of all cheeses, raw and pasteurized, would be … to allow for the use of … antimicrobials."

Answer: Some comments suggested that we evaluate, as a potential mitigation, use of an antimicrobial substance on the surface of cheese to limit the growth of, or reduce, the *L. monocytogenes* bacterial population. Because the scientific literature demonstrates that there are substances that can be reliably used for their antimicrobial effects in a food processing setting, we consider this to be a scientifically sound potential mitigation strategy. We modified the report by adding an analysis of the potential impacts of such a hypothetical mitigation. We examined hypothetical scenarios involving a potential substance (an antimicrobial voluntarily added during the manufacture of the raw-milk cheese) that would reduce the *L. monocytogenes* concentration on the surface of the cheese by 2 $\log_{10}$ cfu, *i.e.* in the order of magnitude of what could be expected for such effect (Guenther and Loessner 2011) (Table 5, this document). The mean risk of invasive listeriosis per serving, at random, of such raw-milk cheeses would be 50 times higher (Canada) and 86 times higher (United States) than the risk per pasteurized-milk cheese serving.

Table 5: Risk of invasive listeriosis per raw-milk cheese serving. Baseline for raw-milk cheese (report) *vs.* addition of a substance reducing the surface contamination by 2 $\log_{10}$.

Raw-milk cheese	Summary statistics	Canada	United States
Baseline (Recall*)	Median	4.36×10^{-11}	9.94×10^{-11}
Baseline (Recall*)	Mean	4.37×10^{-07}	8.42×10^{-7}
Addition of a substance reducing the surface contamination by 2 $\log_{10}$	Median (*dMedian RMC***) (*dMedian PMC*)	1.11×10^{-11} (0.25) (96)	2.38×10^{-11} (0.24) (188)
Addition of a substance reducing the surface contamination by 2 $\log_{10}$	Mean (*dMean RMC*) (*dMean PMC*)	3.63×10^{-7} (0.83) (50)	6.93×10^{-7} (0.82) (86)

* Results might be slightly different than in the draft report, because they were obtained from an updated version of the Analytica™ model.
** Recall: *dMean* (resp. *dMedian*) is the change in the mean (resp. median) risk output with reference to a change in a particular model: PMC: pasteurized-milk cheese, RMC: raw-milk cheese. Example: *dMean PMC* = 96 means that the mean in this alternative is 96 times higher than the mean for the pasteurized-milk cheese alternative.

Milk-filter testing

Example comment: "The efficacy of milk screening as an intervention would assumedly be improved through the more sensitive approach of testing milk filters. This common intervention should be included in the assessment."

Answer: Van Kessel *et al.* (2011) paired results of testing bulk-tank milk and in-line filters in U.S. dairies for various pathogens (Table 6, this document). While the higher sensitivity (meaning higher number of positive sample detected) of in-line filters is significant for *Salmonella* spp. PCR (McNemar test with continuity correction, *p-value*$<10^{-4}$), *Salmonella* spp. culture (*p-value*$< 10^{-4}$), and *Listeria* spp (*p-value* $< 10^{-4}$), it is not significant for *L. monocytogenes* (*p-value* = .133). Therefore, we did not modify the report in response to these comments.

Table 6: Van Kessel *et al.* (2011) testing bulk-tank milk and in-line milk filters in U.S. dairies, *L. monocytogenes*.

Filter \ Milk	-	+	Total
-	470	13	483
+	23	11	34
Total	493	24	517

Individual replies to specific comments not addressed above

In this section, we answer significant individual comments not answered above. We do not identify the commenter. We bolded some words for quick identification of the subject.

