Food Safety Centre

University of Tasmania

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Defining "Short Shelf Life" foods with respect to risk from *Listeria monocytogenes*

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prepared by

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Summary

This report was prepared to provide scientific advice to food safety risk managers in New Zealand Food Safety Authority on the feasibility of defining a "short shelf life" for ready-to-eat (RTE) foods that can support the growth of *Listeria monocytogenes*, but during which growth might be so low as to cause no significant threat to public health. Ready-to-eat foods, particularly those with long, refrigerated shelf lives, are most often implicated in cases of listeriosis (FAO/WHO, 2004). Such a definition might allow regulatory effort to be allocated more appropriately to 'higher-risk' foods with an overall benefit to public health and to reduce the regulatory burden on manufacturers of such short shelf life RTE foods.

Two approaches were adopted. The first was to identify existing regulations or policy that gave exemptions from 'zero tolerance' approaches to RTE foods that support growth of *L. monocytogenes* and to seek to understand the scientific basis of such policy or regulations. This involved literature searches and personal communications with relevant experts, and officers in agencies that had set, or provided advice for the establishment of, such policy or regulations, namely Health Canada and European Food Safety Authority. The second approach, based on predictive microbiology models, sought to estimate the amount of growth of *L. monocytogenes* that could occur under reasonably foreseeable conditions of distribution, storage and consumer use. Interpretation of the results was based on the assumption, now established in several international regulations, that restricting contamination levels of *L. monocytogenes* on food to ≤ 100 CFU.g^{-1} at the time of consumption, provides an appropriate level of consumer protection. For the various approaches and assumptions tested regarding *L. monocytogenes* loads and lag times, and temperatures of storage, it was assumed that the RTE food did not *per se* inhibit growth of *L. monocytogenes* and that the only limitation to growth rate was due to temperature. The interaction of *L. monocytogenes* with other bacteria expected to be on such products, and their influence on *L. monocytogenes* risk from such products, was also explored.

The results from these various approaches suggested that a shelf life of from 3 – 8 days, depending on assumptions made, could usually be expected to limit *L. monocytogenes* to less than 100CFU.g^{-1} on RTE foods up to the time of consumption. These results agree broadly with definitions of "short shelf life" applied to RTE products in current European Union regulations and Canadian policy. The results rest, however, on several key assumptions including that:

- the initial load of *L. monocytogenes* on the product is of the order of a few cells per gram,
- temperatures of distribution, storage and use remain in a range of~5.5 ± 3°C

Where there is temperature abuse, or poor hygiene during manufacture[1], *L. monocytogenes* could attain levels that have been associated with listeriosis outbreaks within the times suggested for 'short shelf life'. Accordingly, the definition of "short shelf life" will depend on the level of probability of 100 CFU.g^{-1} being exceeded. A stochastic model was developed and used in

[1] Subsequent contamination can also contribute to higher risk, but contamination at the plant will be most exacerbated by subsequent time and storage conditions prior to consumption.

the analysis that enables "short shelf life" to be defined for different levels of confidence that products will not exceed the 100CFU.g^{-1} tolerance limit.

A final assumption inherent in definitions of "short shelf life" for RTE foods that support growth of *L. monocytogenes* is that consumers will discard products when the nominal shelf life expires. However, it is proposed that there is reasonable doubt that this will always be the case due to consumer uncertainty of the meaning of "use-by" dates and the possibility that the product will not have spoiled overtly by the "use-by" date, especially if manufacturers place conservative "use-by" dates on their products. An approach for evaluating the probability that *L. monocytogenes* could reach unacceptable levels prior to consumption, including the influence of spoilage bacteria, or lactic acid bacteria, is also presented so that realistic shelf lives based on both the potential growth of spoilage/lactic acid bacteria and *L. monocytogenes* can be estimated.

Existing Codex guidelines and EU regulations for managing the risk of listeriosis from RTE foods provide a mechanism for any food to be defined as a 'non-growth supporting' food and, thus, to be exempted from the 'zero tolerance' regulatory approach (i.e., *L. monocytogenes* not detected in five replicate food samples of 25g each). That approach is to use predictive microbiology models, or challenge studies, or other forms of scientific evidence, to demonstrate that growth in the product does not exceed 0.5 logCFU during the normal shelf life of the product under "foreseeable conditions of storage, distribution, and use". This report demonstrates that using predictive microbiology does not provide an unambiguous generic definition of 'short shelf life' for RTE foods because of the number of variables potentially involved. Challenge tests are likely to be affected by the same variables. Accordingly, any risk management decision will still involve some subjectivity. The intention of this report is to provide information and analyses to reduce the degree of subjectivity required and to assist to make it transparent.

Contents

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Throughout this document reference is made to the Canadian draft policy. The 2010 version of the policy was released in October 2010. For more information on this subject, *see* Health Canada's website:

http://www.hc-sc.gc.ca/fn-an/legislation/pol/policy_listeria_monocytogenes_2010-eng.php

The new policy will come into effect on April 1, 2011 but, until that time, the 2004 version of the Policy on *Listeria monocytogenes* in ready-to-eat foods will remain in effect to allow for a transition period for implementation.

Introduction

Listeriosis is a rare but severe, systemic, infection that results in death in approximately 20-30% of cases. The causative organism is the bacterium *Listeria monocytogenes* and the vast majority of cases of listeriosis are believed to be food-borne (FAO/WHO, 2004). Most cases occur among people with reduced immunity either due to age (neonates or elderly), those with illnesses or receiving therapies causing reduced immune function (HIV, organ transplant recipients, cancer patients), and the foetuses of pregnant women.

Foods associated with listeriosis typically:

i). are ready-to-eat (RTE)
ii). support the growth of *L. monocytogenes*
iii). are stored under refrigeration and, through this and/or their formulation,
iv). have an extended shelf-life before microbiological spoilage is evident.

These associations were originally recognised from epidemiological studies and, with advances in knowledge of the dose-infection relationship for *L. monocytogenes* (*e.g*., Buchanan *et al*., 1997; Chen *et al*., 2003; FAO/WHO, 2004; Smith *et al.*, 2003, 2008; Williams *et al*., 2009) their basis is now better understood. Relatively large doses of *L. monocytogenes* (i.e., of the order of tens of thousands of cells or more) are now believed necessary to cause listeriosis, even among susceptible consumers. Moreover, the risk of infection for any given consumer/strain/food combination, is believed to be directly proportional to the number of cell of *L. monocytogenes* consumed in that meal (FAO/WHO, 2004). In general, at the time of processing only a small percentage of susceptible products become contaminated, and affected foods are contaminated with only low levels of *L. monocytogenes*, even at retail (Gombas *et al*., 2003; Little *et al.,* 2009). Thus, actions to minimise or prevent *growth* of *L. monocytogenes* in foods between the time of their manufacture, or sale, and their consumption should greatly reduce the risk of illness from *L. monocytogenes* on *ready-to-eat* foods[2].

The extent of growth and, accordingly, the risk of listeriosis, will also depend on product formulation *and time.* Recent international requirements (EC 2073/2005; EC 1441/2007) and guidelines (CAC, 2007; CAC, 2009) for control of the risk from food-borne *L. monocytogenes* differentiate foods according to the potential for *L. monocytogenes* growth. Those documents infer:

i). that foods which due to their formulation and processing, prevent *L. monocytogenes* from growing in the product and that,
ii). where *L. monocytogenes* levels do not exceed 100 CFU.g^{-1}

present no significant public health risk. Recognising that some products may support *very limited* growth of *L. monocytogenes*, an additional category is recognized: i.e., foods that during their normal shelf life, and under reasonably foreseeable conditions and duration of distribution

[2] *L. monocytogenes* is not unusually resistant to heat so that foods that are cooked, or fully reheated, prior to eating would not be expected to harbour viable *L. monocytogenes* and, hence, to not pose a significant risk of listeriosis.

and handling, do not support growth of *L. monocytogenes* (CAC, 2009) of more than 0.5 logCFU. In those foods, up to 100 CFU.g^{-1} *L. monocytogenes* at the point of consumption is also considered to present no significant risk. In foods that *do* support its growth[3], *L. monocytogenes* should not be present in the product (articulated as "not detected in 25g", and often described as "zero tolerance", n.b., typically 5 x 25 g samples are assessed and *L. .monocytogenes* should not be detected in any of them).

However, in some regulations (EC 2073/2005) or current (Health Canada, 2004) or draft policy (Health Canada, 2010), foods that may support growth, but that have a stated shelf life of less than five days, are considered exempt from the "zero tolerance" approach or considered to be 'foods that do not support growth' for the purposes of the regulations. The 100 CFU.g^{-1} limit apparently still applies and seems implicit in the text in the Canadian draft policy (*discussed further below*).

This report traces the origin of regulations apparently providing exemption from *L. monocytogenes*-related microbiological criteria for "short shelf life foods", and evaluates their current relevance and utility for application in New Zealand.

Methods

Literature Search and Personal Communications

To identify regulations for food-borne *L. monocytogenes* risk management that include consideration of 'short shelf life" products, internet searches were undertaken using Google and ISI's Web of Knowledge 4.9 (Thomson Reuters, 2010) search engine for published literature using the terms "listeria" and/or "monocytogenes" and "shelf life" or "use by". Professional colleagues involved in food safety risk management in Japan, Denmark, USA and Canada were also consulted, as were relevant officers at the European Food Safety Authority (EFSA) and Directorate General Health and Consumer Protection (DG SANCO) of the European Commission, to try to understand more clearly the genesis of regulations that differentiate risk management approaches for products of 'short shelf life'.

