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## Australian Risk Assessment model for *Listeria monocytogenes* in ready-to-eat meats

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### Introduction

As in other nations, Australian-produced smallgoods (processed meats, “deli” meats) are occasionally contaminated with *Listeria monocytogenes* which, with the exception of most fermented meats and many prosciutto-type products, can increase in number on the product during storage and distribution.

The stochastic simulation model described here (and available for download) was created as part of a study to assess the public health risk due to *Listeria monocytogenes* in Australian made processed meat products. It was jointly developed by Aamir Fazil, Greg Paoli, Tom Ross and Sven Rasmussen in @Risk software, which operates as an “add-in” to Microsoft® Excel. The model is a series of linked spreadsheets involving data and calculations. The model can be described as a process risk model (Cassin *et al.*, 1998) but also has ‘modular’ elements as suggested by Nauta (2002).

The risk manager requested that the model provide decision support to: to:

- i) characterise the nature and size of the microbial food safety risk due to *Listeria* in processed red meat products including comparison with that from other food-borne pathogens;
- ii) identify where critical data and/or knowledge, necessary to characterise that risk, are lacking;
- iii) characterise factors that contribute most significantly to the risk; and

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- iv) assess the effectiveness of potential management strategies to reduce the microbial food safety risk.

The full risk assessment document (249 pp, inc. 12 Appendices) is available upon request from:

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The report provides full details of the model's development, approaches used (including development of novel predictive microbiology models), data sources and analyses used, and results. Aspects of the model and the results have been submitted for publication in the international refereed literature at the time of writing (December, 2008).

### **Model Overview**

In summary, the model predicts the concentrations of *L. monocytogenes* on products at the time of consumption using industry and other survey data augmented by predictive microbiology models. Variables in the model are based on an extensive database of relevant information about Australian processed meat product formulations, levels and frequencies of product contamination with *L. monocytogenes* and other microorganisms, and handling times and temperatures prior to consumption. Using a dose-response model presented in FAO/WHO (2004), and meal size information, the levels of *L. monocytogenes* are 'translated' into a *per serving* risk. From sales and production volume data, and consumption statistics, a number of metrics of total Australian listeriosis risk from processed meats are calculated. The model starts with smallgoods at the plant level after processing and packaging. A schematic overview of the model is shown in Figure 1, which indicates the interplay of factors that may affect the rate and amount of growth of *L. monocytogenes* in processed meats. Included are abiotic factors that will affect the growth rate of both *L. monocytogenes* and spoilage or other microbiota. Those other microbes can, in turn, affect the growth of *L. monocytogenes* a phenomenon termed the Jameson Effect (Stephens *et al.*, 1997; Ross *et al.*, 2000).

The model distinguishes three types of processed meat products: lunch meats, pâtés/liverwursts and cooked sausages. These products were selected on the basis of significant differences in

their formulation, nominal shelf life (“use-by” date), and/or initial contamination levels with *L. monocytogenes* or background microbiota. While more categories of smallgoods could have been modelled, the potential benefits of disaggregation of data (i.e., that important differences in risk between the categories would be able to be distinguished) were considered to be outweighed by insufficient relevant data for formulation, consumption, shelf lives for those product categories etc.

Growth of *L. monocytogenes* on the product prior to consumption is modelled as the sum of growth predicted to occur in different stages of the production to consumption pathway. These stages were able to be modelled discretely because time and temperature data were available to support this disaggregation. This allowed the contribution to risk of each of these stages to be compared. An additional contamination event can be modelled for products that are sliced at retail, prior to sale to the consumer.

