Table A2.13 Nodes for *Growth conditions* module.

Title, Identifier, Structure	Description and Definition
Generation times Generation_times (h) Chance node	Generation times calculated at the exponential growth rates at 5°C. 24 * logten(2)/Growth_rate
Growth, no growth, die-off Growth1 Chance node	Growth, no growth, die-off is a simple summary. if Growth_rate<0 then 'Die-off' else if Growth_rate=0 Or Storage_temperature<Minimum_growth_tempe then 'No growth' else 'Growth'
Growth at maximum Growth_at_maximum Chance node	Growth at maximum tabulates how the maximum population density constrains growth. Sometimes, simulated growth is "Below maximum". Sometimes, simulated growth is constrained by the maximum population density set by the lower Storage temperature range ('At Lower'), set by the middle Storage temperature range ('At Mid'), and set by the upper Storage temperature range ('At Higher'). if 10^Finalconcentration < Maximum_population then 'Below maximum' else if Storage_temperature<5 then 'At Lower' else if Storage_temperature>7 then 'At Higher' else 'At Mid'
Generation times Generation_times1 Chance node	Generation times

A2.2.13 Study indices

The *Study indices* library module stores 4 index nodes that structure results from the exposure assessment (Table A2.14).

Table A2.14 Nodes for *Study indices* module.

Title, Identifier, Structure	Description and Definition
Food groups Food_groups Index node	Food groups lists the food commodities that the exposure assessment addresses. ['Ice cream', 'Fluid milk, pasteurized']
Updates Updates Index node	Updates lets the exposure assessment address different sets of storage, time and growth conditions. ['WHO/FAO 2000.06.17', 'FDA/FSIS 2000.05.19']
Annual meals reporting Annualmealsreporting Index node	Annual meals reporting indexes the Annual meals objective node ['Individual', 'Population']
Contaminated or not Contaminated_or_not Index node	Contaminated or not defines the domain of the Chance node Contaminated or not. ['Not contaminated', 'Contaminated']

A2.3 CONSUMPTION CHARACTERISTICS

A2.3.1 Overview

The exposure assessments characterize consumption by meal size and meal frequency, noting and reporting differences in consumption patterns in the population sub-groups with different susceptibility. The meal or serving size is the estimated portion that people eat and has a

distribution estimated from survey data. Similar surveys derive the frequency of eating specific RTE foods. Sources of consumption data are discussed earlier in Sections 3.1, 4.1.2 and 4.1.4.3 of this rport. For the pasteurized milk and ice cream assessments, data describe the consumption characteristics of adult Canadians.

A2.3.2 The data

Consumption characteristics are derived from 24-hour recall data from Canadian Federal-Provincial Nutrition Surveys (CFPNS, 1992–1995), which addressed the nutritional habits of non-institutionalized adults between 18 and 74 years old in the Provinces of Québec, Nova Scotia, Saskatchewan, Alberta and Prince Edward Island. Results are based on data from a total of 10 162 individual respondents from the 1990 Nova Scotia Nutrition Survey (2212 respondents), the 1990 Québec Survey (2118 respondents), the 1993/94 Saskatchewan Survey (1798 respondents), the 1994 Alberta Survey (2039 respondents) and the 1995 Prince Edward Island Survey (1995 respondents). Detailed one-day 24-hour recall data were used to examine consumption of various foods that would help to describe the consumption frequency and amounts eaten for the food groups relevant to this exposure assessment. All survey estimates are weighted to adjust for the sample design, and balance the ages and provinces according to their representation in the populations of those provinces. It is assumed that the remainder of the Canadian adult population eats like this group.

By using single occasion or daily consumption, estimates represent the fraction of the total population consuming the selected food on a given day, essentially a day at random. Food intakes are subject to day-to-day variation among individuals. Thus, the estimates are not indicative of "usual" intake, but are more indicative of the episodic intake with which would be associated foodborne illness. Distributions of "usual" intakes are unobservable in the 24-hour, one-day recall data that the Nutrition Surveys provide. Bureau of Biostatistics and Computer Applications has developed methods to remove the day-to-day within person variability (Junkins and Laffey, 2000: Junkins, Laffey and Weston, 2001; Hayward, [2001]). Those synthesized distributions of "usual" intakes are less heavily tailed than distributions of intakes that retain inter- and intra-person variability as is appropriate for the consumption distributions for these exposure assessments.

There is some uncertainty due to extrapolation of the results to 365 days' experience, when simulating factors such as annual consumption in the population, or to any reference period. The consumption of milk or ice cream were represented by reference to consumption of any of several foodcode categories, a classification system that the surveys employed. Selection of foodcodes from the nutrition surveys' databases was intended to reflect both consumption frequency and amount consumed on eating occasions. The information from individual all-eating episodes that included the food was used, except when the eating episode involved preparation such as cooking. When the food was an ingredient in the serving, an appropriate amount of the food to include was derived or estimated. There are uncertainties associated with this representation of intended foods by particular foods identified in the surveys. Additionally, there might be underreporting or overreporting errors associated with respondent errors and misclassification errors. Trained interviewers estimated amounts consumed on respondents' eating occasions. This is methodologically preferable to a practice that lets a respondent estimate the amounts consumed. However, it is recognized that the amounts recorded contain reporting errors and variability due to the interviewers' estimation methods.

It was difficult to adequately identify how to appropriately aggregate the sometimes many individual foods into the same eating occasion within the respondent's reference day. Therefore, all eating episodes for a food on the same day were aggregated into a daily consumption amount. That practice loses the distinction that one might wish to strike among occasions when the food was consumed alone, as an ingredient in a recipe or as only one element among several elements in a meal. Although the Nutrition Surveys distinguish consumption at an individual's home from consumption at another establishment outside the home, the distinction has been ignored for this exposure assessment. Past work suggests that consumption frequency differences and consumption amounts differences do exist between home and away consumption. Consequently, the consumption that is incorporated is assumed to represent a combination of all eating occasions. Combined independently with foodborne contamination, it is implicitly assumed that there are no differences in contamination rates and concentrations between food consumed at home and food consumed away from home (E.A. Junkins, pers. comm., 2000; M. Vigneault, pers. comm., 2000).

Gender and Age groups

The Nutrition Surveys do not classify respondents into groups to which might be attributed characteristics like higher susceptibility to foodborne illness. Rather, membership in non-susceptible and susceptible groups is imputed from Gender and Age attributes. Consumption characteristics of susceptible and non-susceptible groups of individuals, then, are different only in the manner that constituent Gender and Age characteristics are present in those groups. A susceptible group that is represented by elderly consumers would therefore possess consumption characteristics that differ from the non-susceptible group, solely because of differences between the consumption characteristics of elderly consumers and other consumers.

Simulating consumption amounts

To make it easier to specify the consumption distribution, some conventions were followed. Distributions were described by sampling in the correct proportions, from distributions that describe consumption in 4 Age $\times$ 2 Gender ranges, both for frequency of consumption and consumption amount. Eating episodes, both at home and away from home, were combined into the same distribution, capturing some variability but not distinguishing their separate influences. Non-susceptible and susceptible populations were defined by assuming that some fraction of each Gender $\times$ Age group is more susceptible. It is assumed also that the consumption characteristics of all persons of the same age and gender are the same, whether the person is in the susceptible or non-

Table A2.15 Fraction of population in Non-susceptible and Susceptible risk groups attributed to Gender $\times$ Age groups.

Susceptible group			
Age	Female	Male	Total
18–34	0.16	0.04	0.20
35–49	0.08	0.03	0.12
50–64	0.02	0.02	0.04
65–74	0.35	0.29	0.64
	0.61	0.39	1.00
Non-susceptible group			
Age	Female	Male	Total
18–34	0.20	0.23	0.43
35–49	0.17	0.18	0.35
50–64	0.11	0.11	0.22
65–74	0.00	0.00	0.00
	0.48	0.52	1.00

susceptible group. So, consumption in the susceptible and non-susceptible groups can be correctly simulated by sampling in the correct proportions from consumption characteristics captured for the Gender × Age groups. However, differences in the distributions of consumption for persons from the non-susceptible and susceptible groups are attributable only to the different Gender × Age group make-up of the groups. The values used are presented in Tables A2.15 and A2.16.