Mathematical Modelling

On the basis of criteria found and comments and information received, definitions of short shelf life were evaluated using predictive microbiology models against the current EC and CAC "tolerance" limits of up to 100 CFU.g^{-1} *L. monocytogenes.* Predictions were made on a relative basis, and also using initial contamination levels derived from large and systematic surveys of *L. monocytogenes* in foods, within the definitions of "short" shelf-life available, and under foreseeable conditions of storage and handling as well as other more extreme scenarios.

The predictive models used include:

i). the *L. monocytogenes* growth rate model of Mejlholm and Dalgaard (2009), as implemented in the Seafood Spoilage and Safety Predictor (SSSP) software

[3] where growth is defined CAC (2008) as greater than 0.5 logCFU increase of *L. monocytogenes* during the stated shelf life and under reasonably foreseeable conditions of storage.

(http://sssp.dtuaqua.dk/). The model is arguably the most extensive and extensively evaluated model currently available for *L. monocytogenes* growth in foods and, in a recent publication (Mejlholm *et al*., 2010), has been shown to have the most reliable predictions of a range of models currently available for *L. monocytogenes* growth rate estimation.

ii). ComBase growth predictor, for *L. monocytogenes* and psychrotrophic pseudomonad growth rates, based on data contained in the ComBase international collaborative predictive microbiology database (http://www.combase.cc/)

iii). the United States Department of Agriculture's widely used and distributed Pathogen Modelling Program software (V. 6.1) for *L. monocytogenes* growth rate http://www.ars.usda.gov/Services/docs.htm?docid=11550).

iv). Pseudomonas model of Neumeyer *et al*. (1997a, b),

v). Models for lactic acid bacteria growth rate of Devlieghere *et al*. (2000) and Wijtzes *et al*. (2001), as modified by Ross *et al*. (2004).

Due to the numerous possible scenarios that could be envisaged to evaluate the implications of short shelf life definitions, analyses were further refined by development and application of stochastic simulation model. The model is able to quantify the effects of variable temperature of storage, variable initial levels of contamination and variable lag times of *L. monocytogenes* on the time to development of unacceptable levels of *L. monocytogenes* on the product. The model and its development are described in detail Appendix A. In the stochastic model growth is modeled deterministically (as a function of the previously mentioned variables) using a simplified version of the model of Mejlholm and Dalgaard (2009) only, i.e., not using the other available models. The choice of the Mejlholm and Dalgaard (2009) model was because, as noted earlier, it has been much more extensively validated than either of the other models mentioned above. The stochastic model was also used to evaluate a number of other, putative, scenarios to help to communicate the results of the analysis.

Results

Three sets of microbiological criteria were found that explicitly exempt foods with short shelf life, but that otherwise would support growth of *L. monocytogenes*, from the 'zero tolerance' approach. Those criteria relate to Canada (Farber and Hartwig, 1996; Health Canada, 2004; Health Canada, 2010) and the European Union (EC 2073/2005; EC 1441/2007). Earlier regulations in Denmark (Nørrung *et al*., 1999) also regarded heat-treated products that could be recontaminated, as "stabilized" with respect to *L. monocytogenes* growth if the shelf life were less than one week. Those Danish regulations have, however, been superseded by current EU legislation (Dalgaard, *pers. comm*., 2010). Specifically, European Commission regulation No 1441 of 2007 amending EC Regulation No. 2073/2005 states (EC 1441/2007; footnote 8 in Annex 1) that foods with a shelf life of less than five days are automatically considered to belong to the category: "ready-to-eat foods unable to support the growth of *L. monocytogenes*". It should be noted, however, that the regulation does not specifically exempt such products from the 100 $CFU.g^{-1}$ limit that applies to foods that do not support *L. monocytogenes* growth.

Similarly, current Canadian policy for regulatory/inspection priorities to manage food-borne listeriosis places greatest regulatory scrutiny on RTE foods that support growth of *L. monocytogenes*, with the highest priority given to RTE foods that have caused listeriosis and those with shelf-lives of greater than 10 days.

Further information concerning the basis of the EU and Canadian regulations/policy was sought by correspondence with relevant officers in either organization. Dr. Jeffrey Farber is Director, Bureau of Microbial Hazards, of the Food Directorate of Health Canada. Dr. Marta Hugas is Scientific Coordinator, Head of Biological Hazards Unit, EFSA, in Parma, Italy. Dr. Leena Rasanen is Legislative Officer for DG-SANCO.

It should perhaps be noted that there is some ambiguity between the various pieces of legislation about whether "short shelf life" products are considered to pose insignificant risk or whether, due to their short shelf life, they are considered only to represent significantly *lower* risks than equivalent products with longer shelf lives, i.e., so that, if resources are limited, regulatory attention should be focused on the long shelf life products. In the Canadian policy (Health Canada, 2010) it is apparent that the short shelf life definition is for pragmatic reasons and for prioritization of regulatory attention. The EU regulation is presumably based on consideration of the potential for growth of *L. monocytogenes* on foods held under "reasonably foreseeable" storage conditions and for realistic initial contamination levels. Relevant calculations and/or discussion were not found to demonstrate this low growth potential in either EU or Canadian documents, but were undertaken and are presented as part of this report.

Examples of 'short shelf life' definitions

Canada

In the current Canadian approach (Farber and Hartwig, 1996, Health Canada, 2004), which are under review (*see below*) three food categories are differentiated upon listeriosis risk. Products in Category 1 have been:

i) causally linked to documented outbreaks of listeriosis and/or

ii) listed as "high risk" products in the USDA/CFSAN/FSIS (2003) risk ranking of RTE foods,

and receive the highest priority for inspection and compliance assessment. Detection (i.e., presence in a sample, irrespective of contamination level) of *L. monocytogenes* in these RTE foods "will likely result in" a product recall with consideration of a 'public alert' (if the product is still in the marketplace).

Category 2 includes all other RTE foods which are capable of supporting growth of *L. monocytogenes* and have a shelf life > 10 days. These products receive the second highest priority for inspection and compliance assessment.

The third category contains both RTE foods supporting growth but with a shelf life of ≤10 days, and those RTE foods not supporting *L. monocytogenes* growth. These products receive the lowest priority in terms of inspection and compliance action. For Category 3 foods, an action level of 100 CFU.g^{-1} is applied but others factors considered in determining the compliance action taken include the actual levels of *L. monocytogenes* in the food and whether there is evidence of

adherence to Good Manufacturing Practices.

The Canadian policy is currently under review (J. Farber, *pers. comm*., 2010; Health Canada 2010) and the new *draft* policy proposes that foods with five days shelf life or less be considered a lower risk to public health and, therefore, be subject to less regulatory reaction if *L monocytogenes* is detected. The rationale for this decision is that: "*The latter time period would not allow sufficient time, under reasonably foreseeable conditions of distribution, storage and use, for L. monocytogenes to grow to levels above 100 CFU/g by the end of the stated shelf-life*". In relation to Category 2A foods (those foods that offer limited potential for *L. monocytogenes* growth), the Canadian draft policy continues: "*Notwithstanding that these foods can support the growth of L. monocytogenes, the growth is generally limited because of a number of factors such as short refrigerated shelf-life, a large background microflora containing anti*-Listeria *lactic acid and/or other bacteria, etc*."

Dr. Farber also indicated that another part of the reason for the short shelf life considerations was a pragmatic one, i.e., the time taken to analyse a sample. Concerning the five day "short shelf life" definition in the draft Canadian policy (Health Canada, 2010) Dr. Farber noted that using prescribed Canadian methods, a minimum of five days are required to enumerate 'confirmed' *L. monocytogenes* from foods and that, as such, these (Category 2A) products would no longer be available for sale, or usable, before test results were available. (J. Farber, *pers. comm*., 2010). The conclusion was that in practical terms, even if such products support the growth of *L. monocytogenes*, a lower level of oversight can be given compared to other RTE foods that support the growth of *L. monocytogenes,* because they have longer shelf lives and that *L. monocytogenes* could potentially grow to very high levels.

European Union

In correspondence, Dr. Leena Rasanen (*pers. comm.*, 2010) observed that the current EU regulations were based on the 1999 scientific opinion on *Listeria monocytogenes* of the European Commission Scientific Committee on Veterinary Measures relating to Public Health (ECSCVMPH, 1999). That expert opinion has since been revised (EFSA/BH, 2007), but not in time to be included in the most recent EU legislation (EC 1441/2007) concerning *L. monocytogenes* in foods.

The EFSA 2007 opinion document (EFSA/BH, 2007) notes, however, without criticism that the previous EU legislation (i.e., EC 2073/2005) considers foods with <5 days shelf life as not able to support the growth of *L. monocytogenes.* The 2007 opinion also notes the requirement for food business operators "*to conduct studies to investigate compliance with criteria throughout shelf life, for ready-to-eat foods able to support growth of* L. monocytogenes" and adds that: "*These investigations should take into account the "reasonably foreseeable storage conditions" (in particular temperature and shelf life) and should consider the important variability in refrigeration temperatures observed in Europe, particularly in domestic refrigerators*". That is, there is tacit recognition in the opinion that there is much subjectivity about the idea of "reasonably foreseeable conditions".

The 2007 opinion document also emphasises that microbiological criteria are only one of a set of activities needed to assure food safety and stresses the roles of HACCP and GMP in the overall

management of food safety.

Theoretical Analysis: Predictive Microbiology

From the above discussion it would seem that any definition of a short shelf-life food must require that the product to which it relates will have a finite shelf life beyond which, due to quality deterioration, the product would not be consumed (or beyond which regulators or industry cannot be considered to be liable if the product is consumed and illness ensues). If product quality does not markedly deteriorate after the "use-by" date, common experience suggests that many consumers will judge for themselves whether the product is edible, rather than relying on the advice implicit in the 'use-by' date[4] given on the product. (Note also that high levels of *L. monocytogenes* do not cause overt signs of food spoilage).