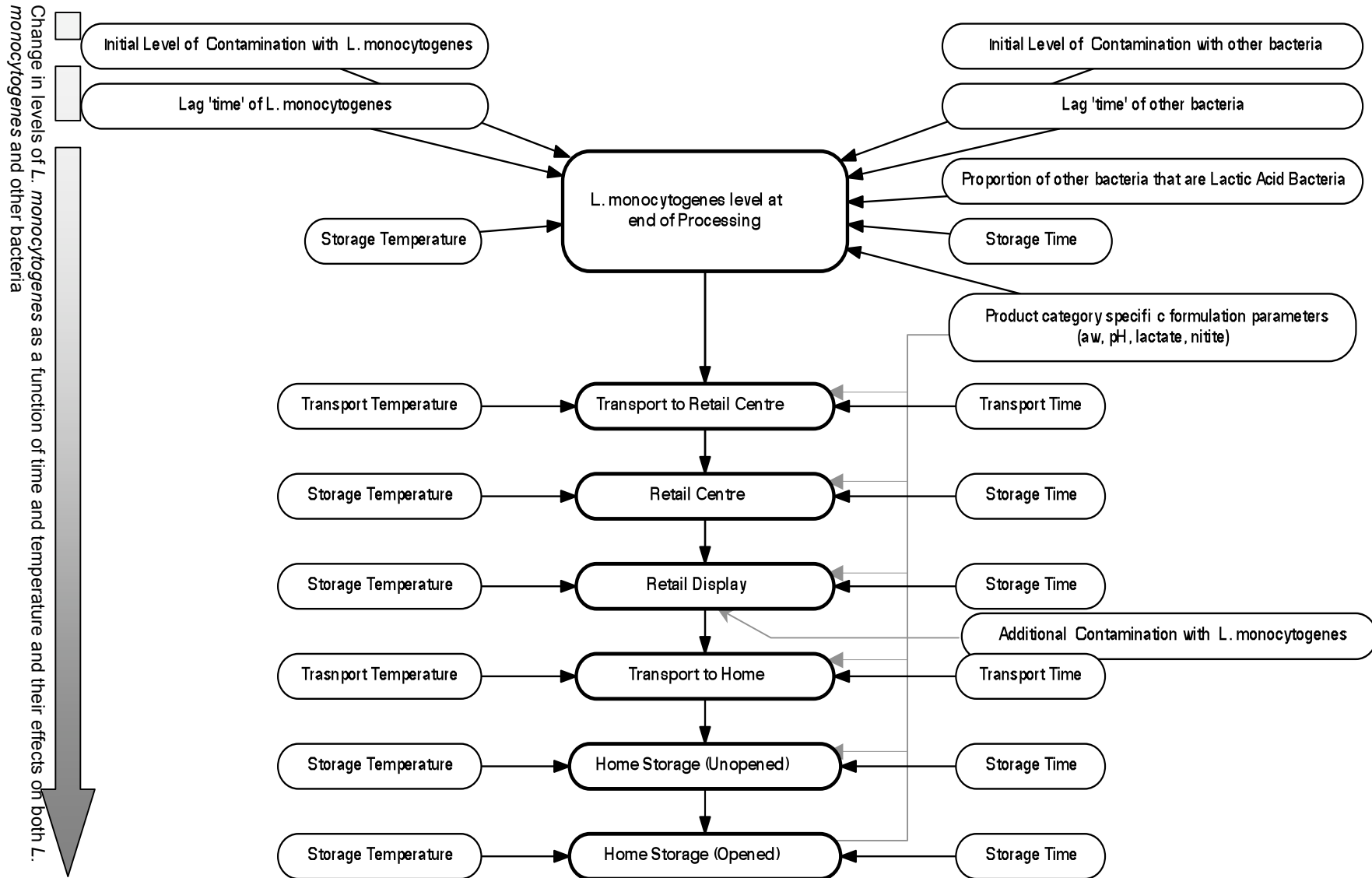


Figure 1 Overview of the stages modelled between processor and consumer.

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## Model Execution

### Modelling *L. monocytogenes* levels at the time of consumption

In each iteration of the model one serving of one type of product is considered. Thus, the first step in the simulation is the selection (by the user of the model) of a product category. The categories are “Luncheon meats”, “Pâtés/Liverwursts” and “Sausages”. Only vacuum-packed (VP), or in modified-atmosphere packaged (MAP) products are considered. These represent almost the entire production of processed meats in Australia. Based on the choice of product type, product formulation and storage and distribution characteristics appropriate to that product type are sampled from the distributions in the model. After the initial product category selection and characterization, the model performs calculations in seven stages:

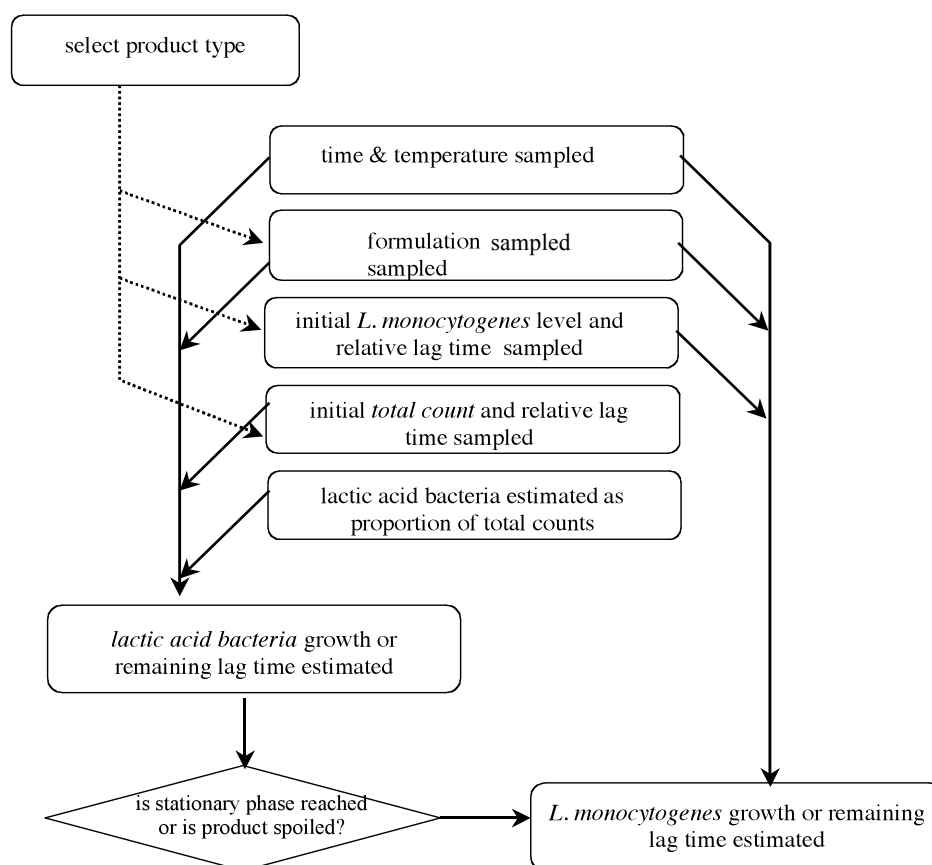
- Storage at the processing plant
- Transport: Processor to Retail Distribution Centre
- Storage at Retail Distribution Centre
- Retail Display
- Transport: Retail - Consumer
- Storage by Consumer (Package unopened)
- Consumer by Consumer after Package opened

At each of these stages the growth rate for both lactic acid bacteria (LAB) and *L. monocytogenes* given by the sampled product characteristics and the temperature is estimated from predictive models as described below. Based on the calculated growth rate and the time spent at that stage, resolution (i.e., completion) of the lag phase is assessed. Consequently, the number of generations of growth predicted to have been possible (based on the growth rate and the time expired) is subtracted from the ‘relative lag time’ (RLT). When the cumulative potential generations are in excess of the RLT generations, population growth is modelled to proceed. Use of relative lag time simplifies the process of calculating the resolution of lag time under changing temperature conditions (*see* Ross and McMeekin, 2003).

Predicted growth during each stage is calculated based on the time remaining at the stage after the lag phase is completed, with the growth calculated by multiplying the growth rate by the time remaining after the resolution of lag, if it occurred in that stage. At the subsequent stage a check is performed to determine if bacteria were growing at the preceding stage (i.e., whether lag was

resolved). If so, growth is modelled to continue at this next stage i.e. the growth rate at the new conditions is calculated and multiplied by the time spent at the stage. If the lag time is not completed at the previous stage the lag time continues to be “depleted” (potential generations continue to be subtracted from the RLT generations). These steps are performed independently for both spoilage bacteria (*see below*) and *L. monocytogenes*. Thus, spoilage bacteria may be modelled to have begun growing while the *L. monocytogenes* remain in the lag phase, or *vice versa*.

The shelf life of the product under ideal conditions is derived from “use-by” data used by industry. However, because each product can experience other than ideal conditions during the processor-to-consumption pathway, the time to spoilage is also modelled by assuming the product will be spoiled by growth of lactic acid bacteria (LAB). Spoilage of the product is modelled to take ~2.3 times as long as the time modelled for the LAB to reach their MPD. If that time is exceeded the product is considered to have spoiled and to be discarded and is not ‘available’ for consumption. This process is depicted in Figure 2, below.



**Figure 2** Influence diagram showing factors affecting the calculation of *L. monocytogenes* growth or lag time remaining at the time of leaving the processing plant

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The growth of both the *L. monocytogenes* and LAB populations is predicted from predictive models. Two models are used to predict the growth rate of each population. Under the conditions of temperature simulated to occur at each stage and for the levels of water activity, pH, lactic acid and nitrite sampled for the product, the faster growth rate predicted by either of the two *L. monocytogenes* models, or two LAB models, is combined with the time of storage at that stage to estimate the growth during that stage.

During each iteration of the simulation, at each of the seven stages identified above, the level of LAB in the product is estimated and compared with the maximum population density (MPD) sampled for that iteration/scenario. If the LAB level reaches the MPD then the *L. monocytogenes* growth rate is adjusted to  $1/10^{\text{th}}$ , or less, of the rate it would otherwise be in those environmental conditions. This is done to simulate the Jameson effect.