Comment	Response
"The **impact of warning labels** and education for at-risk populations, as implemented in several other countries, should be considered."	The effectiveness of labeling to address a public health problem like that presented by the consumption of raw milk and raw milk products was discussed in the preamble to 21 CFR 1240.61 (52 Federal Register 29509, at 29513).
"The impact of animal health monitoring to reduce the already rare incidence of ***Listeria* mastitis** should also be considered. The present assessment addresses this in discussing Bemrah *et al.*, where eliminating high levels of *L. monocytogenes* from mastitic cows significantly reduced the frequency of milk batches with high levels of *L. monocytogenes* and resulted in a 5-fold reduction in predicted annual illnesses."	The impact of animal health monitoring to reduce the already rare incidence of *Listeria* mastitis is considered in the risk assessment: The risk assessment's *On farm* section describes how the distribution of *L. monocytogenes* concentration in *L. monocytogenes*-positive milk changes with progressive reduction of the prevalence of *L. monocytogenes* shedding by a clinical or sub-clinical *L. monocytogenes*-mastitic cow. Describing the effect on risk of illness is a natural extension. Under conditions in which the prevalence of *Listeria* mastitis is exactly 0, the risk of invasive listeriosis from consumption of raw-milk cheese reduces by a factor of 2.8 (elderly, Canada) and 1.4 (elderly, United States) compared with the baseline raw-milk cheese case, with mean risk per serving, at random, still higher than the mean risk per serving, at random, from the consumption of pasteurized-milk cheese. The sensitivity of the model to this parameter is lower than the one obtained by Bemrah *et al.* (1998).

Comment	Response
"**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."	Pooling milk from many individual cows in multiple herds is considered in the risk assessment. The increase in prevalence and the decrease of concentration by dilution is modeled and is consistent with other applications that modeled (Steele *et al.* 1997) or observed (Jackson *et al.* 2012) this effect.
"QRA authors define soft-ripened cheese made with pasteurized milk as a **baseline** against which to compare risk analyses for such cheeses from unpasteurized milk. In contrast to other aspects of the QRA, which are generally of high quality and consistent with scientific practice, this rubric choice is out of line with international QRA standards, quantitatively problematic, and misleading in the interpretation. Risk calculations are typically measured against a standard quantitative baseline in order to appropriately characterize the frequency and severity of a given foodborne hazard."	We disagree with this comment. Classifying or categorizing risk results with labels and making value judgments in risk assessments is inconsistent with *Codex Alimentarius* (1999), the Health Canada Decision-Making Framework (2000), and U.S. FDA CFSAN frameworks (2002). Rather, risk characterizations describe how the risk varies among conditions and circumstances, and, in doing so, invite comparisons among the risks under those different conditions and circumstances. It is common to use *a baseline* case to facilitate comparisons, without implying absolute accuracy of one case versus another or attributing more fundamental appropriateness to one case over another. In this risk assessment, we actually use two baselines - a pasteurized milk cheese baseline and a raw milk cheese baseline.
"Claeys et al suggest raw milk's **commensal bacteria as important mitigators** of invasive listeriosis via competitive exclusion… In their 2008 study of soft-ripened cheesemaking from unpasteurized milk (an important omission from the current QRA), Henri-Dubernet and colleagues report a strong microbiological variability across such cheeses, but note that *Lactobacillus paracasei* (known to have pathogen mitigating action) is most frequently present. … Further, as the report authors are certainly aware, the presence of lactic acid bacteria in fermented foods is known to enhance food safety via production of various antimicrobial metabolites such as lactic and	As discussed above in the section of this document titled "*L. monocytogenes* growth in raw-milk cheese and pasteurized-milk cheese," our general reply to these statements is that the applicable literature does not reveal *L. monocytogenes* growth-rate differences at the same pH, a_w, and temperature among raw milk, unpasteurized milk, and pasteurized milk Camembert cheeses during aging [(Genigeorgis *et al.* 1991; D'Amico *et al.* 2008a); our appendix]. Additionally, even if beneficial antimicrobial bacteria were able to provide a sufficient hurdle to reduce *L. monocytogenes* growth, their random presence in cheeses would still prevent it