Some food safety risk managers may reject this assumption, and some regulatory authorities expect that some consumers would eat 'spoiled' product, (R.L. Buchanan, *pers. comm*., 2010) and therefore may be at risk if the product also supports the growth of pathogens. Thus, a regulatory response for presence of *L. monocytogenes* on the basis of a "short" shelf life or one that assumes that contamination levels won't reach 100 CFU.g^{-1} before consumption must require that:

i). the product becomes inedible before *L. monocytogenes* levels reach 100 CFU.g^{-1} or greater; or that consumers will not eat a product once it is beyond the 'use by' date and

ii). that the existing chill chain is reliable enough to prevent growth of *L. monocytogenes* in such products to levels of concern.

To have reasonable assurance that *L. monocytogenes* growth to high levels is not possible before the product spoils it is necessary, as articulated by Koutsoumanis (2009), to know the organisms responsible for spoilage ('specific spoilage organisms', or 'SSOs'), the level of those organisms at which spoilage occurs, the initial level of the SSOs, and how quickly the SSOs will grow in the product under different storage conditions. Moreover, the growth of *L. monocytogenes* that occurs in that time, relative to the growth of the SSO, can vary due to differential sensitivity to temperature of *L. monocytogenes* and SSO growth rates. A proposed approach for determination of "use-by" dates, that takes spoilage into account, is presented below.

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[4] "use-by" date in many jurisdictions indicates the time beyond which the product will become *unsafe* to eat. While this is a strict definition, and is communicated to consumers by a variety of means, it is reasonable to believe that many consumers do not realise that "use-by" date is not related to quality, but to safety. It is also reasonable to assume that few food processors' "use-by" dates are scientifically determined. In USA "expiration" date is analogous to "use-by" date. As a means of demonstrating the likely confusion of consumers regarding the interpretation of such terms, and their credibility, see "http://shelflifeadvice.com" a US web-site that promotes the idea that "Food product dates encourage food waste". In a related article (http:// http://www.msnbc.msn.com/id/38582178/ns/health-food_safety/), various prominent food safety scientists, including FDA spokesperson Ira Allen, Prof. Mike Doyle (University of Georgia Centre for Food Safety), and Prof Joe Regenstein (Food Science, Cornell University) are cited as giving advice that (US) food date codes, including expiration dates, do not equate to safety, i.e., that they are too conservative. Given these "mixed messages" it seems reasonable to consider that consumers may not understand the significance of various date codes on foods, and therefore, that they could often disregard them in the absence of overt deterioration of the product itself.

Simple Case

To explore the problem posed (i.e., scientifically based definition of short shelf life), it is assumed that for *short* shelf life foods there are no impediments to microbial growth other than temperature. Assuming that a level of <100 CFU *L. monocytogenes*.g^{-1} at consumption is an appropriate level of protection, to begin to determine a "use-by" date it is necessary to estimate how much growth of *L. monocytogenes* could occur before the 100 CFU.g^{-1} limit was reached. Numerous published studies suggest that initial contamination levels are usually low, in the range of a few cells per gram or less. Two relatively recent, large, systematic studies have been undertaken, one in USA (Gombas *et al*., 2003) involving 31705 samples taken from retail displays, and one in the United Kingdom (Little *et al*., 2009) also at retail and involving 6818 samples. An overview of the distribution of contamination rates and contamination levels reported in those studies is presented in Appendix A. In the USA study (Gombas *et al.*, 2003) the weighted mean contamination rate among eight product types in each of two regions (proportion of samples with *L. monocytogenes* detected in 25g) was 1.8% of which the median concentration was <1 CFU per 10g. The 95th percentile concentration level, however, was~41 CFU.g^{-1} due to the long "right hand tail" of the distribution of logCFU contamination levels. In the UK survey (Little *et al.*, 2009) the median contamination level among the 2.4% of contaminated samples was < 2 CFU.g^{-1} and the 95th percentile ~27,000 CFU.g^{-1}. Based on the median values, up to 2 logs of growth would be 'tolerable'. Based on the 95th percentile values, *no* growth could be tolerated. In this situation, it was considered more useful to base calculations on median values, as these are more representative of the "normal" situation, remembering that only a small percentage of samples are contaminated at all.

Thus, as a first estimate, the time required for a 100-fold increase in *L. monocytogenes* numbers at various temperatures was estimated using the model of Mejlholm and Dalgaard (2009) as implemented in Seafood Spoilage and Safety Predictor V. 3,1 assuming pH 7.0 and 0.5% salt in the aqueous phase in one case, and pH 6.2 and 3% salt (water activity ~0.983). The first case represents a food in which there are no constraints on *L. monocytogenes* growth other than temperature of storage. The second case is typical of the physico-chemical parameters of a range of RTEs of extended, refrigerated shelf-life, e.g., processed meats or cold-smoked salmon. In both cases the lag time was assumed to be zero to generate conservative estimates, and all other potential growth inhibitors in the model were set to zero. Analogous estimates were generated using Pathogen Modeling Program (V.6.1) and ComBase Predictor with the same assumptions concerning product formulation. The results are presented in Table 1, overleaf.

Table 1. Predicted times (days) for a 100-fold increase in concentration of *L. monocytogenes* derived from selected predictive models

	Time (d)* for 100-fold increase in *L. monocytogenes*			
	pH 7.0, 0.5% NaCl		pH 6.2, 3 % NaCl	
Temperature (°C)	**SSSP**	**PMP/ComBase**	**SSSP**	**PMP/ComBase**
4	8	3/5	10	4/6
5	6	3/4	8	4/5
6	5	2/3	6	3/4
8	2	2/2	4	2/3
10	1	1/2	3	1/2
15	< 1	<1/<1	2	<1/<1
25	Beyond model range	<<1/<<1	Beyond model range	<<1/<<1

* All estimates are rounded to the nearest day

From Table 1, it would appear that a shelf-life of from five to eight days at 4°C would be adequate to limit the growth of *L. monocytogenes* to 'tolerable' levels but, clearly, less time would be tolerable at higher temperatures.

Temperature abuse might result in a much *greater* risk, however, if the rate of growth of *L. monocytogenes* compared to the rate of spoilage is relatively faster at higher temperature. Thus, the question arises whether the product would normally become overtly spoiled prior to *L. monocytogenes,* if present, increasing 100-fold in numbers under all conditions, including overt temperature abuse. To put that into context, it is necessary to consider the growth rate of the SSO and the extent of growth of the SSO required on the product before spoilage is evident.

A product with a short shelf life at 4°C (e.g., a few days to a week) is, by inference, either very perishable or has a high level of spoilage microorganisms present before it is presented for sale. In the first case, such short shelf lives are likely to be characterized by high water activity, near neutral pH, freely available and readily assimilable nutrients, and aerobic storage such as might occur with fresh meat, or fish, or milk. In such products, spoilage is usually due to psychrotrophic pseudomonads, or similar, species. To put the *L. monocytogenes* growth rate predictions into context, the times to spoilage of an imaginary product were predicted using published predictive models. It was assumed that the product has a shelf life governed by the time taken for psychrotrophic pseudomonads to reach a level of 10^8 to 10^9 CFU.g^{-1}, corresponding to overt, severe, spoilage. It was further assumed that the product was of good initial quality and with a starting contamination level of 10^2 to 10^3 CFU.g^{-1}, i.e. that six ten-fold increases in pseudomonad levels would occur before spoilage was overt. For consistency with the predictions presented in Table 1, spoilage time estimates for two sets of product characteristics were calculated, *viz*. pH 7.0 and 0.5% salt in the aqueous phase in one case, and

pH 6.2 and 3% salt (water activity ~0.983) in the other.

The predicted times to overt spoilage are presented in Table 2, overleaf.

From comparison of Table 1 and Table 2 it is apparent that at temperatures that are higher than those typically recommended for perishable foods (e.g. 4 – 5°C), and particularly above 8°C, *L. monocytogenes* could grow to unacceptable levels before microbiological spoilage is evident. It should be noted however, that the above calculations are based on the food being based on materials of good microbiological quality. Some 'short' shelf life products (e.g., fish, crustacea in northern Europe) have short shelf lives because initial microbial counts are high. In these cases the product would be more likely to be overtly spoiled before *L. monocytogenes* levels became unacceptably high. Another exception might be products that lose quality due to non-microbial spoilage, e.g. the staling, or drying, of bread in sandwiches.

For completeness, an additional set of predictions was generated based on growth inhibition of *L. monocytogenes* by lactic acid bacteria, such as might occur in an MAP or vacuum-packed product. This suppression has been termed the "Jameson Effect "(*see* Ross *et al*., 2000). From the data in Table 3 and Table 1 it is predicted that *L. monocytogenes* could reach unacceptable levels before growth inhibition by lactic acid bacteria began to occur.

Table 2. Predicted times for product spoilage (assuming spoilage requires six ten-fold increases in concentration of aerobic psychrotrophic spoilage organisms) derived from selected predictive models

	Time (d)* for overt spoilage by psychrotrophic Pseudomonads based on predictions of two mathematical model			
	pH 7.0, 0.5% NaCl		pH 6.2, 3 % NaCl	
Temperature (°C)	**Neumeyer *et al*. (1997a)**	**"ComBase predictor"**	**Neumeyer *et al*. (1997a)**	**"ComBase predictor"**
4	5	6	6	7
5	4	5	5	6
6	3	5	4	5
8	3	4	3	4
10	2	3	3	3
15	1	2	2	2
25	1	Beyond model range	1	Beyond model range

* All estimates are rounded to the nearest day

Table 3. Predicted times for *L. monocytogenes* growth suppression (assuming suppression requires six ten-fold increases in concentration of psychrotrophic lactic acid bacteria) derived from selected predictive models

	Time (d)* for growth suppression (10^6-fold increase) by LAB			
	pH 7.0, 0.5% NaCl		pH 6.2, 3 % NaCl	
Temperature (°C)	***L. sake***	***L. curvatus***	***L. sake***	***L. curvatus***
4	9	14	12	17
5	8	11	11	13
6	7	8	9	11
8	5	6	7	7
10	4	4	6	5
15	3	2	4	3
25	1.5	1	2	1

* All estimates are rounded to the nearest day.