Table A2.16 Fraction of population in Gender × Age group attributed to Susceptible group.

Age	Female	Male
18–34	0.12	0.03
35–49	0.08	0.03
50–64	0.03	0.03
65–74	1.00	1.00

A2.4 NON-SUSCEPTIBLE AND SUSCEPTIBLE POPULATIONS

Among adults, susceptible groups are defined to include all adults 65 and older, pregnant women (1.3% of the population) and individuals with suppressed immune systems and certain medical conditions, such as cancer and recent organ transplantation (3.3% of the population) (Miller, Whiting and Smith, 1997). Different fractions of the population in Age and Gender groups are attributed to the susceptible group (Table A2.15), so non-susceptible and susceptible groups include gender and age groups in different fractions (Table A2.16). Fractions attributed to a Gender × Age group depend on the population size for that group, and are criteria for attributing risk categorization to that group. When individual food products are considered, the fraction of individuals who are susceptible depends, too, on consumption characteristics for the population. Susceptible groups would include also all children under 6 months, or perhaps 0-4 years old (J.M. Farber, pers. comm., 2000), some fraction of individuals under 18 years old, and all persons older than 74 years. Among adults aged 18–74, 15% would fit into the susceptible group and 85% would fit into the non-susceptible group when the definition described here is applied (Tables A2.15, A2.16 and A2.17).

An alternative approach follows one suggested in FDA/FSIS (2001). Observations from the FoodNet database describe listeriosis in the United States of America by age group. One might hypothesize that the incidence in an age group depends on, particularly, susceptibility, consumption characteristics and population representation. United States of America incidence data were used and combined with Australian populations in different age groups. Figure A2.11 scales those incidence data so that the incidence in the 10–19-year-old age group corresponds to 1. The relative incidence in the <30 days age group is 300 times the incidence in the 10–19 age group. Similarly, consumption characteristics among populations could be used to account for consumption differences. The remaining differences would affect the different age groups' susceptibility to listeriosis, perhaps forming a surrogate representation for the contrast between susceptible and non-susceptible populations.

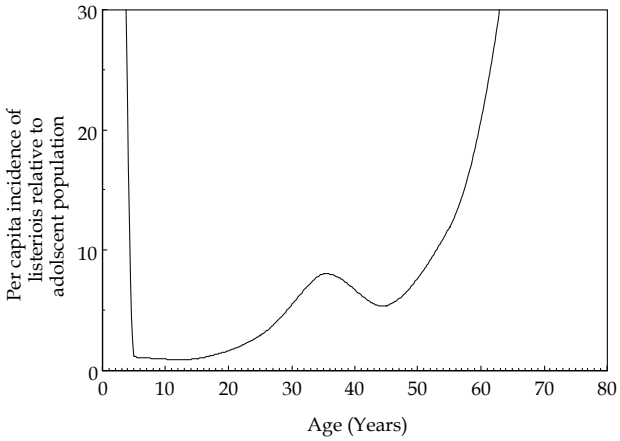

Figure A2.11 Relative per capita incidence of listeriosis (See text for details of calculation).

Table A2.17 Population of Canada allocated to susceptible and non-susceptible groups, using factors from Table A2.15.

Population of Canada				Susceptible population size				Non-susceptible population size			
Age	Male	Female	Total	Age	Male	Female	Total	Age	Male	Female	Total
0-4	911 028	866 302	1 177 330	0-4	911 028	866 302	1 777 330	0-4	0	0	0
5-17	2 738 162	2 598 365	5 336 526	5-17	82 145	77 951	160 096	5-17	2 656 017	2 520 414	5 176 431
18-34	3 711 154	3 591 613	7 302 768	18-34	111 335	430 994	542 328	18-34	3 599 820	3 160 620	6 760 439
35-49	3 823 789	3 802 863	7 626 652	35-49	114 714	304 229	418 943	35-49	3 709 075	3 498 634	7 207 709
50-64	2 403 311	2 453 603	4 856 914	50-64	72 099	73 608	145 707	50-64	2 331 212	2 379 995	4 711 207
65-74	1 000 723	1 134 443	2 135 166	65-74	1 000 723	1 134 443	2 135 166	65-74	0	0	0
74+	644 742	1 069 989	1 714 731	74+	644 742	1 069 989	1 714 731	74+	0	0	0
	15 232 909	15 517 178	30 750 087		2 936 785	3 957 516	6 894 301		12 296 124	11 559 662	23 855 786

SOURCE: Adapted from: Statistics Canada, www.statcan.ca/english/Pgdb/People/Population/demo10a.htm (November 2000), except for 15–19 years age group prorated into 5–17 years and 18–34 years age groups in table above.

A2.5 HOME STORAGE CHARACTERISTICS

A2.5.1 Home refrigeration temperatures

Four studies[1] contributed information about the distribution of refrigeration temperatures, important as one of the main determinants of growth of *L. monocytogenes* during storage. Audits International (2000) surveyed homes in the United States. Johnson et al. (1998) surveyed persons 65 years and older in the United Kingdom. Sergelidis et al. (1997) published results from a survey of homes in Athens, Greece. O'Brien (1997) also considered homes in the United States of America (Figure A2.12). Quantiles from Johnson et al. (1998)

1. A comment made on a late draft of this report pointed to two other references. Notermans et al. (1997) report refrigerator temperatures for households in the Netherlands, for pasteurized milk. Willocx, Hendrickx and Tobback (1993) report, *inter alia*, refrigerator temperatures for Belgian residences.

are approximately 3ºC higher in the middle of the distribution than the ones in Audits International (2000). Based on limited presentation, though, the quantiles from Sergelidis et al. (1997) are approximately 3ºC higher still in the middle of the distribution. O'Brien (1997) and Sergelidis et al. (1997) report 1 or 2 quantiles from the core of their temperature distributions (Table A2.8). It has been assumed that the same storage temperature distribution is appropriate for all RTE foods of interest. Further, it has been assumed that the food is stored at the same temperature throughout its shelf life.

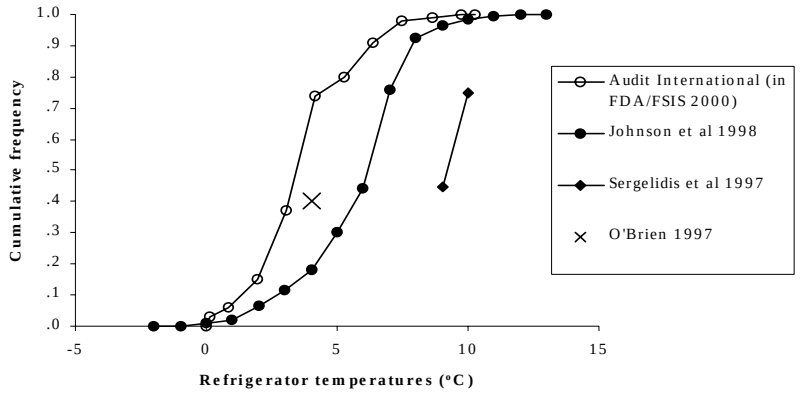

Figure A2.12 Four storage temperature distributions.

A2.5.2 Home storage times

No specific references that describe the length of time that consumers store foods in the home before eating were found[2]. Several characteristics that simulated results should try to emulate, at least qualitatively, might be considered. First, there should be at least a short, minimum storage time associated with all food consumed, representing, at least, the time from retail purchase to the individual's home. Second, storage time distributions that describe variability should have some maximum time that should be constrained by when the consumer would no longer accept the product. The maximum time would be related to the product's shelf-life, but should also reflect variability among individuals' practices of choosing whether to consume foods that have been stored beyond recommended limits. Some authors have studied the relationship between food spoilage, as represented by growth of some spoilage bacteria to high concentrations, and the organoleptic qualities of the food – qualities that help individuals to decide whether to eat a food or not (Priepke, Wei and Nelson, 1976; King, Henderson and Lill, 1986; Garcia-Gimeno and Zurera-Cosana, 1997). Several countries use 10^6 CFU/g concentrations of mesophiles as a guideline for acceptability.