This spoilage modeling approach described above applies for the first six stages (processor, processor to retail distribution centre, retail distribution centre, retail display, retail to consumer and consumer unopened).

In the final stage (consumer opened) the product is assumed to be able to be consumed at any point during the time sampled for this stage. In this stage growth is modelled in finer time intervals to allow the level of *L. monocytogenes* on the product to be estimated at any point during this period when a portion could be potentially consumed prior to product spoilage. The potential spoilage of the product is also monitored during this stage and, similar to the other stages, the product could be discarded at any point during this stage. The time before spoilage after opening is limited to ~7 days at refrigeration temperatures, and adjusted accordingly for higher or lower temperatures.

### **Modelling Risk of Listeriosis**

In each iteration of the model the risk of infection leading to systemic listeriosis is estimated for a consumer of the contaminated product at a certain point. It is assumed that consumption is equally likely at any time after the product is opened and before it is overtly spoiled. In some cases the modelled time of consumption may be after the product has already been modelled to have spoiled. In these cases the product is assumed to have been discarded prior to consumption and is omitted from further calculation.

To estimate the risk of listeriosis, the portion size is combined with the estimated concentration of *L. monocytogenes* on the product at the time of consumption to estimate the dose ingested. The estimate of the dose is then combined with a dose-response function to estimate the

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probability of listeriosis for the specific consumer sampled from that specific serving of Australian processed meat under the specific time and temperature conditions sampled during that iteration of the model.

The dose-response model used is that presented in FAO/WHO (2004). It considers the risk to both healthy “average” members of the population and those more susceptible to listeriosis by virtue of age, pregnancy or underlying illness. In each iteration the consumer is modelled to be either healthy or in the ‘susceptible group and the dose-response is adjusted accordingly. The probability of selecting a healthy or susceptible consumer in any iteration is based on Australian epidemiological data, including uncertainty.

In the execution of the model, it is assumed that all products are contaminated with *L. monocytogenes* at a level  $\sim \geq 1$  cell per package. In reality, approximately 5-10% of units are contaminated. If a package is not contaminated, the risk is always zero and no further calculations are needed. Thus, to allow more iterations to be modelled and thereby produce statistically better estimates the model initially only estimates the distribution of risk from contaminated servings. The prevalence of contamination data is incorporated at the end of calculations. Effectively by modifying (i.e., decreasing) the risk per serving estimate to account for the prevalence. The probability of smallgoods being contaminated at the plant was estimated from the prevalence of contaminated products reported in industry data, government surveys and routing monitoring and academic studies.

The risk of listeriosis from consumption of a serving of luncheon meats and pâtés/liverwursts was, therefore, estimated by multiplying the *per serving* estimate from contaminated servings by the probability of contamination. The risk of listeriosis from cooked sausages intended for re-heating before consumption was estimated as for the other categories but also included consideration of the proportion of product that might be eaten without further cooking, arbitrarily assumed to 5% of all (pre-)cooked sausage product consumed.

Finally, the annual number of cases of listeriosis in Australia due to consumption of smallgoods was estimated by multiplying the estimated number of servings per year of each product type by the risk per serving estimate. The number of servings was estimated from government diet/nutrition surveys, supermarket sales data and industry production data, and the risk per serving as described above.

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## Bacterial Growth Modelling

A feature of this model is the simulation of growth of both lactic acid bacteria (LAB), which eventually dominate the microbiota of VP and MAP processed meats and *L. monocytogenes*. This process brings a level of realism to this model rarely used in microbiological risk assessment simulation models yet presented.

The influence of LAB is incorporated to dictate the likelihood that the product is discarded due to organoleptic (i.e. taste, smell, appearance) changes. In addition, the growth of LAB is also simulated to play an important role in limiting the potential for growth of *L. monocytogenes*, through the Jameson effect. Details of the growth of spoilage bacteria including LAB, as well as the Jameson effect, are described in the full report alluded to earlier, available on request. Details of the models and the genesis are also detailed in the full report.

### *Growth Models for Lactic Acid Bacteria*

Modifications of the models of Devlieghere *et al.* (2000) for *Lactobacillus sake* and Wijtzes *et al.* (2001) for *Lactobacillus curvatus* are used to model the growth of LAB in processed meats. *L. sake* and *L. curvatus* are often the dominant organisms on VP or MAP processed meat products stored at refrigeration temperatures. The models were modified by inclusion of terms, as needed, so that each model predicted the combined effects of temperature, water activity, pH, lactic acid/sodium lactate and nitrite. Terms were adopted from other published models as described below. The model of Devlieghere *et al.* (2000) was modified by addition of a term for the effect of pH on growth rate. The model of Wijtzes *et al.* (2001) was modified by addition of a term for the effect of sodium lactate.