Stochastic Model

The processes described above for estimation of times within which no significant growth of *L. monocytogenes* occurs do not offer unequivocal support for decisions. In particular, an assumption was made that the median contamination level be used as the starting point for estimates.

As described in Appendix A, a stochastic simulation model was developed in Analytica® software (Analytica Release, 4.2.3.7, www.lumina.com). The model predicts the concentration of *L. monocytogenes* ($log_{10}CFU.g^{-1}$ or $log_{10}CFU.ml^{-1}$ or $log_{10}CFU.cm^{-2}$, etc) that might be expected after different time intervals in a food product in which temperature is the only limitation to growth of *L. monocytogenes*, i.e., the food's composition and/or packaging cause no other inhibition of growth. The model does not consider the effect of other microorganisms that may be present, whether to cause spoilage or to cause growth suppression.

The temperature of storage is based on both the expected distribution of storage temperatures in retail display (*see* Appendix A) and the expected distribution of temperatures in domestic refrigerators derived from a 2004-5 study in New Zealand (Gilbert *et al*., 2007). The relative time in retail or home storage is modeled as a random variable and the proportion of time in each is used to calculate a weighted average of the temperatures drawn from each of the respective distributions. The temperatures are translated into growth rate predictions using a simplified version of the model of Mejlholm and Dalgaard (2007), and taking into account bacterial lag times. Full details of the model structure and data inputs are given in Appendix A.

The model was used to estimate distributions of *increases* in $log_{10}CFU$(*L. monocytogenes)* (i.e., relative growth), or expected distributions of $log_{10}CFU$(*L. monocytogenes)* (i.e., absolute levels at the time of consumption). The latter are based on predicted growth from initial levels of contamination observed at retail derived from either USA data (Gombas *et al*., 2003) or UK data (Little *et al*., 2009).

Figure 1 is an output of the stochastic model. It shows expected relative growth, at different levels of confidence, for realistic temperatures of storage in New Zealand. The growth predictions shown assume no lag time.

The 'median' growth curve shown in Figure 1 (i.e. Probability= 0.50) is analogous to the predictions given in Table 1 for time to reach 2 log CFU increase, based on predictions of the SSSP model for a temperature of 5.5°C[5]. Given the assumptions, as in Table 1, a short shelf life might be defined as ≤ 5 days after first display at retail, assuming that the distribution of storage temperatures are as expected and modeled. Higher temperatures of storage would be expected to reduce the time taken for a 2 log CFU increase. Less than or equal to 5 days is the *median* expected duration. However, if a higher degree of confidence is required, i.e. that a lower proportion of the products would exceed a 2 log CFU increase, the acceptable shelf life would be shorter. Selecting a 95% confidence level (i.e., 95% of food contaminated with *L. monocytogenes*

[5] 5.5°C approximates the average temperature calculated in the scenario modeled for combinations of relative times in retail and home storage.

did not experience a >2 log CFU increase), for example, would require that 'short shelf life' be defined as ≤3 days (rounding to the nearest day).

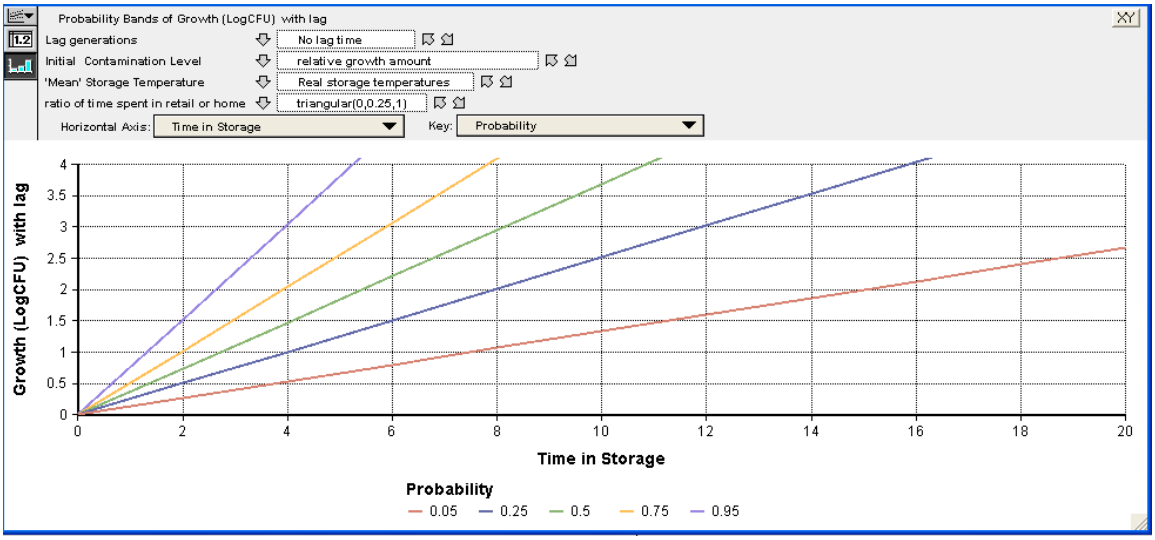

Figure 1. Baseline predictions of relative amount of growth (i.e., logCFU increase) of *L. monocytogenes* in highly perishable food stored under temperatures representative of New Zealand retail and home storage. The plot shows predictions for different levels of confidence, i.e., the proportion (='probability') of products that would be expected to have *L. monocytogenes* levels below the levels indicated after different durations in storage.

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In Figures 2 - 4, the influence of various different approaches/assumptions to estimation of a "short shelf life" for the purposes of management of *L. monocytogenes* are illustrated, where "short shelf life" is the time taken for products to achieve a 2 logCFU increase, or for initial contamination level to increase to 2 logCFU.

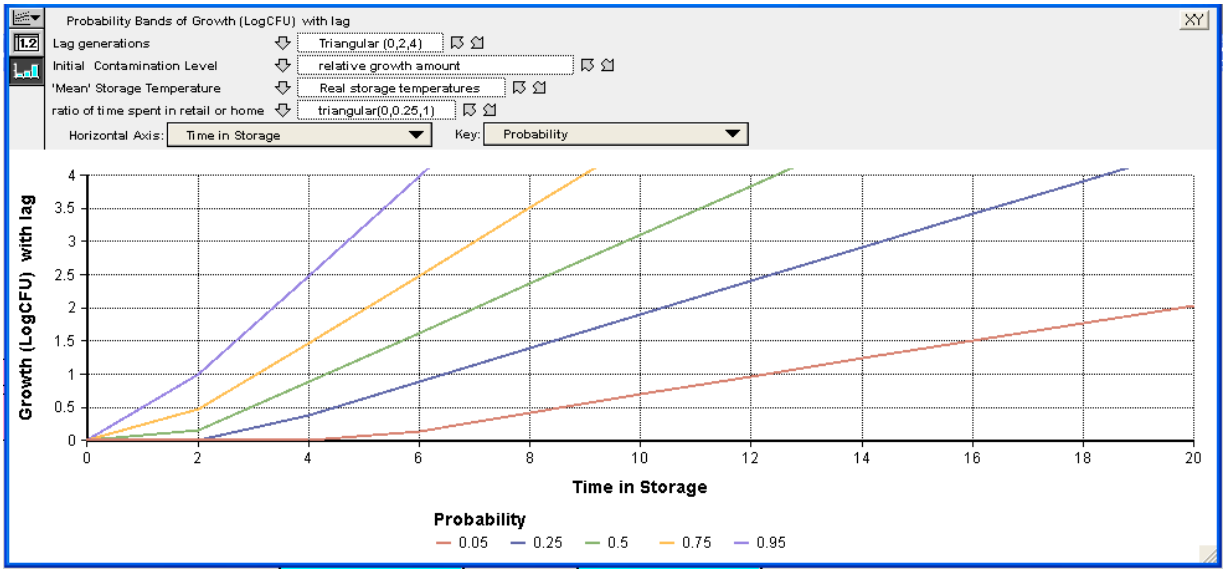


Figure 2. The effect of including consideration of probable lag times on the predictions of relative amount of growth (i.e., logCFU increase) of *L. monocytogenes* in highly perishable foods stored under temperatures representative of New Zealand retail and home storage (*see* Figure 1 for 'baseline" values, i.e. assuming no lag time). The plot shows predictions for different levels of confidence, i.e., the proportion (= 'probability') of products that would be expected to have *L. monocytogenes* levels below the levels indicated after different durations in storage. (Note also that 'bend' in the curve is an artifact of the plotting because values are generated at one day intervals only).