2. A comment on a late draft of this report pointed to Notermans et al. (1997), which reports summary results for the storage time (after pasteurization of the milk) for pasteurized milk in households in the Netherlands.

In another manner, FDA/FSIS (2001) provides some useful information about storage time to consumption among individuals (Table A2.18). For the present, this exposure assessment uses those same criteria. FDA/FSIS (2001) accounts for individual variability in the storage time by describing a distribution specified by Triangular(Minimum, Mode, Maximum). That work also introduces variability and uncertainty in their representation by varying the Mode uniformly in the interval Mode ±20% and the Maximum in the interval Maximum ±50%.

Table A2.18 Storage time distribution parameters.

	Minimum	Mode	Maximum
Ice cream	0.5	7	30
Fluid milk, pasteurized	1	5	12

A2.5.3 Storage time and temperature

Clearly, storage time and storage temperature are not independent. Spoilage actions would severely truncate storage time, forcing shorter storage times to happen with higher storage temperatures. No studies that describe such a relationship directly were found. Studies that directly relate spoilage bacteria to organoleptic qualities might be useful, but have not been explored. A simple implementation for pasteurized milk assumes that organoleptic preferences that would truncate storage time can be related to storage life. Storage life for pasteurized milk depends on the growth of spoilage bacteria, which depends on temperatures. The storage life for pasteurized milk is assumed to be 12 days at 4°C, with storage life at other temperatures determined by the relationship $Life(T) = 12 \times \left[\frac{4+7.7}{T+7.7}\right]$ (Neumeyer, Ross and McMeekin, 1997; Neumeyer et al., 1997). To account for variability among individuals, the relationship time $\propto$ temperature^{-1} would not be deterministic and this relationship would be directed only to constrain the most extreme storage length at a given temperature.

A2.6 GROWTH CHARACTERISTICS

A2.6.1 Introduction

Extensive discussions about the microbial ecology of *L. monocytogenes*, predictive microbiology and growth characteristics are discussed elsewhere in this report. For the assessment examples, it has been assumed that the *L. monocytogenes* organisms were in the food sufficiently long for the lag phase to have passed. With that assumption, growth dynamics can be described with the exponential growth rate alone. It is assumed, further, that growth characteristics that must be explicitly accounted for are: the exponential growth rates, the minimum growth temperature and the stationary phase population.

A2.6.2 Growth rates

It is assumed that there is no growth or decline of *L. monocytogenes* populations in contaminated ice cream. For the milk exposure assessment example, growth rates reported in FDA/FSIS (2001) have been used to capture level and variability in growth of *L. monocytogenes*. Specific parameterization is given in Table A2.19, where growth rates

refer to growth at 5°C. FDA/FSIS (2001) reports that in their referenced studies, study-specific growth rates were converted from the growth rate at a specific study temperature other than 5°C using relationships from McMeekin et al., 1993. At a temperature T°C other than 5°C, growth is calculated using the relationship $\sqrt{\mu_T} = \sqrt{\mu_5}(T - T_{\min})(5 - T_{\min})^{-1}$, where μ is the growth rate and T_{min} is the minimum growth temperature. Storage temperature as a dependent condition for the growth rate has been explicitly accounted for. However, it is assumed that the assumed distribution of growth rates effectively samples among the other dependent conditions (a_W, pH, NaCl, NO_3) in the same proportions that would occur in real environments. Bovill et al. (2000) note that competitive flora in the growth environment and the physiological state of the *L. monocytogenes* organisms might also be considered to be growth conditions. There is additional uncertainty in the estimated growth rates, not explicitly accounted for. Study methods and measurements contribute generally random effects that increase variability in replicated results for a given set of conditions, and therefore contribute uncertainty regarding what the true rate would be at those conditions.

Growth rates in Table A2.19 have been converted to refer to growth at 5°C, from the growth rate at a specific study temperature, using the square root relationship (McMeekin et al., 1993). So, growth rates at 5°C, as a baseline, do not explicitly account for variability and uncertainty in model extrapolation (or interpolation) from a study temperature back to 5°C.

A2.6.3 Stationary phase population

This implementation of maximum population densities is straightforward. FDA/FSIS (2001) reports stationary phase population values that change with storage temperature (Table A2.19). The stationary phase population is viewed as one of many constraints on the growth of *L. monocytogenes*. Other characteristics include competition with other microorganisms and growth of total spoilage bacteria populations to the extent that the food is not organoleptically acceptable, but these other characteristics are not accounted for.

A2.6.4 Minimum growth temperature

Based on Farber and Peterkin (2000), minimum growth temperature is implemented as Triangular(1°C, 1.1°C, 2°C) for this exposure assessment. Alternatives, such as -1.18°C (FDA/FSIS, 2001), set lower minimum growth temperature for *L. monocytogenes* than are implemented here. It is assumed also that minimum growth temperature is the same for the example foods to which it is applied.

A2.6.5 Implementation of microbial growth

The amount of growth using *daily growth* $\times$ *days storage* is calculated and applied to initial concentrations using $Concentration_{Final} = Concentration_{Initial} + Growth$ to get final concentrations, when quantities are expressed on a $\log_{10}$ scale. Growth rates at a stochastic storage temperature are adjusted for, using the relationship $\sqrt{\mu_T} = \sqrt{\mu_5}(T - T_{\min})(5 - T_{\min})^{-1}$ (McMeekin et al., 1993). Doing so incorporates variability associated with storage temperature, but does not explicitly incorporate uncertainty in extrapolating from a growth rate at 5°C to a growth rate at another temperature. The amount of growth until consumption of a portion is the simple product of the daily growth rate and the length of the storage time. This incorporates variability associated with the storage time, but assumes constant growth

rate over the whole storage time. Growth is constrained so that final concentrations cannot be simulated to exceed the maximum population density.

Table A2.19 Population growth characteristics for *L. monocytogenes*, giving growth rates at 5°C and stationary phase populations at various temperatures.

	Growth rate distribution		Stationary population		
			<5°C	5°C–7°C	>7°C
Ice cream	0				
Milks	Uniform(0.092, 0.434)	Milks	10^7	$10^{7.5}$	10^8

SOURCE: FDA/FSIS, 2001.

A2.7 PREVALENCE AND CONCENTRATION

This exposure assessment model copies the practice that separates prevalence of contaminated servings and the concentration of *L. monocytogenes* in contaminated servings. This practice is similar to some published quantitative risk assessments (Cassin, Paoli and Lammerding, 1998; Lindqvist and Westöö, 2000), but differs from others (Bemrah et al., 1998, in part; FDA/FSIS, 2001). The practice separates concentration zeros (non-prevalence) from concentration non-zeros. First, the literature presents large data sets that count qualitatively positive and qualitatively negative samples. Concentrations, when presented, come from the typically small number of qualitatively positive samples. Second, it makes the simulation more efficient. The same 10^k iterations can define the probability of contaminated product and the distribution of concentrations, given contaminated product. Were both zero and non-zero concentrations combined, then that 10^k simulated observations would generate $\sim 10^{k-m}$, with *m* generally smaller than 1, number of zeros and only $\sim 10^m$ non-zeros, reducing the amount of precision that the simulation generates about the concentration distribution.

This implementation acts as if the declarations that positive and negative samples make are exact. Hence it calls the concentration in qualitatively negative samples exactly 0 CFU/g. The concentration in qualitatively negative samples should be modelled as random variables on $[0, \infty)$.

A2.8 COMBINING INDEPENDENT PREVALENCE ESTIMATES

A2.8.1 Introduction

Prevalence estimates for the presence of *L. monocytogenes* in foods come from surveys that typically provide summary information that includes the number of samples detected positive and the total number of samples tested. In some cases, though rarely, a detailed study design is also provided. Most studies give some context for the source of the samples – geography, food types or textures, points of origin, raw materials used or motivation. Most studies describe the methods used to test for *L. monocytogenes* presence. Most often, research has come via the microbiological literature. Some research has come from reports issued by national agencies. The food industry has also provided data sets. One could include prevalence estimates whose source is a modelled estimate, as is common in a quantitative risk

assessment. When several studies are available, it is useful to take advantage of the observed variability between study estimates to provide a proxy model for including uncertainty and variability in a probabilistic risk assessment. To this end, it is assumed that k *combinable* studies are available, providing summary data $\{(Y_i, n_i); i = 1, \ldots, k\}$, where Y is the number of samples positive for *L. monocytogenes* and n is the number of samples. This assumes that, within studies, sample designs behave as simple random samples, that samples are independent, and that there is constant probability of a positive sample.