Both of the models can generate negative growth rate predictions if the parameters sampled within an iteration are outside of the growth ranges for the organisms that are implicit in the fitted parameters of the equations. In practice, the sampled values for temperature and product formulation parameters will not exceed the growth ranges listed but might be below lower limiting pH, water activity or temperature. Accordingly, logical tests were applied during every growth rate calculation of every iteration of the model and, if the sampled values were below the lower limits of temperature, pH or water activity for the respective models, the predicted generation time was set to one million hours, equivalent to  $0.000001 \text{ generations.h}^{-1}$  even though the “true” growth rate under such conditions would be zero. This approach was necessary because during the calculations the logarithm of the growth rate is required to be calculated. If a zero growth rate were used the software would be unable to complete the intermediate

calculation and stop. The default value approximates a zero growth rate but eliminates the calculation problem.

During simulation modelling the LAB growth rates predicted from the models were calculated during each producer-to-consumer step of each iteration. The fastest predicted growth rate was selected as the rate of growth of all lactic acid bacteria during that stage of the processor to consumer chain for that iteration of the model.

### ***L. monocytogenes* growth**

Two models were used to predict the growth rate of *L. monocytogenes* at every step in every iteration of the model. These were the modified model of Ross (1999):

$$\begin{aligned}
 \text{growth rate} &= 0.02349 \times [Temp - 0.5973]^2 \times [1 - \exp(0.1285 \times [Temp - 50. \\
 (\text{generations.h}^{-1}) & \times [a_w - 0.925] \\
 & \times [1 - 10^{(4.94 - pH)}] \\
 & \times \left[ 1 - \left( \frac{Lactate}{4.55 \times (1 + 10^{[pH - 3.86]})} \right) \right] \\
 & \times \left[ 1 - \left( \frac{Lactate}{1821.9 \times (1 + 10^{[3.86 - pH]})} \right) \right] \\
 & \times \left[ 1 - \left( \frac{NO_2 \times \left( 1 + \left( \frac{6.5 - pH}{2} \right) \right)}{391.3} \right) \right]
 \end{aligned} \tag{Eqn. 1}$$

where: *Temp* = temperature (°C)  
*a<sub>w</sub>* = water activity  
*pH* has its usual meaning  
*Lactate* = concentration of total lactic acid (mM)  
*NO<sub>2</sub>* = concentration of nitrite (mg/kg)

Sampled values in any iteration were evaluated to determine whether they were beyond the growth limits implicit in the above model. Thus, if:

- the sampled temperature was less than 0°C, or
- the sampled water activity was less than 0.9251, or
- the sampled pH was less than 4.95, or
- the combination of the sampled pH and lactic acid caused the lactic acid term to have a value less than zero, or

- the combination of the sampled pH and nitrite caused the nitrite acid term to have a value less than zero

the predicted generation time was assumed to be one million hours for the reasons described above.

Similarly, the model of Devlieghere *et al.* (2001) was modified to include terms for pH and nitrite as follows:

$$\begin{aligned}
 \text{growth rate} &= 7.33 \times 10^{-7} \times [Temp + 3.542]^2 \\
 (\text{generations, h}^{-1}) & \times (a_w - 0.9295) \\
 & \times (5.9547 - NaL) \\
 & \times (3140 - CO_2) \\
 & \times \left[ \left( 1 - 10^{(4.94 - pH)} \right) 0.9454 \right] \\
 & \times \left[ 1 - \left( \left[ \frac{NO_2 \times \left( 1 + \left( \frac{6.5 - pH}{2} \right) \right)}{391.3} \right] \right) \right]
 \end{aligned} \tag{Eqn. 2}$$

where  $CO_2$  is the concentration ( $\text{mg.l}^{-1}$ ) of dissolved carbon dioxide and was assumed to be zero for all calculations, and all other terms are as described above.

Sampled values in any iteration were evaluated to determine whether they were beyond the growth limits implicit in the above model. Thus, if:

- the sampled temperature was less than  $-3.5^\circ\text{C}$ , or
- the sampled water activity was less than 0.9296, or
- the sampled pH was less than 4.95, or
- the sampled sodium lactate value was greater than 5.87 (mM)
- the combination of the sampled pH and nitrite caused the nitrite acid term to have a value less than zero

the predicted generation time was assumed to be one million hours, for reasons described above.

During simulation modelling the *L. monocytogenes* generation rates predicted from both Equations 1 and 2 were calculated during each producer-to-consumer step of each iteration.

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