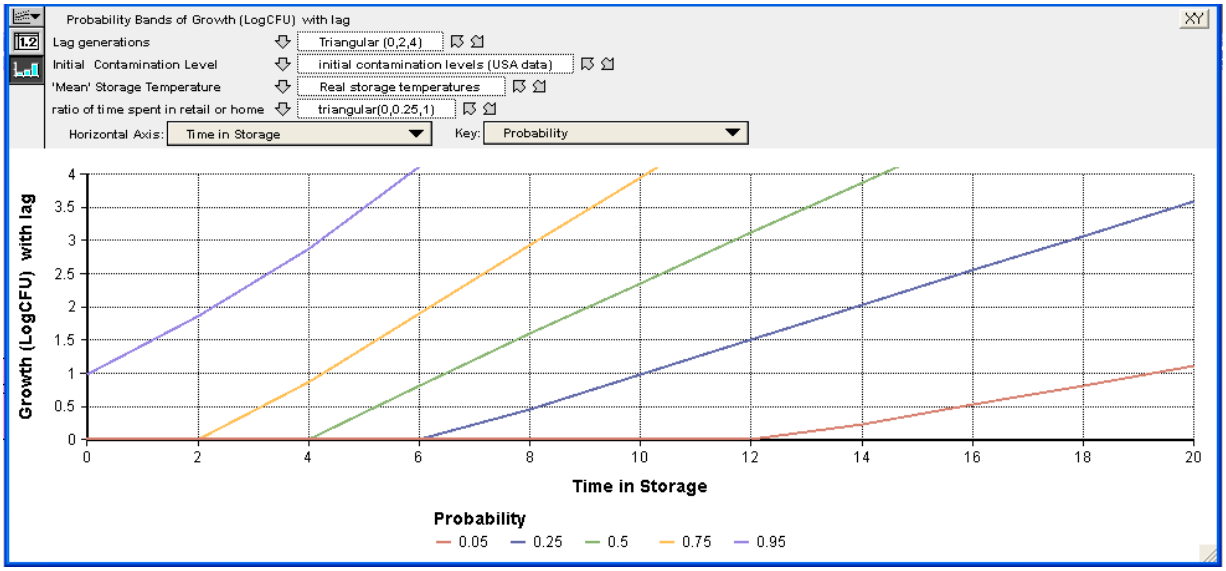
From Figure 2, above, it can be seen that consideration of lag times increases the estimate of "short shelf life" that can be tolerated, according to the criterion of time to a 2 log CFU increase in a highly perishable product. For the 50th percentile estimate (=median), the definition could be increased to ≤7 days, but at the 95th percentile, while the time is slightly longer, the definition, to the nearest whole day, would not change.

The effect of inclusion of realistic distributions of initial contamination at retail are illustrated In Figures 3a and 3b, overleaf. In these scenarios, the criterion is no longer 'time for 2 logCFU increase' but is based on predicted absolute contamination levels, i.e., time for 2 logCFU to be reached, based on the distribution of initial contamination levels, and time and temperature of storage. In Figures 3, the same lag time assumption as in Figure 2 is made.

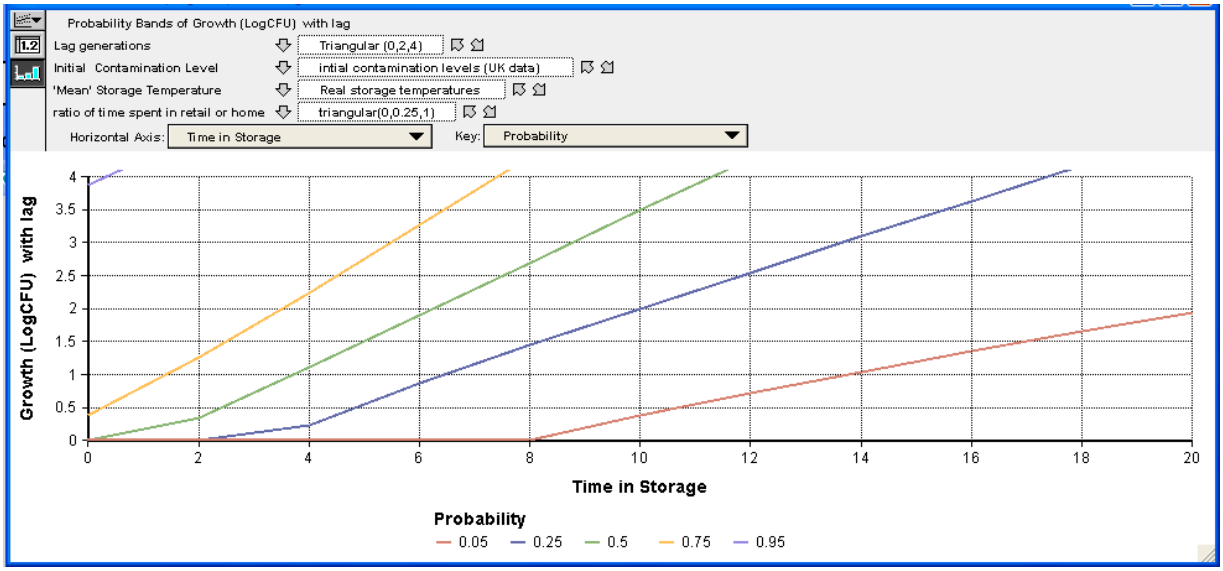
To illustrate the effect of the lag time assumption, Figure 4 shows the same scenario as Figure 3a, but with the assumption that there is no lag time.

Table 4 presents a summary of the storage periods under New Zealand conditions that might be interpreted to be acceptable definitions of "short shelf life" from the data in Figures 1 – 4.

a)



b)



Figures 3. The effect of survey data for initial contamination levels on the probability of *L. monocytogenes* loads on highly perishable foods after certain periods at temperatures representative of New Zealand retail display and home storage. Figure 3a) uses USA data for contamination levels with *L. monocytogenes* of a wide range of ready-to-eat foods purchased at retail (data of Gombas *et al.*, 2003). Figure 3b) uses UK data for contamination levels with *L. monocytogenes* of a wide range of ready-to-eat foods purchased at retail (data of Little *et al.*, 2009). Comparison of Figures 2 and 3a shows the effect of using real contamination data, describing a *distribution* of contamination levels, as opposed to selecting a single representative value. (Note also that in Figure 3b the 95th percentile value is so high, i.e., logCFU = 3.9 at time 0, that the growth curve barely appears on the Figure).

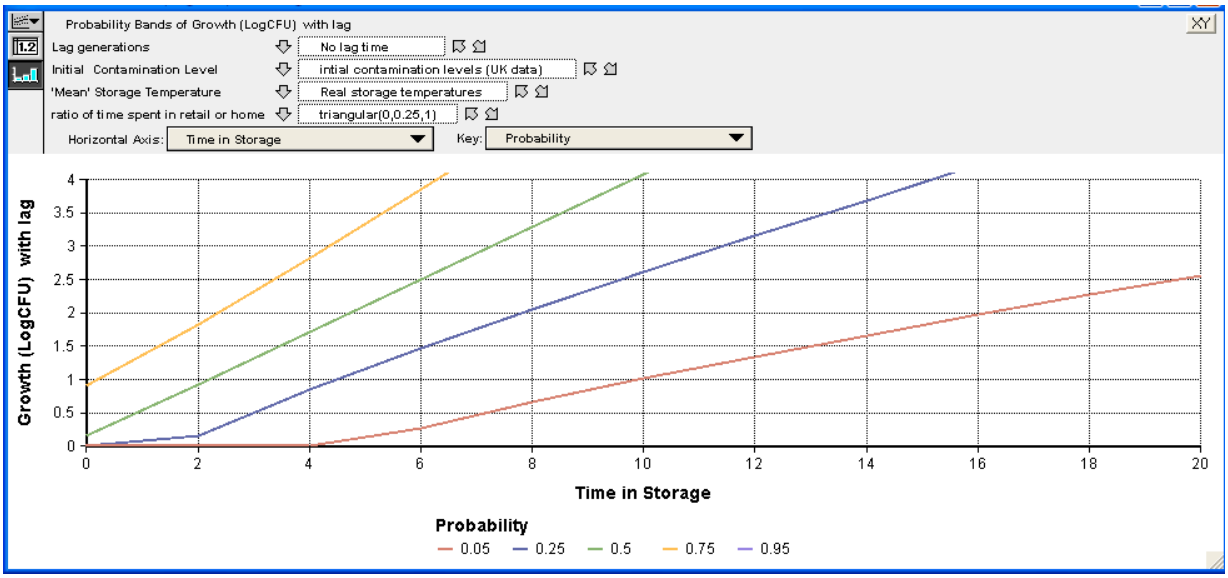


Figure 4. The effect of lag time assumptions on the probability of *L. monocytogenes* loads on highly perishable foods, after certain periods of storage, at temperatures representative of New Zealand retail display and home storage. Figure 4 shows predictions based on UK data for contamination levels at retail (Little *et al*., 2009) but assuming that there is no lag time. Hence, comparison of Figures 3b and 4 shows the effect of the lag time assumption combined with other assumptions/data. Note also that in Figure 4 the 95th percentile value is so high (logCFU = 4.5 at time 0) that the growth curve does not appear on the Figure.

Table 4. Predicted acceptable storage times at 'realistic' storage temperatures of RTE foods in New Zealand based on predicted growth of *L. monocytogenes* limited to ≤ 2 log CFU

Scenario	Time (days) for *L. monocytogenes* levels to reach 100CFU.g^{-1}at specified confidence	
	50% of samples below limit	95% of samples below limit
Figure 1 (no lag, time to 2 logCFU increase)	5	3
Figure 2 (lag time 0 – 4 days)	7	3
Figure 3a (USA initial contamination data, lag time 0 – 4 days)	9	2
Figure 3b (UK initial contamination data, lag time 0 – 4 days)	6	0
Figure 4 (USA initial contamination data, no lag time)	5	0

Discussion

From the analyses presented above it could be concluded that a "short shelf life", defined as from 3 to 8 days, and loosely consistent with regulations/policy existing in Europe and Canada, is credible *provided that reasonable temperature control is maintained* throughout the life of the product and that the product has low initial contamination levels (e.g., < 1-3 CFU.g^{-1}). These times correspond to potential increases in *Listeria monocytogenes* levels on the foods, given the stated conditions, such that they would likely remain below the 100 CFU.g^{-1} level agreed, in several international regulations or guidelines (e.g., EC, 2073/2005, CAC, 2007; CAC, 2007; USFDA, 2008), to represent a tolerable level of public health risk. The results rely on the assumption of low initial contamination levels and, while this appears to be valid in USA and UK, no data were presented for typical contamination levels in relevant products in New Zealand. This assumption should be tested.