Comment	Response
acetic acid, hydrogen peroxide, and others; these factors contribute to a 'hurdle effect' towards reducing the presence of milk-borne LM, an effect which authors should attempt to model, if even vaguely."	from being a scientifically sound mitigation strategy. Responding more specifically to this comment, we note that Claeys *et al.* (2013) noted milk's commensal bacteria, notably lactic acid bacteria, as mitigators (not of invasive listeriosis but of pathogen growth), but only when milk is stored at high enough temperatures to let lactic acid bacteria grow to high enough levels to produce lactic acid in sufficient quantities, which would happen only at temperatures that lead to rapid milk degradation – not at refrigerator temperatures: "Many lactic acid bacteria are capable of producing bacteriocins, but it is unlikely that they would reach levels necessary for the production of bacteriocins in refrigerated milk as they would not grow. Raw milk contains negligible levels of nisin." [(Claeys *et al.* 2013); pg. 257]. We carefully checked the Henri-Dubernet *et al.* (2008) reference that the comment provided. Henri-Dubernet *et al.*[(2008), page 226] cited Schwenninger *et al.* (2005) for some *Lactobacillus* spp.'s antimicrobial properties, and Schwenninger *et al.* (2005) examined antifungal (yeasts) properties. Henri-Dubernet et al (2008) specifically report a great variability in terms of *Lactobacilli* populations.
"To employ in the QRA an earlier conceptual model which neither incorporates the sophistication of that used by Schvartzman et al, (2011) nor acknowledges these researchers' indication that yet additional factors are impacting growth patterns, positions the current report as out-of-date in its conceptualization…The largest body of literature on bacteriostatic and bactericidal factors for reducing LM in raw milk involves the **lactoperoxidase hydrocyanate (LP) system**, which is particularly active at ambient	Schvartzman *et al.* (2011) showed, in a specific product (smeared cheese, which is not a soft-ripened cheese and is therefore outside the scope of the risk assessment), that "No growth of *L. monocytogenes* occurred during raw milk cheese-making, whereas growth did occur in pasteurised milk. During ripening, growth occurred in raw milk cheese, but inactivation occurred in pasteurised milk cheese" and concluded that "The general results indicate that as the

Comment	Response
temperatures… such as those involved in cheese making. Raw milk's indigenous lactic acid bacteria will produce hydrogen peroxide for this purpose during aging… whereas such will not spontaneously occur in pasteurized milk without the addition of lactic cultures."	cheese enters the market place the relative risk from cheeses made from pasteurized milk are almost 100-fold less than those made from raw milk, if contaminated with *L. monocytogenes*." The study by Schvartzman *et al.* (2011) addressed growth during cheese *ripening*, during which changes in cheese pH govern *L. monocytogenes* growth; differences attributable to the cheese-making process, not the type of milk (raw or pasteurized). International authorities (FAO/WHO 2006) have stated that the natural antibacterial activity of the lactoperoxidase system (LPS) is quite weak, because milk contains only suboptimal levels of the thiocyanate (rather than hydrocyanate) ion and hydrogen peroxide. LPS is only activated, to any significant degree, by the addition of exogenous thiocyanate ion and an exogenous source of hydrogen peroxide (FAO/WHO 2006).
"The Draft QRA suffers from a **lack of field data**. The study includes almost no experimental or other data, particularly in regard to the cheese-making phase."	We disagree that the QRA lacks sufficient data. We made the choice to gather all the available published, relevant literature on *L. monocytogenes* in soft-ripened cheese that met necessary criteria to perform the QRA, rather than using a single specific dataset. While we acknowledge that it would be helpful to have additional data, we are not aware of other published data beyond that used in the QRA that met the necessary criteria. It is, to our knowledge, the QRA that gathers the largest literature knowledge. Most published QRAs use a single dataset, specific to the situation under study [see (Sanaa *et al.* 2004) for "Camembert of Normandy and Brie de Meaux" as an example], selecting one dataset among many datasets to be the representative one or averaging over the datasets.
"The Draft QRA is confusing with regard to the **60-day rule**. [Our] view is that a 60 day aging	While some other countries may not apply aging requirements to soft-ripened cheeses,