A2.8.2 Beta-binomial model for combining prevalence estimates

A simple assumption about the stochastic structure of a collection of studies gives binomial variability to the individual study estimates and a Beta distribution to the between-study variability of the true study prevalences. Also, this assumes that there are no overriding factors that are present that would group the studies into subsets, part of the assumption that the studies can be combined with a simple mixing distribution. More formally, the following two-stage model is assumed: $Y_i|n_i,\pi_i \sim$ Binomial (n_i,π_i), $i = 1, \ldots, k$ and $\pi_i \sim$ Beta(α,β). For risk assessments, the mixing distribution is of importance. The role played by the distribution of the true study prevalence values is understood in the following way. If the mixing distribution is primarily a description of uncertainty, with a common fixed underlying prevalence value, then information from these several studies could be simply combined to give a more precise estimate of that single, fixed, true prevalence than the individual studies give. In this case, the Beta distribution plays the role of a prior density on the prevalence parameter. However, if the distribution of true study prevalence values also reflects variability in the prevalence value, then increasing the number of observations does not reduce this variability, though it can improve knowledge of the underlying distribution. In this case, the Beta distribution is an intrinsic component of the variability of the phenomenon under consideration among circumstances, situations or scenarios. Information from the studies can be appropriately combined to estimate the unknown parameters of that Beta distribution (Ross, pers. comm., 2000). There are a number of approaches available for estimating the parameters α and β of the Beta mixing distribution (Vose, 2000).

A2.8.3 Other alternatives appropriate to some circumstances

Alternatives can be found appropriate to some circumstances that Lindqvist and Westöö (2000) and Vose (2000) illustrate. Lindqvist and Westöö (2000) present prevalence data that are proportions $\{p_j, j = 1, \ldots, k\}$, where the sample sizes and the numbers of positive samples are either ignored or not reported. Those authors pool the observed data, treating them as independently and identically distributed from a distribution that they describe by the quantiles of that pooled sample. There, quantiles are defined by associating the *j*th largest observed fraction p_j with the $j(k+1)^{-1}$ th point of the distribution. Such a derivation is appropriate when the sample sizes used to estimate the individual fractions are the same or nearly the same, so that they are ignorable. Retaining the sample sizes, nevertheless, is useful to properly account for the uncertainty that one would associate with the fractions as estimates of true fraction values drawn from that empirically defined distribution. The true fraction values play the same role as described above. They can be understood as describing the variability among the true values of the fraction obtained under the conditions that the pooled sample describes. Alternatively, they can be understood as an expression of the uncertainty about the single true prevalence for that same population, from which the samples

form independent observations. Vose (2000) discusses several methods to use with an example data set of fractions $\{p_j, j = 1, \ldots, k\}$, where the sample sizes and the numbers of positive samples are not reported. He specifies a Beta mixing distribution for the true values that the data set observes and describes how to estimate the parameters, α and β, by maximum likelihood methods and by the method of moments. He also describes in brief a procedure similar to the one that Lindqvist and Westöö (2000) carry out with their pooled data.

Alternatively, some knowledge of the population might be constructed by considering how to appropriately mix the various conditions of the studies sampled from, rather than basing the mixing distribution for the variability or uncertainty about the true prevalence values on the Beta distribution. One might consider mixing distributions for recognizable parts of a food supply: geographical, food type, point of origin, or raw materials used.

A2.9 DISTRIBUTIONS FOR *L. MONOCYTOGENES* CONCENTRATIONS IN FOODS

A2.9.1 Empirical distribution functions and fitted distributions

Small samples of observations for *L. monocytogenes* concentration in contaminated samples capture the distribution only with some uncertainty, both in the centre of the distribution and in the tails. Of particular concern is the upper tail of the distribution, where large concentrations sit. Studies that were reviewed seldom record high concentrations, or only under exceptional circumstances, making it difficult to model the thickness of the tail. Theoretical constraints on the length of the tail probably can be derived from predictive microbiology, but these require knowledge of growth conditions such as temperature and medium, and might be so much larger than empirical data produce that they would be somewhat unrealistic for practical use. Empirical distributions, too, are somewhat limited in their ability to capture the distribution very precisely in the upper tails. Confidence intervals can capture some notion of uncertainty, but will be a constant width in the tails, above the largest recorded observation. Uncertainty about the whole distribution can be captured non-parametrically by determining confidence intervals about the empirical density function or the empirical distribution function, or as a summary of the empirical distribution at selected quantiles. Last, given some assumptions, one can capture the shape of the distribution by fitting parametric distributions to the data. Uncertainty can be captured by varying the parameters among a confidence set, encompassing all combinations of the parameters that produce distributions that are consistent with the data. The distributions themselves capture variability among *L. monocytogenes* concentrations in different conditions. Parameter uncertainty and confidence intervals may be considered to describe some combination of that variability and uncertainty about that variability.

A2.9.2 Families of distributions

One alternative is to fit an analytical distribution to the data. The families of distributions considered as candidates for describing the concentration distribution should, first, respect the domain of the distribution. As used here, concentrations in contaminated foods have support on $(0, \infty)$ or on a subset, truncating $(0, \infty)$ at a minimum and at a maximum value. Second, consideration of candidate probability distributions would be restricted to ones that refer to a continuous random variable, and not a discrete random variable. FDA/FSIS (2001), for

example, considered candidates like the lognormal distribution, the logistic distribution (folded or half-logistic, since the logistic distribution is defined on (-∞, ∞)) and the Beta distribution. Method of moments could be used to estimate parameters for the analytical distributions. Preferable would be to use maximum likelihood methods. Nevertheless, some attention to the fit that the analytical distribution provides for the data, and in which parts of the domain, and goodness-of-fit criteria for the fit over the whole range of the data, should be considered. Distributions selected should also represent what is known about the sampling methods. That is, a point estimate is made about the concentration in an amount of product based on a small sample.

Minimum and maximum concentrations

Setting limits on the length of upper and lower tails can be straightforward and heuristic. When working with concentration distributions in this exposure assessment, the extent of the lower limit of contamination has been set to 0.04 CFU/g (1 CFU per 25 g), a lower detection limit, in effect, for every foodstuff. Upper limits are often set based on authors' suggestions, or set a judged limit larger than the largest observation. In some studies, the largest observed concentration stands as the upper limit, though this might be considered to be unrealistic. A more rigorous approach to setting maxima would consider the operating characteristic curve that is associated with the sample size and sample design of the studies that form the data sets. Minimum and maximum concentrations might also be used in conjunction with fitted distribution functions. The distribution function would define the shape of the distribution; the limits would define the domain of the distribution.

Heterogeneity of the organism in the package

Data collection and organization from referenced studies provide concentration distributions that represent levels of concentrations in recognizable packages or units of products, or give measurements from which one makes an inference about the concentration in the package or unit of product.

A2.10 REFERENCES CITED IN APPENDIX 2

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Appendix 3. Predictive microbiology: concepts, application and sources

Predictive microbiology involves the systematic study and quantification of microbial responses to environments in foods and may be considered as the application of research concerned with the quantitative microbial ecology of foods. It is based upon the premise that the responses of populations of microorganisms in a defined environment are reproducible. By characterizing environments in terms of those factors that most affect microbial growth and survival, it is possible from past observations and experience to predict the responses of those microorganisms in other, similar environments. Ideally, the patterns of microbial behaviour are integrated with knowledge of the physiology of microbes. This knowledge can be expressed very succinctly using the language of mathematics, in the form of mathematical models. Those models can be considered as "condensed knowledge".

Predictive microbiology models provide a way to estimate changes in *L. monocytogenes* levels in foods as the product moves through the production-to-consumption chain. To make those estimates, periods, temperatures, product composition and concentrations of *L. monocytogenes* at some other point in the chain are required.