A 'risk-averse' risk manager might reject these caveats, however, because they are not always satisfied, i.e., that the conditions of storage experienced by any particular unit of food are variable and uncertain, as are initial contamination levels. The upper time limits referred to above are based on average conditions: if a greater level of assurance is needed that concentrations of *L. monocytogenes* on products will not exceed 100 CFU.g^{-1} at the time of consumption, or if this level is deemed inappropriate, or if there is evidence of temperature abuse, or poor hygiene during processing, lower estimates of acceptable "short shelf life" would need to be selected. For this reason, the results presented in Figures 1 – 4 include consideration of the influence of variability and the uncertainty that temperature and initial contamination conditions would be satisfied, and could give rise to levels greater than 100 CFU.g^{-1} at the time of consumption. Table 4 summarises the results of the stochastic modeling and reinforces that, for average circumstances, a "short shelf life" consistent with an acceptable level of public health protection might be defined as from 5 – 9 days. Nonetheless, that definition would have to be markedly shorter if higher levels of confidence, accounting for a greater range of scenarios of product contamination and storage temperatures, were deemed necessary. In those examples, 95th percentile levels were from 0 to 3 days[6].

To illustrate this further, the scenario represented in Figure 3a was repeated, first with the "mean" temperature calculation increased by 1°C in all iterations of the simulation, then by 2°C in all iterations of the simulation, and then reduced by 1°C in all iterations. The results are summarized in Table 5, overleaf, and indicate the magnitude of the change, due to relatively small average temperature differences, that could be expected in the resulting tolerable shelf life with respect to potential for growth of *L. monocytogenes*. A 2°C increase in average temperature during the life of the product (i.e. from about 5.5°C to 7.5°C) would require a 30% reduction in the definition of "short shelf life" based on the median growth predictions. The effect is less pronounced, however, for higher percentiles levels.

[6] It should be noted that the lower limits of these estimates were strongly influenced by the relatively high proportion of samples contaminated at >100 CFU.g^{-1} in the UK survey, i.e., > 5% of contaminated samples at retail exceeded the 100 CFU.g^{-1} threshold before any growth was modeled.

It is clear that the required level of confidence (i.e., proportion of foods required not to exceed the microbiological criterion) will influence an appropriate definition of "short shelf life" for the purposes of risk management of *L. monocytogenes* in such RTE foods. Similarly, the microbiological criterion chosen (e.g., 100 CFU.g^{-1} at point of consumption, or 2 log CFU increase above initial contamination level, or 1 log increase in initial contamination level) will affect the time between production and consumption that could be considered "tolerable" in terms of risk of listeriosis, in turn influencing the definition of 'short' shelf life.

Table 5. Effects of small changes in temperature on predicted time to 2 log CFU.g^{-1}, based on USA contamination levels at retail

Temperature shift over entire storage period (°C)	Time (days) for *L. monocytogenes* levels to reach 100CFU.g^{-1}at specified confidence	
	50% of samples below limit	95% of samples below limit
-1	11	3
0	9	2
1	7	2
2	6	1

Dr. Jeffrey Farber of Health Canada (*pers. comm.*, 2010) explained that, in deliberations involved in proposing a 'short shelf life' definition for the draft Canadian policy, predictive microbiology was used to estimate an appropriate duration. The Pathogen Modeling Program and ComBase were used to predict the extent of growth at 4, 5 and 6°C in an *an*aerobic environment at pH 6.0 with water activity of 0.985. Those conditions inhibit growth more than the product formulation assumed in the stochastic model (*see* Table 1), and would be expected to result in a longer estimated tolerable shelf life with regard to *L. monocytogenes* risk management. This suggests that the results of the stochastic model are more conservative, even using the median values of the predicted distributions, than those used by Health Canada. Health Canada also assumed growth starting from 1 CFU.g^{-1}, analogous to the scenario summarized in Figure 1.

In interpreting these analyses it should also be noted that the results of Gombas *et al*. (2003) and Little *et al*. (2009) indicate approximately 98% of ready-to-eat foods are *not* contaminated with *L. monocytogenes*. Taking this into account, the 50th percentile predictions for contaminated samples (*see* Table 4) represent approximately the 99th percentile for all ready-to-eat foods, and the 95th percentile values for contaminated samples represent ~99.9th percentiles when applied to all ready-to-eat foods.

A potential criticism of the approach presented here is that contamination levels used were taken from a wide range of products at retail including those, such as processed meats, with long shelf lives (e.g., weeks to months). As such, it is possible that the average contamination levels used are *lower* than what might be observed in short shelf life products where microbial growth might be expected to be faster. To attempt to evaluate the validity of this criticism, the data of

Little *et al.* (2009) were examined in more detail. That survey included prepared sandwiches, which would be expected to have a short shelf life (e.g., a few days). If contamination levels in sandwiches at retail were higher than other RTE foods examined, the results of the stochastic modeling based on average contamination levels derived from all RTE foods could *over*estimate the extent of growth that could be tolerated before the 100 CFU.g^{-1} limit were exceeded leading to *over*prediction of the tolerable "short shelf life". While the Little *et al*. (2009) data are rather 'coarse' (i.e., few distinct categories of contamination level) it is apparent that the contamination *prevalence* is higher (~4 – 6%) in sandwiches than the other categories of RTE foods that they surveyed. This may be a consequence of the number of ingredients and the extent of manual handling expected to be associated with these products and which might increase the incidence of cross-contamination. However, the distribution of contamination levels is shifted "to the left", indicating *lower* contamination levels than other kinds of RTE foods considered in the Little *et al.* (2009) study. This could derive from the short shelf life of the product and may suggest, in fact, that the contamination distributions used in the stochastic model are biased towards unrealistically *high* contamination levels because the datasets used contained data from 'old' products of long shelf life. For example, Little *et al*. (2009) included a large number of processed meat samples that were at the end of their shelf life and which could systematically bias the data toward higher-than-representative contamination levels. Similarly, the data in the USA and UK surveys were taken at any point during the products shelf life but, in the modeling, the levels are assumed to represent those at the moment that the product is made available for retail sale. The consequence is that the model, and results based on it, could be biased towards lower estimates of overall tolerable growth than actually occurs and, thus, *under*predict tolerable "short shelf life". This potential problem will not apply when using the calculation of relative growth (i.e., 0 log CFU as initial contamination level).

Conversely, several studies have shown that the apparent proportion of contaminated samples can increase slowly over storage time (Jorgensen and Huss, 1997; Little *et al.*, 2009) not due to increased prevalence of contamination *per se* but because, as *L. monocytogenes* grows in foods, the probability of detection in the food increases. The apparent increase in prevalence is, however, minor.

Whilst this analysis has explored factors to consider when estimating whether a food can support growth of *L. monocytogenes* to unacceptable levels within a relatively short shelf life, it clearly cannot unequivocally nominate an appropriate definition of "short shelf life". This will require a risk management decision concerning the proportion of products that, at the time of consumption, should satisfy the microbiological criterion. Inevitably, determining that level of confidence will be a somewhat subjective decision. This report has attempted to provide information and analyses that will help to advise that decision by providing quantitative estimates of the potential for growth of *L. monocytogenes* under various sets of assumptions and different scenarios relevant to the question.

In deciding the required level of confidence, it may be helpful to a revisit the reasons for attempting to define "short shelf life". In terms of food safety risk, there now appears to be reasonable consensus internationally that *L. monocytogenes* in foods at ≤ 100 CFU.g^{-1} provides an appropriate level of public health protection. In foods that do not support the growth of

L. monocytogenes, this criterion can be readily assessed, but the issue is more difficult when growth is possible, and for many years many nations adopted a 'zero tolerance' policy for all foods that support growth of *L. monocytogenes*. Recent international regulations and guidelines (EC 2073/2005; EC 1441/2007; CAC, 2007) provide flexibility for risk management of foods that support only limited growth of *L. monocytogenes* ***within their nominal shelf life*** and consider that if growth is less than 0.5 log $CFU.g^{-1}$ over the shelf life of the product and within "***reasonably foreseeable conditions of distribution, storage and use***", levels of up to $100CFU.g^{-1}$ are tolerable. In both the EU legislation and Codex guidelines, either predictive microbiology or challenge studies are nominated as acceptable means to demonstrate that these criteria are satisfied for a given product. Thus, in the EU regulations, it seems that definition of a short shelf life product is redundant because the criteria for acceptability encompass foods *of any shelf life* and that equating short shelf life foods with no-growth foods (e.g. pH <4.4, a_w <0.92 etc) seems incongruous. However, EC 1441/2007 (footnote 8) indicates that the same exemption can be applied to other foods "subject to scientific justification". Thus, the exemption for short shelf life food can be seen a pre-emptive use of "scientific justification" for this category of product. However, as shown in this report, if initial contamination levels at retail are high, but "acceptable" (e.g., 100 $CFU.g^{-1}$) they could increase within 5 days to levels as high as 10,000 $CFU.g^{-1}$, which would clearly be considered unacceptable. This seems to be acknowledged by EFSA/BH (2007) who observed that: "*The criteria of below 100 cfu/g during shelf life will likely be applied during retail. For foods supporting growth of* L. monocytogenes*, depending on the conditions between retail and consumption, the limit of 100 cfu/g might be exceeded at consumption."* This reinforces the emphasis in EFSA/BH (2007) on the need for GMP and HACCP, i.e. to limit the risk by limiting initial contamination that might be present on growth-permissive products.