Comment	Response
rule is relevant for hard cheeses, but not for soft-ripened cheeses."	the existence of a rule in Canada and in the United States makes a 60-day aging rule relevant for soft-ripened cheeses for the purposes of the risk assessment. As stated on page 19 of the report, "The U.S. definition of soft-ripened cheese also states that "if 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 as defined by B.08.030; i.e. "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."
"The **physicochemical parameters** throughout the process, particularly for the raw milk Camembert, are based on the 2004 Sanaa model, itself based on Ryser and Marth's data, which goes back to 1987. Moreover, hypotheses were made concerning pH levels at the start of the ripening stage for standardised soft cheeses. In general, measuring pH levels, water activity, lactic acids and temperature throughout the production process would have allowed for more reliable results."	While we acknowledge that it would be helpful to have additional data of the kind described in the comment, we are not aware of other published data beyond that used in the risk assessment. The data from Ryser and Marth (1987), as modeled by Sanaa *et al.* (2004), are in good agreement with the description by Lawrence *et al.* (1987) and with the exhaustive study by Liu and Puri (2004).
"Concerning raw milk cheeses, it must be noted that Marielle Gay and Albert Amgar published **latency period** data in 2005. The study estimates the mean latency period for a raw milk Camembert at 31.1+/-10.5 days."	Gay & Amgar's (2005) study reported three observations, with a very large variability in the latency period. We did not use the results from this study, but those from Ross *et al.* (2009), because of this low number of observations. Note that the authors do not provide any statistical test, although one can approximate a formal test from their Figure 3. From that Figure, null hypotheses like H_0:$\lambda_{PMC}=\lambda_{RMC}$ (the lag time in PMC: pasteurized milk cheese equal the lag time in RMC: raw milk cheese) and H_0: $T_{PMC}=T_{RMC}$ (the time to a 10^3 – fold increase in population in PMC is equal to the time to a 10^3 – fold increase in RMC) would not be rejected against simple one-sided alternatives in the direction that favors those authors' premise.

Comment	Response
	Three observation data sets would be too small to detect even large differences, regarding the large among cheeses variance in *L. monocytogenes* lag time that they measure. Note that the lag time for raw-milk cheeses in our risk assessment is at least as large as those reported in this publication (see next comment).
"Latency periods used for re-contaminant cells are taken from Ross *et al.*, 2009. Yet Guillier *et al.* (2005) assess **latency period data that reproduce the environmental conditions** (disinfection, lack of nutrients, for example) bacteria typically go through on the production site, and which contaminate the surfaces of cheeses made from pasteurised milk."	Our definition and evaluation of the latency period lead to potentially long lag times before growth following environmental contamination. In the risk assessment, the actual lag time is a function of K_ξ and the growth rate, this latter parameter being a complex function of the cheese environment. Overall, for classical ripening cheese-making, the median of the actual lag time distribution was 34 days, with 25^{th} percentile point 13.8 days and 75^{th} percentile 113 days. For stabilized cheeses, the median of the actual lag time was 14.1 days, with 25^{th} percentile point 5.1 days and 75^{th} percentile 64 days. Little is known about the stress of *L. monocytogenes* before cheese contamination, and we considered a complete distribution of K_ξ parameters, as proposed by Ross *et al.* (2009), to be more effective for describing the variability of the stress condition, compared with the six situations described by Guillier *et al.* (2005). The Ross *et al.* way of modeling this parameter actually includes, but is not limited to, the Guillier *et al.* (2005) results.
"The level of contamination in portions of cheeses made from pasteurized milk for the general population estimated at the moment of consumption is given in the table below: US: Average prevalence = 0.49% Average number of bacteria/ portion = 16 bacteria (ave ≈ 10^6) Canada: Average prevalence = 0.49% Average number of bacteria/ portion = 16 bacteria (ave ≈ 10^6) Exposure in the ranges of 10^6 cells per portion, in regard to concentration extremes, seems particularly high to us. The 2010 results of	Johnsen *et al.* (2010) recently reported an outbreak linked to Camembert with up to 6 million cfu per gram in unopened packages. Growth studies also suggest that the maximum achievable bacteria concentration in Camembert is high. This parameter has a big impact on the predicted arithmetic mean (Pouillot and Lubran 2011). Indeed, the level of bacteria follows a highly skewed distribution. Its arithmetic mean is, then, very unstable and probably cannot be robustly evaluated through such a survey.

Comment	Response
national monitoring plans for soft-ripened cheeses made from pasteurized cow milk for *L. monocytogenes* by [our] Directorate-General for Competition, Consumption and Fraud Control show us 6 contaminated samples over 1453 (.4%), with prevalences all inferior to 10 cfu/g."	Moreover, while we do not have the complete reference for the French study to which the commenter alluded, these studies are usually done at the manufacturer or retail level. The risk assessment clearly suggests that home storage dramatically increases the bacterial concentration in some rare cases of long storage at abusive temperature, and then the distribution's mean concentration. (Correction to the comment: *16 bacteria* refers to the median of the distribution of the number of *L. monocytogenes* per contaminated serving, not the distribution mean.)