This section provides practical guidance for the application of predictive microbiology models in exposure assessment. Predictive microbiology has been extensively reviewed (Farber, 1986; Ross and McMeekin, 1994; Buchanan and Whiting, 1997; Ross, Baranyi and McMeekin, 1999; McDonald and Sun, 1999). McMeekin et al. (1993) provide a good introduction to the concept and its practical application.

The information below is drawn largely from Ross, Baranyi and McMeekin (1999).

A3.1 SOURCES OF GROWTH RATE MODELS AND DATA

Many data (*see* ICMSF, 1996) and many models for prediction of the growth rate of *L monocytogenes* are available (see Table A3.1, at the end of this appendix). In general, *L. monocytogenes* responds to environmental factors with the same patterns of response as other vegetative microorganisms and can be described by the same forms of model that describe growth rate responses of other organisms (Ross, 1993; Wijtzes et al., 1993; Tienungoon, 1998). However, it is reported that the temperature–growth rate relationship of *L. monocytogenes* is not as well described by existing models as it is described for other organisms, particularly at low temperatures that cause slow growth rates (Bajard et al., 1996; Ross, 1999). Generation times of *L. monocytogenes* under a range of conditions can be estimated easily using models such as most of those listed in Table A3.1. One can easily incorporate a published model into spreadsheet software.

A3.2 PRACTICAL CONSIDERATIONS

Models used in risk assessment must adequately reflect reality. Thus, before predictive models are used in exposure assessment, their appropriateness to that exposure assessment and overall reliability should be assessed. Users of models must be aware of the predictive limits of models, both in terms of the range of conditions that a model's interpolation region encompasses (Baranyi et al., 1996) and the variables that the model considers. Completeness error arises in model predictions when the model does not explicitly consider the effect of factors in a food that will affect the growth response of the microorganism modelled. The models referred to in Table A3.1 were developed to test different modelling strategies or, in the later published models, to include the effect of specific variables not included in earlier models. Ideally, a single model could encompass all the variables of relevance in all foods and is the ultimate aim of the scientific approach to predictive microbiology as the basis of a quantitative understanding of the microbial ecology of foods. However, creating such a model and scientific framework is time consuming. Alternatively, an iterative approach for development of product-oriented models i.e. based on observations in a system closely related to the food of interest, may satisfy the current technological needs of the food industry (Dalgaard, 1997; Dalgaard, Mejlholm and Huss, 1997).

Where completely appropriate models are not available, the limitations of the models should be documented and the implications of those limitations discussed as sources of uncertainty.

This section will consider assessment of model performance and limits. The discussion will be presented under the following headings:

- limits to application (i.e. interpolation or extrapolation);
- sources of variability and uncertainty; and
- performance evaluation.

A3.3 INTERPOLATION OR EXTRAPOLATION

No predictive models currently in use have a sound basis in theory, i.e. they are empirical descriptions and summaries of observations. A simple rule of modelling is that models without theoretical bases cannot be used reliably to make predictions by extrapolation, but only by interpolation. Interpolation relates to prediction made "between" the observations that the model is based on, while extrapolation is when predictions are made for conditions outside the range of those studied in the development of the models**.** A common interpretation of the interpolation region is that any combination of variables (e.g. temperature, water activity, pH, phenol, nitrite, etc.) that falls within the respective ranges of variables tested in the development of the model is within the interpolation region.

Certainly, microbial growth or death in a food cannot be predicted reliably when the conditions are *outside* the range of any individual factor tested in the model. However, the interpolation region is usually smaller than the simple interpretation suggested above. Few models are based on full factorial experimental designs. Unfortunately, the regions with fewest observations are usually those at the extremes of the ranges, where growth is slowest or may not occur at all due to the interaction of inhibitory factors (this is considered further in the section below on growth/no-growth models). However, these regions are often of most relevance when modelling because they are the conditions normally used to extend the shelf-

life and safety of foods. As a result, users of models may inadvertently make predictions by extrapolation, particularly for conditions under which growth may be slow but of direct relevance in determining exposure to *L. monocytogenes* in RTE foods.

The determination of the true interpolation region, and the consequences of extrapolation, were discussed by Baranyi et al. (1996). Those authors concluded that models using a large number of parameters, e.g. higher order polynomial models, were more prone to unreliability resulting from inadvertent extrapolation because the predictions of the model often changed dramatically near the limits of the interpolation region.

Inadvertent extrapolation can also occur when using stochastic modelling techniques to describe effects of fluctuating temperature. Inadvertent extrapolation may also occur for other factors, but temperature is the factor most likely to fluctuate. Distributions can have infinitely long "tails", so it is important that the tails of the distributions used to model temperatures are truncated to match the interpolation range of the predictive microbiology model used.

A3.4 GROWTH/NO-GROWTH MODELS

Growth/no-growth models are a relatively new area of predictive microbiology. They aim to define the sets of combinations of factors that permit the growth of a modelled organism and those that do not. While there are absolute limits to growth of *L. monocytogenes* (see Table 3.1 in the main report) combinations of inhibitory factors can also prevent growth under milder conditions of each factor, a phenomenon widely employed in the food industry. These combinations of growth-preventing factors form a smooth surface in multi-dimensional space, or a smooth curve if one considers the interaction of two factors at a time as shown in Figure A3.2. There are relatively few growth/no-growth models currently available (Table A3.1). On the growth side of the interface, models can predict growth. On the no-growth side of the boundary, death occurs. Thus, growth/no-growth models provide additional information on the interpolation region of models.

A3.5 SOURCES OF VARIABILITY AND UNCERTAINTY

Model predictions can never perfectly match observations. Each step in the model construction process introduces some error, as outlined below, and presented in order of the magnitude of their contribution to the overall error in the models predictions.

- **Homogeneity error** arises because either some foods are clearly not homogeneous, or, at the scale of a microorganism, foods of apparently uniform consistency may comprise many different microenvironments. Current predictive models do not account for this non-homogeneity of foods.
- **Completeness error** arises because the model is a simplification, i.e., in practice, not all relevant factors can be included in the model.
- **Model function error** is similar to completeness error, and arises mainly from the compromise made when using empirical models, i.e. that the model is only an approximation to reality.
- **Measurement error** originates from inaccuracy in the raw data used to estimate the parameters of a model, i.e. due to methodological limitations in our ability to measure accurately the environment and the microbial response.

- **Numerical procedure error** includes all errors that are the consequences of the numerical procedures used for model fitting and evaluation, some of which are methods of approximation only. Generally, numerical procedure errors are negligible in comparison with the other types of errors.

The error in the estimate of maximum specific growth rate (or doubling time) of an organism determined from measurement of growth in laboratory media is ~10% per independent variable. As a rule of thumb, each additional environmental factor (pH, a_w, etc.) adds at least another 10% relative error to the model, assuming that the interpolation region of the model is comparable to the whole growth region. (Models with a small interpolation region have smaller error). An example of the interaction of factors limiting the growth of *L. monocytogenes* and the use of a model to predict those interactions is presented in Figure A3.1. Thus, the best performance that might be expected from a kinetic model encompassing the effect of three environmental factors on growth rate is ~30%.

A3.6 DISTRIBUTION OF RESPONSE TIMES

It is recognized that there is variation in the ecology of strains of *L. monocytogenes.* Begot, Lebert and Lebert (1997) reported variability in the growth rate responses of 58 strains. Peleg and Cole (1998) hypothesized that non-linear inactivation curves result from the natural variability that exists in microbial populations.

There has been lively discussion in the literature concerning the variability of bacterial growth rates. Using the limited amount of replicated published data concerning growth rate estimates under varying environmental conditions, Ratkowsky et al. (1991) concluded that growth rate responses became increasingly variable at slower growth rates, an observation confirmed by others (Fehlhaber and Krüger, 1998).