Draft Canadian policy (Health Canada, 2010) offers a similar basis for exemption, but more overtly identifies risk management prioritization as the reason for the distinction. In reference to short shelf life foods, included in Category 2A, (i.e., those foods that offer limited potential for *L. monocytogenes* growth), it is stated: "*Notwithstanding that these foods can support the growth of* L. monocytogenes*, the growth is generally limited because of a number of factors such as short refrigerated shelf-life, a large background microflora containing anti-Listeria lactic acid and/or other bacteria, etc. These Category 2A foods should receive a medium-low priority, with regards to the level of inspection and compliance activity*". Importantly, the draft policy also explicitly identifies that the relatively lower attention to short shelf life foods is predicated on the assumption of good hygienic practice. The draft policy adds: "*For these foods, operators would need to validate that the levels of* L. monocytogenes *are consistently equal to or less than 100 CFU/g during the whole shelf-life of these products*".

Finally, in the preceding discussion the focus has been on the potential growth, and attendant public health risk, of *L. monocytogenes* in foods of short shelf life that support its growth. As discussed in the "Results" section, the overall safety of the approaches described rely on the consumer discarding product that has exceeded its "use-by" date. It is beyond the scope of this report to assess the validity of that assumption, but it is important to note that it *is* an assumption. If unfounded or suspected to be unfounded, the consequences of that assumption must be considered in the management of risk of "short shelf life" foods and interpretation of the

results and discussion above. It could be anticipated that the longer the relative time difference between the "use-by" date and onset of overt spoilage, the greater the risk to consumers. It could also be anticipated that if the declaration of a shelf-life of <5 days, for example, meant lower levels of regulatory scrutiny and lower frequency of product testing, many manufacturers might choose to adopt a nominal shelf life compliant with that criterion, even if their product had a greater shelf life. This response, if it occurred, would exacerbate the problem of differences between nominal "use-by" and actual time of overt product spoilage by allowing greater than anticipated growth of *L. monocytogenes* on the product before consumption.

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Appendix A. Description of the stochastic simulation model.

Overview

A stochastic simulation model was developed in Analytica® software (Release 4.2.3.7, www.lumina.com). The model predicts the concentration of *L. monocytogenes* ($log_{10}CFU.g^{-1}$ or $log_{10}CFU.ml^{-1}$ or $log_{10}CFU.cm^{-2}$, etc) that might be expected after different time intervals in a food product in which temperature is the only limitation to growth of *L. monocytogenes*, i.e. the food's composition and/or packaging cause no other inhibition of growth.

The temperature of storage is based on both the expected distribution of storage temperatures in retail display and the expected distribution of temperatures in domestic refrigerators. The relative time in retail or home storage is modeled as a random variable and the proportion of time in each used to calculate a weighted average of the temperatures drawn from each of the respective distributions. The temperatures are translated into growth rate predictions using a simplified version of the model of Mejlholm and Dalgaard (2007).

The model can be used to estimate distributions of *increases* in $log_{10}CFU$(*L. monocytogenes).*g^{-1} (i.e., relative growth), or expected distributions of $log_{10}CFU$(*L. monocytogenes).*g^{-1} (i.e. levels at the time of consumption) based on expected levels of contamination at retail. The distribution of contamination levels at retail is based on published studies involving very large numbers of samples. The model can also accommodate different assumptions regarding lag times of *L. monocytogenes* on the product.

Model Structure

Figure A1 gives an overview of the structure of the model. Each shape or 'cell' in the model contains, or produces, data needed for estimation of growth amount after different periods of time. Pale blue cells contain data; dark blue cells contain formulae that use those data to generate new results. Pink cells contain distributions used to generate other variables in the

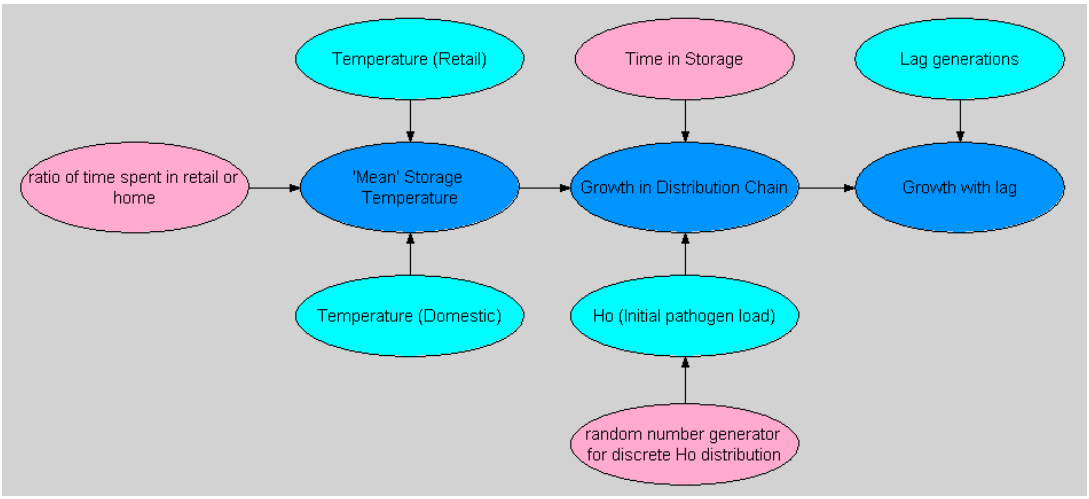

Figure A1. Influence diagram of the model used for estimation of maximal growth of *Listeria monocytogenes*. (*See text for explanation*).

model, as discussed below. Arrows indicate that the data from one cell (*origin* of the arrow) is used to calculate the result in the cell *receiving* the arrow, i.e. that the first cell influences the value in the second cell.

Variables in the model

The following describes the information/data manipulation in each cell in the model.

"ratio of time spent in retail or home"

To simplify the modeling (i.e., rather than modeling growth in retail storage and growth in domestic storage separately and having to correlate time in each) the ratio of times in each situation is modeled and used to generate a representative temperature of storage for that iteration of the model. (The calculation of that temperature is described below).

In the current model, two alternatives are used to model this ratio. The first is a "uniform" distribution between 0 and 1, inferring that the food is purchased with equal probability at any time within its nominal shelf life, and then stored for a further period in the consumer's home. The value is used to simplify the calculation of an overall (weighted average) temperature for the period of storage. The uniform distribution is used because it involves the fewest assumption about the relative amount of time a product would experience under retail *vs*. domestic refrigeration conditions. The food is more likely to be purchased early in its period of retail display and that the probability of purchase later declines in direct proportion to the time since first display. This is modeled by a Triangular (0,0,1) distribution (*n.b.,* a simulation to compare the influence of a "uniform(0,1)" distribution *vs.* a triangular(0,0,1)" distribution indicated either assumption leads to < 10% difference in the time estimated to reach any specified level of growth, at the 95th percentile level).

"Temperature (retail)"

This models distribution of temperatures in retail storage. Many published reports of retail refrigeration temperatures point to both temporal and spatial variability in temperatures in the chilled display cabinets. Most studies report that average temperatures are higher than the recommended storage temperatures. Kotsoumanis *et al.* (2010) undertook a survey of retail chiller temperatures in which milk was stored but also provided a summary of other such surveys in Europe. In Greece, the mean storage temperature was 4.98°C (SD = 2.90°C). Pierre (1996; cited in Kotsoumanis *et al.*, 2010) reported a mean temperature of 4°C in France, while Likar and Jevsnik (1996; *cited in* Kotsoumanis *et al.*, 2010) reported average temperatures of 4.6°C in Slovenia. Based on data reported by Microtech (1998) and Alliance (1998), Ross *et al*.

(2004) calculated that the mean temperature in four types of retail display cabinets in Australia was 6.0°C. Possibly the most extensive data set is that from USA developed by Audits International (1999) in collaboration with the US Food and Drug Administration. That survey included data from ~900 food retails premises throughout USA, and temperature distributions for chilled products from that survey are summarized in Table A1.

Table A1. Analysis of retail temperatures of refrigerated ready-to-eat foods in USA (1999 data)

Type of Product	Mean temperature (°C)	Standard Deviation (°C)	Number of observations
pre-packaged lunch meat product	6.46	3.41	954
"deli-counter" product	7.14	3.29	905
"liquid dairy product"	4.95	2.81	955
semi-solid dairy product	4.85	4.83	277
pre-packaged deli product	5.72	3.08	921
total number of observations			4012
weighted mean temperature	5.97		
average SD		3.39	

It is perhaps noteworthy that the survey also recorded temperature data for semi-solid dairy product in a "backroom refrigerator". The average temperature of those 954 observations was 3.25°C (SD 2.94°C), supporting that suggestion in other reports that the problem lies with the design of the retail display cabinets rather than lack of commitment or knowledge on the part of store managers and staff.

On the basis of the above, the temperature in retail display used in the model is based on the American and Australian data, on the assumption that New Zealand retail systems and practices are more likely to be similar to them, than to Greece or Slovenia. The data is described by a normal distribution with mean 5.97°C and SD 3.39°C. It is noted, however, that both data sets used are at least 10 years old and that improvement in refrigeration technology is likely to have occurred. More recently, Pointon *et al*. (2009) reported that the average temperatures of poultry meats surveyed in South Australia in 2005/2006 (n= 155) was 3.8°C in butcher shops and 2.0°C in supermarkets.

The distribution was also modified slightly to limit temperature to be in the range 0 to 25°C, thus implemented as "truncate(normal(5.97, 3.39),0, 25).

"Temperature (domestic)"

This distribution is used to model the distribution of temperatures in retail storage. The default values in the model are "normal(5.2, 2.5)" which is based on a 2004-2005 study of New Zealand domestic refrigerators (Gilbert *et al*., 2007). The distribution was modified slightly to limit temperature to be in the range 0 to 25°C, implemented as "truncate(normal(5.2, 2.5),0, 25).