References

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.

CDC. (2013a, September 24, 2013). "Multistate Outbreak of Listeriosis Linked to Crave Brothers Farmstead Cheeses (Final Update)." Retrieved 12/4, 2013, from http://www.cdc.gov/listeria/outbreaks/cheese-07-13/index.html.

CDC (2013b). "Vital signs: Listeria illnesses, deaths, and outbreaks--United States, 2009-2011." MMWR Morb Mortal Wkly Rep **62**(22): 448-452.

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

Claeys, W. L., S. Cardoen, G. Daube, J. De Block, K. Dewettinck, K. Dierick, L. De Zutter, A. Huyghebaert, H. Imberechts, P. Thiange, Y. Vandenplas and L. Herman (2013). "Raw or heated cow milk consumption: Review of risks and benefits." Food Control **31**(1): 251-262.

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

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.

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.

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.

FAO/WHO (2006). Benefits and Potential Risks of the Lactoperoxidase system of Raw Milk Preservation - Reports of an FAO/WHO technical meeting. FAO/WHO.

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.

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.

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.

Guenther, S. and M. J. Loessner (2011). "Bacteriophage biocontrol of *Listeria monocytogenes* on soft ripened white mold and red-smear cheeses." Bacteriophage **1**(2): 94-100.

Guillier, L., P. Pardon and J. C. Augustin (2005). "Influence of stress on individual lag time distributions of *Listeria monocytogenes*." Appl Environ Microbiol **71**(6): 2940-2948.

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

Henri-Dubernet, S., N. Desmasures and M. Gueguen (2008). "Diversity and dynamics of lactobacilli populations during ripening of RDO Camembert cheese." Can J Microbiol **54**(3): 218-228.

Jackson, E. E., E. S. Erten, N. Maddi, T. E. Graham, J. W. Larkin, R. J. Blodgett, J. E. Schlesser and R. M. Reddy (2012). "Detection and enumeration of four foodborne pathogens in raw commingled silo milk in the United States." J Food Prot **75**(8): 1382-1393.

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.

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

Latorre, A. A., A. K. Pradhan, J. A. S. Van Kessel, J. S. Karns, K. J. Boor, D. H. Rice, K. J. Mangione, Y. Grohn and Y. H. Schukken (2011). "Quantitative Risk Assessment of Listeriosis Due to Consumption of Raw Milk." Journal of Food Protection **74**(8): 1268-1281.

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

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.

Pouillot, R. and M. B. Lubran (2011). "Predictive microbiology models *vs*. modeling microbial growth within *Listeria monocytogenes* risk assessment: What parameters matter and why." Food Microbiol **28**(4): 720-726.

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.

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.

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.

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.

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.

Schwenninger, S. M., U. von Ah, B. Niederer, M. Teuber and L. Meile (2005). "Detection of antifungal properties in *Lactobacillus paracasei* subsp. *paracasei* SM20, SM29, and SM63 and molecular typing of the strains." Journal of Food Protection **68**(1): 111-119.

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.

Tenenhaus-Aziza, F., J. J. Daudin, A. Maffre and M. Sanaa (2013). "Risk-Based Approach for Microbiological Food Safety Management in the Dairy Industry: The Case of *Listeria monocytogenes* in Soft Cheese Made from Pasteurized Milk." Risk Anal: n/a-n/a.

Van Kessel, J. A. S., J. S. Karns, J. E. Lombard and C. A. Kopral (2011). "Prevalence of *Salmonella enterica*, *Listeria monocytogenes*, and *Escherichia coli* Virulence Factors in Bulk Tank Milk and In-Line Filters from US Dairies." Journal of Food Protection **74**(5): 759-768.

Varma, J. K., M. C. Samuel, R. Marcus, R. M. Hoekstra, C. Medus, S. Segler, B. J. Anderson, T. F. Jones, B. Shiferaw, N. Haubert, M. Megginson, P. V. McCarthy, L. Graves, T. V. Gilder and F. J. Angulo (2007). "*Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States." Clin Infect Dis **44**(4): 521-528.