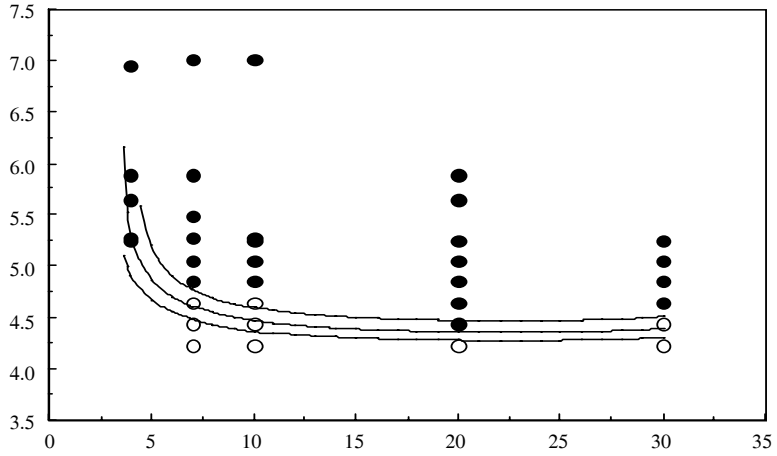


Figure A3.1 An example of the interaction of factors limiting the growth of *Listeria monocytogenes* and the use of a model to predict those interactions. The smooth lines are predictions of a model for the probability of growth of *Listeria monocytogenes* L5 (Tienungoon et al., 2000). The symbols (filled circles: growth observed; open circles, no growth observed) are the data of George, Lund and Brocklehurst (1988) for the effect of temperature and pH on the growth of *L. monocytogenes* NCTC 10357. In the figure, the lines indicate the predicted limits of growth at various levels of confidence (P = 0.9: lower curve; P = 0.5: middle curve; P = 0.1: lower curve).

In the data presented by Ratkowsky et al. (1991) variance in the square root of growth rate (var$\sqrt{}$*rate*) is constant, regardless of the magnitude of *rate*. Alber and Schaffner (1992) showed that for a strain of *Yersinia enterocolitica* (serotype 08), a logarithmic transformation of rate better "homogenizes" or "stabilizes" the variance. Dalgaard et al. (1994) reported that a transformation intermediate between the square root of rate and the logarithm of rate was required to normalize the variability in growth rate responses. Ratkowsky et al. (1996) reported similar observations, depending on the data used.

Ratkowsky (1992) presented the following general relationship between the variance in growth response times and the mean of those responses for a range of possible distribution types:

$$V = c\mu^n$$

where μ is the mean of the probability distribution, V is the variance of the probability distribution, *n* is an integer exponent having values 0, 1, 2 or 3, corresponding to the normal, Poisson, Gamma (logarithm of rate) and Inverse Gaussian (square root of rate) distributions, respectively, and c is a constant.

It is important to characterize the variability in responses, and to recognize that those responses are not normally distributed if that information is to be used within stochastic models for risk assessment.

A3.7 EVALUATION OF MODEL PERFORMANCE

A number of authors (Buchanan and Phillips, 1990; Wijtzes et al., 1993; George, Richardson and Peck, 1996; Fernandez, George and Peck, 1997; Walls and Scott, 1997; McClure et al., 1997; te Giffel and Zwietering, 1999) have evaluated the reliability of predictive microbiology models for *L. monocytogenes* growth rate and many have concluded that the models perform satisfactorily. However, in most of those assessments, no objective criterion for "satisfactory" was given.

Evaluation of model performance typically involves the comparison of model predictions to analogous observations not used to develop the model. Various measures have been used. Wijtzes et al. (1993) plotted literature values for the generation time of *L. monocytogenes* against the corresponding predictions of a model derived from studies in laboratory broth. From this plot, predictions that would be unsafe in practice could be visualized readily, and the overall reliability of the model assessed. Duh and Schaffner (1993) developed predictive equations for *Listeria* growth rate based on measurements in brain-heart infusion broth. Complementary literature values for the growth of the organism in food were then added to the data set and regression analysis of the supplemented dataset performed. The close similarity in mean square error (MSE**)** and correlation coefficient (r^2) values of the equations fitted to either data set were taken as an indication of the reliability of the models when applied to foods. Another measure of the accuracy of predictive equations was introduced by McClure, Zwietering and Roberts (1993), who compared their models on the basis of the sum of the squares of the differences of the natural logarithm of observed and predicted values. Zwietering et al. (1994) introduced the use of the *F*-ratio test. In this method the MSE of the models when assessed against data that are not used to generate the model was compared to the measurement error of the model itself, i.e. the model compared to the data used to generate it. If the measurement error is not significantly different from the prediction error,

the model is considered to be satisfactory. te Giffel and Zwietering (1999) reviewed these measures in greater detail.

Two additional complementary measures of model performance can be used to assess the "validity" of models and are claimed to have the advantage of being readily interpretable (Ross, 1996), namely a bias factor and an accuracy factor.

The "bias factor" (B_f) is a multiplicative factor by which the model, on average, over- or under-predicts the response time. Thus, a bias factor of 1.1 indicates not only that a growth model is "fail-dangerous" because it predicts longer generation times than are observed, but also that the predictions exceed the observations, on average, by 10%. Conversely, a bias factor less than unity indicates that a model is, in general, "fail-safe", but a bias factor of 0.5 indicates a poor model that is overly conservative because it predicts generation times, on average, half of that actually observed. Perfect agreement between predictions and observations would lead to a bias factor of 1.

The "accuracy factor" (A_f) is also a simple multiplicative factor indicating the *spread* of observations about the model's predictions. An accuracy factor of two, for example, indicates that the prediction is, on average, a factor of two different from the observed value, i.e. either half as large or twice as large. The bias and accuracy factors can equally well be used for any time-based response, e.g. lag time, time to an *n*-fold increase, death rate, D value, etc. Modifications to the factors were proposed by Baranyi, Pin and Ross (1999).

Ideally, predictive models would have $A_f = B_f = 1$, but, typically, the accuracy factor will increase by 0.10–0.15 for every variable in the model. Thus, an acceptable model that predicts the effect of temperature, pH and water activity on *Listeria* growth rate could be expected to have A_f = 1.3–1.5. Satisfactory B_f limits are more difficult to specify because limits of acceptability are related to the specific application of the model. B_f is a measure of the extent of under- or over-prediction of the observed response rates by the model. Thus, a bias factor of 1.1 indicates not only that a generation time model is "fail-dangerous" not only because it predicts longer generation times than are observed, but also because the observations exceed the predictions, on average, by 10% in terms of $\log_{10}$ CFU. Conversely, B_f <1 indicates that a model is, in general, "fail-safe". Note, however, that when applied to rate-based data, $B_f > 1$ indicates the model *under*-predicts the observed rate, potentially leading to "fail-dangerous" predictions.

Armas, Wynne and Sutherland (1996) considered that B_f values in the range 0.6–3.99 were acceptable for the growth rates of pathogens and spoilage organisms when compared with independently published data. te Giffel and Zwietering (1999) assessed the performance of many models for *L. monocytogenes* against seven datasets, and found Bias factors in the range 2–4, which they considered to be acceptable, allowing predictions of the order of magnitude of changes to be made.

Other workers have adopted higher standards. Dalgaard (2000) suggested that B_f values for successful validations of seafood *spoilage* models should be in the range 0.8–1.3. Ross (1999) considered that, for pathogens, less tolerance should be allowed for $B_f > 1$ because that corresponds to under-predictions of the extent of growth and could lead to "fail-dangerous" predictions. Thus, Ross (1999) recommended that for models describing *pathogen* growth rate, B_f in the range 0.9–1.05 could be considered good, in the range 0.7–0.9 or 1.06–1.15 considered acceptable, and less than ~0.7 or greater than 1.15 considered unacceptable.

A3.8 SPECIFIC MODELS VERSUS GENERAL MODELS

The results of te Giffel and Zwietering (1999) and Ross (1999) showed that model performance is dependent on the data used to assess them. Differences in the performance of individual models were observed when the test datasets were disaggregated into food groups, or into ranges of growth rates. Some of these differences stem from the quality of the data used to assess the models, and the shortcomings of assessing models against data derived from the published literature have been commented on in several studies (Sutherland, Bayliss and Roberts, 1994; Ross, 1996; Walls and Scott, 1997; te Giffel and Zwietering, 1999). A second reason for poor performance may stem from completeness error. While te Giffel and Zwietering (1999) endorsed the performance of general models, Dalgaard (1997) and Dalgaard, Mejlholm and Huss (1997) proposed that strategies for model development based on observations in a system closely related to the food of interest will provide better performance for that specific product.