"'Mean" Storage Temperature"

Based on the ratio of times in retail and domestic storage, a representative temperature of storage for each iteration of the model is calculated using the following equation:

'Mean' Storage Temperature (°C) = "ratio of time spent in retail or home" x "Temperature (Retail)" + (1-"ratio of time spent in retail or home" x "Temperature (Domestic)"

i.e. 'Mean' Storage Temperature is the mean of the two temperatures weighted according to relative amount of time in each storage situation (retail, or domestic).

"Time in Storage"

This set of values is used as a device to generate a graph of growth as a function of time. The structure of the model/software does not allow the predicted growth to be shown as a function of time unless multiple discrete values are included in the variable definition. (Note that, when graphed, the values for time are labels, not numbers. As such, the time values selected must be at equal intervals to generate a graph that can be interpreted as 'growth over time, i.e., as a growth curve).

"Initial Contamination Level"

The values in this variable are used to specify the initial concentration (Log_{10}CFU) of *L. monocytogenes* on the food. In the current model, two options are provided.

The first is simply "0". In that case the output of the model is interpreted as the predicted increase in log_{10}CFU, whatever the initial level, as a function of time and temperature.

The second is a choice of distributions of actual concentrations reported in two large surveys of contamination levels at retail, namely Little *et al*. (2009) for ready to eat foods in the United Kingdom, and Gombas *et al*. (2003) for ready to eat foods sampled at retail in USA. Those surveys generated 'categories' of contamination levels, e.g. *L. monocytogenes* detected in 25 g,

but $<10^{-1}$, $>10^{-1}$ but<100 g^{-1}, etc. As such, the distribution is described as a series of ranges of values corresponding to the categories given. In the stochastic model the probability of a value being selected from each of those categories is based on proportions reported, e.g., in the case of Little *et al.* (2009) three ranges of contamination level and the percentage of contaminated units in each of those categories reported. Thus, that distribution can be described by the mathematical function such that in 77% of the iterations of the model a concentration in the range 1/25g to 1 per gram is selected at random, in 9.75% of iterations a value in the range 10 to 100 CFU.g^{-1} is selected at random and in the remaining cases a contamination level in the range 100 to 1 million CFU.g^{-1} is selected at random. The upper limit on the range is derived from study of Gombas *et al.* (2003), which reported contamination at this level in some samples. A similar approach was adopted to describe the observations of Gombas *et al.* (2003). The predicted distributions of contamination levels in contaminated samples for the two data sets are shown in Figure A2.

In the model, only contaminated samples are modeled, so that it is also necessary to consider the proportion of samples that have detectable contamination in 25g. In the USA study involving

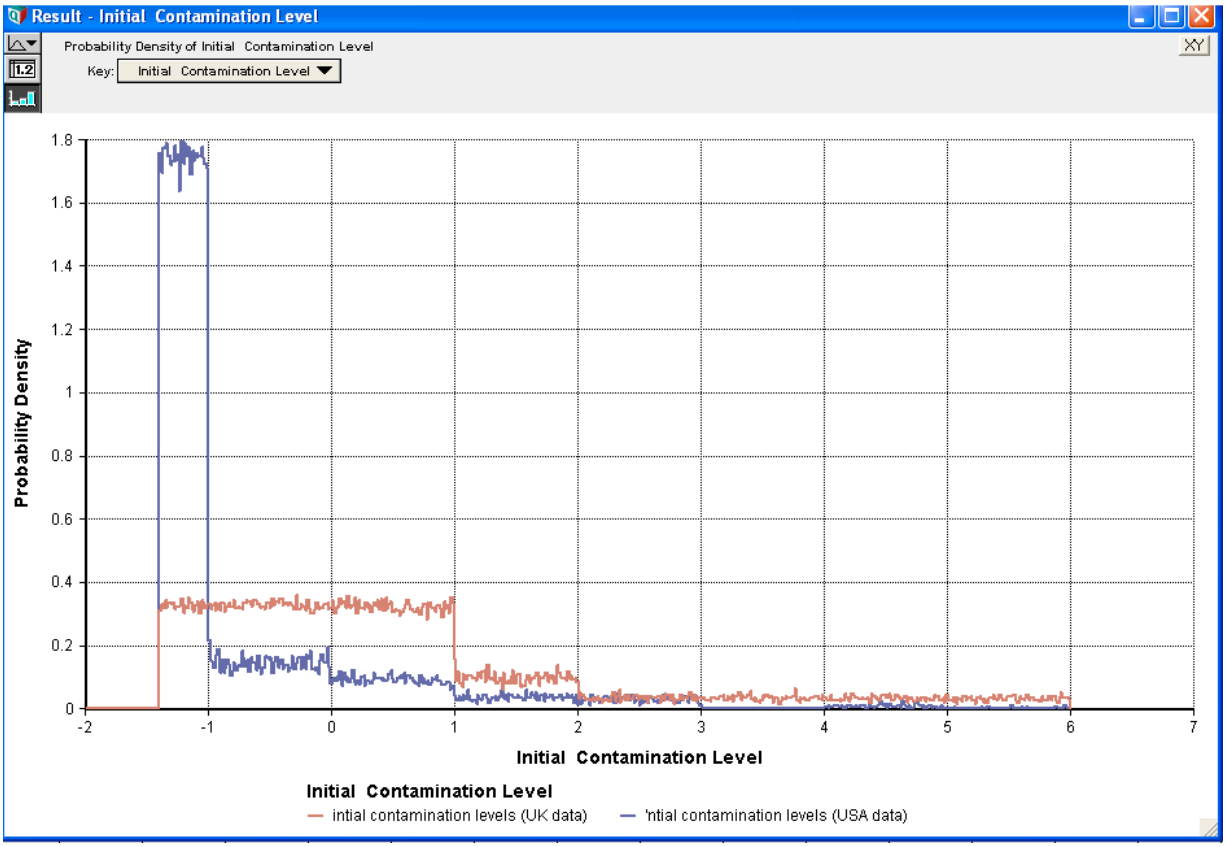

Figure A2. Modelled distributions of *L. monocytogenes* contamination levels (LogCFU. g^{-1}) in contaminated ready-to-eat foods sampled at retail. The results are descriptions of the observations of Little *et al.* (2009; labeled "UK data") and Gombas *et al.* (2003; labeled "USA data").

31705 samples, 1.8% of samples were found to have *L. monocytogenes* at levels of at least 1/25g. In the UK study, 2.4% of 6818 samples were found to contain *L. monocytogenes* at levels of at least 1/25g. Importantly, however, the UK study reported much higher average levels of contamination in the contaminated products, *viz*. in the UK study not only were more samples found to be contaminated, b*ut more contaminated samples were reported to have higher contamination levels.* This is apparent in Figure 2A, above.

"Growth in Distribution Chain"

In this cell, the predicted growth is calculated for each of the time periods specified in "Time in Storage". The calculation for each time is based on the 'mean' storage temperature and the initial contamination level options. The model used to predict the extent of growth is a simplified form of the Mejlholm and Dalgaard (2007) model. The simplification arises from using the model only to estimate the growth rate at each temperature, assuming optimal level of all other factors in the model[7]. Predictions of the maximum specific growth rate from the model of Mejlholm and Dalgaard (2007) model as implemented in the SSSP (V3.1) software package (*download from*: sssp.dtuaqua.dk/) were generated and refitted. The resultant growth rate model is:

$$\mu_{max}\ (h^{-1}) = 0.0228 \times (\text{temperature } (°C) + 2.83))^2 \qquad (R^2 = 1.000)$$

To calculate growth amount (logCFU) the following equation is used:

$$\text{Growth } (\log_{10}\text{CFU}) = 0.4343 \times \mu_{max}\ (h^{-1}) \times \text{time elapsed (h)}$$

"Lag Generations"

Bacterial cells transferred to a new environment, e.g., from a relatively hostile environment in a food processing plant to a food product ,often experience a delay before commencing growth. This is referred to as the 'lag time'. The lag time is variable but responds to temperature quantitatively in the same manner as generation time responds to temperature (Robinson *et al.*, 1998; Mellefont *et al*., 2003), i.e., if the lag time at one temperature is '*x*' hours, and changing the temperature increases the generation time by a factor of two, the lag time duration will also increase by the same relative amount. Thus, the lag time can be relatively easily included in predictions of bacterial growth by deducting a number of generations of growth equivalent to the duration of the lag time at some known temperature. This translation of lag time in to equivalent generations of growth is termed the 'relative lag time' (RLT).

Ross (1999) presented an analysis of literature data that suggested that bacterial lag times are typically in the range of 2 – 6 generations times, with a mean value equivalent to approximately four generation times.

The effect of lag time on predicted growth is included in the stochastic model, with three options given:

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[7] The values for variables in the model were 0.3% aqueous phase salt, pH 7.0, and all other variables values set to 0.

i). no lag time (used for determination of 'worst case' estimates)
ii). some lag consumed between processing and retail
iii). lag time distribution based on the analysis of Ross (1999).

Growth calculations in the model are all in units of log(CFU). Accordingly, the above 'generation time equivalents' are converted into equivalent amount of logCFU growth (1 log = 3.322 doublings, or generations of growth), so that the actual values in the model are:

i). 0
ii). Triangular (0, 0.602, 1.204)
iii). Triangular (0.602, 1.204, 1.806)

In option ii), the selection of two generation time equivalents (RLT =2, = 0.601 logCFU) of growth being "used up" as lag time between time of production and time first on retail display is based on the following assumptions:

i). that the fastest growth rate at 4°C, as indicated in Table 1 (n.b., 4°C was chosen on the assumption that the temperature control during processing and warehousing etc, is better than in retail display or the home - see discussion above under "Temperature (retail)")
ii). that two days elapse between production of the food and its first appearance in retail display and availability for purchase by domestic consumers. (n.b., the time is an assumption included for comparative purposes and can be readily altered if more information becomes available, or there is a desire to investigate other scenarios.

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