A3.9 PRACTICAL MICROBIAL ECOLOGY MODELLING IN RISK ASSESSMENTS

A3.9.1 Temperature distributions

Foods are rarely held under completely controlled temperature during their entire shelf-life. A common technique is to model the average temperature, based on temperature records obtained from surveys. The growth rate response of bacteria to temperature is complex and is not directly proportional to temperature. As noted by Cassin et al. (1998) the question arises whether the use of the average temperature over a time interval systematically biases the estimate of growth. This issue was addressed by Ross (1999) who used 246 temperature histories obtained using electronic temperature data-loggers for meat processing, transport and storage in Australia. Typically, the time interval between temperature recordings was a few minutes long.

Three methods were used to calculate the amount of microbial growth for each temperature history. In the first, the estimate of growth was based on the average temperature of all the temperatures recorded over the monitoring period. In addition, estimates were also generated for the *worst* case, i.e. based on the highest temperature recorded in each temperature record. The average and highest temperature values were substituted into models to predict the number of generations of pseudomonads and *E. coli* for each temperature history, respectively, by the two approaches. In the third method, the growth was determined using "time temperature function integration". For each time interval in the temperature history the growth rate of both pseudomonads and *E. coli* at the beginning and end of each time interval was calculated. The average of those growth rates was substituted into predictive models to calculate the number of generations over each recording interval, and the calculated number of generations for each time interval added to estimate the growth (i.e. number of generations) over the *entire* time monitored for each of the 246 temperature histories used.

In all methods, any temperature outside the ranges specified for each model were calculated to correspond to no growth, whether based on the average temperature over the interval, or full time-temperature integration.

For each organism-sector combination, the histograms of the distributions predicted by each method were plotted on a single graph to enable direct comparison of the effect of the three calculation methods. Representative plots of pseudomonad growth are shown in Figure A3.2.

The relationship between specific sets of predictions is lost in the preparation and presentation of the frequency distribution graph.

Ross (1999) showed mathematically that the average rate of growth at two temperatures *in the sub-optimal temperature region* is always greater than or equal to the growth rate at the average of two temperatures and that the difference between the two calculation methods is a function of the magnitude of the difference between the two temperatures. Using the dataset described the results indicated that *in practice* the difference between the two estimation methods is typically of the order of -0.1 to 0.2 $\log_{10}$ CFU, presumably because in most cases the range of temperatures experienced is small. This is a very small difference, particularly bearing in mind that the limits of accuracy of current microbial enumeration methods is approximately 0.3 $\log_{10}$ CFU (Jarvis, 1989).

However, there are certain situations and temperature ranges in which differences due to estimation method become more pronounced. If the temperatures experienced transcend growth boundary values, e.g. maximum or minimum temperatures for growth, estimates of the predicted growth by the two methods can differ significantly and lead to different frequency distributions of predicted growth. They are unlikely to be important for prediction of the growth of *Listeria monocytogenes* in RTE foods, however, because the lower (= 0°C) or upper (= 45°C) temperature thresholds for *L. monocytogenes* are unlikely to be experienced in normal refrigerated storage.

Thus, the results of that study (Ross, 1999) suggest that the use of the average temperature approach can provide a reasonable prediction of the extent of the growth of *L. monocytogenes* under real conditions of storage and distribution.

A3.9.2 Upper and lower limits

When distributions of temperatures are defined, they should reflect reality, i.e. the distributions should be truncated at realistic values. Similarly, when the range of temperature defined in the exposure model exceeds the minimum and optimum or maximum temperatures for growth of the organism, the growth model used must model the response of *L. monocytogenes*, i.e. the decline in growth rate as temperatures increase above that optimal for growth rate; and the cessation of growth at temperatures above or below the limits for growth.

Further pitfalls may occur in the use of unbounded temperature distributions. If the temperature distribution exceeds the range of the predictive model, nonsense predictions can occur, and may not be revealed by the simulation software used. While the effects might be subtle, they are likely to increase the range of uncertainty in the final model prediction.

A3.9.3 Lag time response

Microbial lag time is dependent both on the environment and its effect on growth rate, and the amount of "work" the cell has to do before it can initiate growth. This has presented problems for modellers, because models are developed under sets of constant conditions and

it has been difficult to relate one set of conditions to another. The use of the relative lag time (RLT) concept, and RLT distributions, provides a way to overcome these problems in developing exposure assessments.

Ross (1999), Mellefont, McMeekin and Ross (2003) and Mellefont and Ross (2003) combined lag time data from experiments deliberately intended to induce long lag times with the published observations of other workers to investigate the distribution of lag times that are observed. When the lag time is expressed as an equivalent number of generation times of the organism in the same environment, i.e. lag time divided by generation time, or RLT, the distribution of RLTs observed has a sharp peak in the range 3–6. Augustin and Carlier (2000) also collated relative lag time distributions. The results are shown in Figures A3.3 and suggest that in many situations there is a practical upper limit to the lag time duration.

The number of generations of growth is predicted from the time and environmental conditions. The relative lag time is sampled from the RLT distribution and deducted from the predicted growth. If the predicted generations of growth do not exceed the lag time, no growth is predicted. If it does, the growth predicted to have occurred is given by the difference between the predicted generations of growth less the RLT.

A3.9.4 Jameson Effect

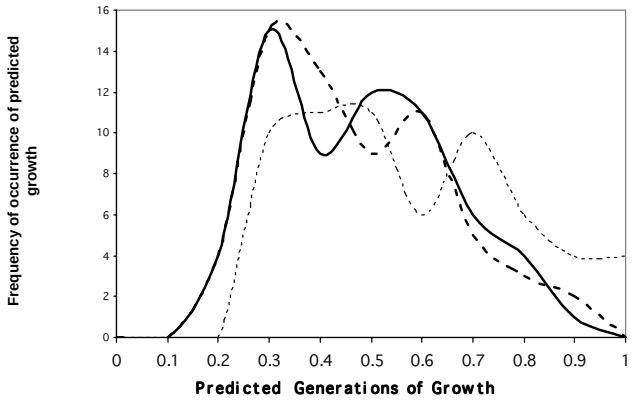
There has been very little work done to include in predictive models factors that contribute to the "Jameson effect" (Stephens et al., 1997), i.e. the suppression of growth of all micro-organisms in the food by high *total* microbial loads. In some products, this effect may greatly reduce the health risk from *L. monocytogenes* predicted on the basis of models currently available. Example 4 in this report (cold-smoked fish) introduces a method for inclusion of the Jameson effect in exposure assessment modelling. It models the increase in spoilage or other microorganisms, or both, on the product simultaneously with the growth of *L. monocytogenes*. If the predicted growth of other microbiota is predicted to exceed 10^9 CFU/g, the predicted growth of *L. monocytogenes* is modified accordingly. Full details are given in the example.

A3.9.5 Physiological state of cells

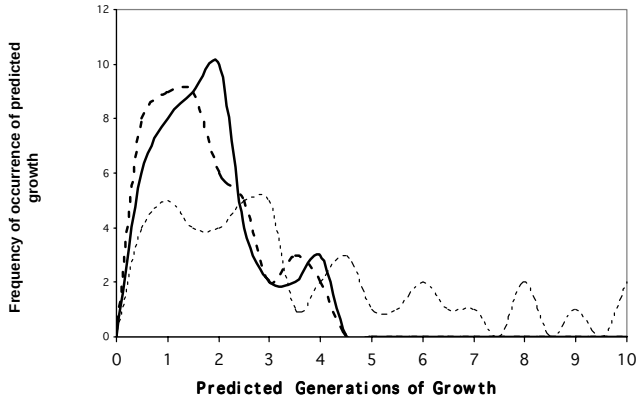
Environmental and physiological factors during food processing or present in foods are reported to affect the infectivity or virulence, or both, of *L. monocytogenes* (Buchanan et al., 1994; Zemser and Martin, 1998). These have been reviewed (Rees et al., 1995; Archer, 1996; Rowan, 1999), and also specifically in relation to *L. monocytogenes* in foods (Lou and Yousef, 1999). Conversely other workers (Conte et al., 1994; Gahan and Hill, 1999) have found little effect of environmental conditions on virulence.

Harsh environments in foods that stress the microbial cell produce a response that makes the cell more resistant to subsequent stressful or potentially lethal conditions, extending the survival of the cell under those conditions. Cells that are in stationary phase will also have increased tolerance to potentially lethal environments. This phenomenon has been suggested as increasing the chance that pathogenic bacteria, including *L. monocytogenes*, will survive passage through the acid environment of the stomach, thereby effectively increasing their virulence.

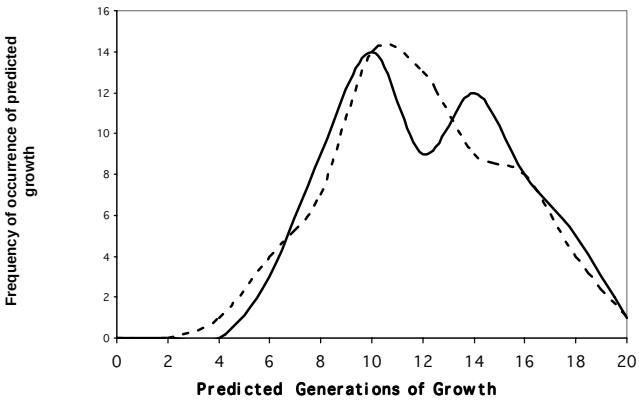
(a)



(b)

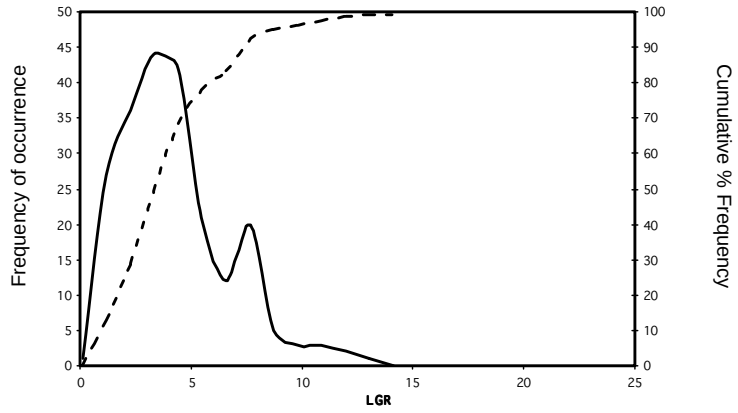


(c)

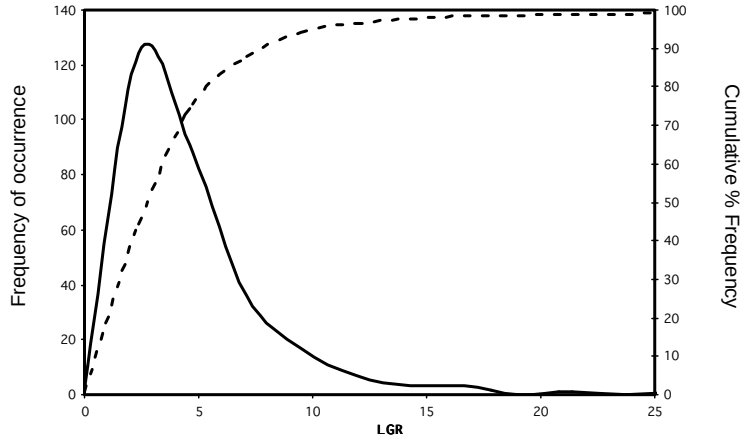


Figures A3.2 Graphs showing the distribution of the predicted number of generations of growth of pseudomonads during (a) transport from retail to home, (b) foodservice, and (c) domestic storage (home refrigerators) The heavy dashed line represents the predictions based on the average temperature; the solid line represents predictions based on time-temperature function integration; and the light dotted line represents predictions based on the maximum temperature recorded.

(a)



(b)



(c)

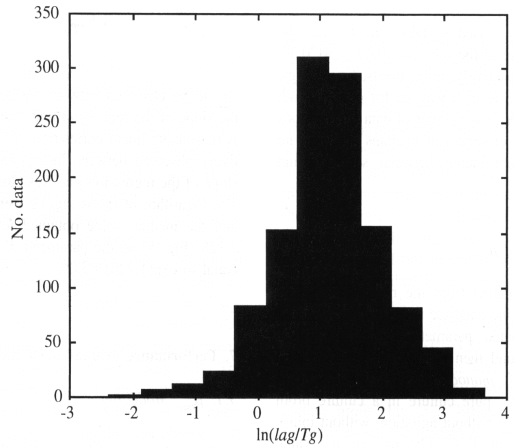


Figure A3.3 Distribution of relative lag times reported for *Listeria monocytogenes*: (a) grown in broth under laboratory conditions (collated in Ross, 1999); (b) in foods (collated in Ross, 1999); and (c) collated by Augustin and Carlier (2000) for all sources and plotted as ln(RLT). In figures (a) and (b), the dotted lines represent the cumulative frequency.

While these effects on virulence, and the chance of infection, are recognized, they are not well characterized and may be specific to strains and conditions (Buncic and Avery, 1996; Buncic, Avery and Rogers, 1996).

There is also uncertainty about the virulence of foodborne strains of *L. monocytogenes*. Other than that most cases of foodborne illness have been associated with serotypes 4a and 1/2a/b, specifically virulent strains cannot yet be differentiated. Notermans et al. (1998) studied the infectivity of more than 20 foodborne strains of *L. monocytogenes*, using a mouse bioassay and chick embryo test. Despite observing differences in virulence between strains, they concluded that almost all *L. monocytogenes* serovars present in foods have clear virulent properties, and should be considered potentially pathogenic, a view shared by McLauchlin (1996). Conversely, some exposure assessments (Farber, Ross and Harwig, 1996; Bemrah et al., 1998) have assumed that only 1–10% of foodborne *L. monocytogenes* are pathogenic.

The above issues are also discussed in detail in Part 2 of the main report.

A3.10 MODELLING CONTAMINATION AND RE-CONTAMINATION

There is little data available upon which to enable cross-contamination, or its effects, to be modelled quantitatively. There are a number of variables that might be considered:

(i) If contact with contaminated material occurs, how often does cross-contamination result?

(ii) At what point in the food chain does it occur?

(iii) What is the potential for growth on fomites, such as cutting equipment?

(iv) How much material is transferred and does the nature of the source affect the amount transferred?

A3.10.1 Source and amount of material transferred

FAO/WHO (2002) cites the results of Zhao et al. (1998), who developed a model system to enumerate bacteria transferred during common food preparation practices. Zhao et al. (1998) found that chicken meat and skin inoculated with 10^6 bacteria transferred 10^5 to a chopping board and hands and then 10^3–10^4 to vegetables chopped on the unclean board. It should be noted that chicken skin is likely to be wet and this might facilitate the transfer of bacteria, suspended in a surface film of moisture, compared with what might be transferred from RTE foods, which are often "drier" to the touch, e.g. cheeses, processed meats, smoked fish, etc.

A3.10.2 Potential for growth

In risk assessments, because of the assumption in some dose-response models that the risk of infection is directly proportional to dose for the low- to medium-dose range, the estimate of the microbiological risk to a population is largely governed by the estimate of the total numbers of the pathogen in the food supply. How that number of pathogens is distributed among individual packages of foods has less effect on the risk estimate. Accordingly, simple transfer of contamination from one unit of food to another will not affect the risk estimate, unless that transfer is subsequently accompanied by growth (i.e. multiplication) of the pathogen on a contact surface that contaminates uncontaminated material or growth in the (now) contaminated product itself.

In many RTE products, *L. monocytogenes* cells that contaminate the product will remain effectively immobilized at the site of contamination unless there is free liquid in the package, or other modes of transfer, to transport them to other parts of the package. Consequently, *L. monocytogenes* in some foods may be highly localized and exist as discrete pockets of contamination. This may limit the potential for growth of the organism, as nutrients are utilized and wastes accumulate at that site of contamination. Transfer to a new environment provides new potential for growth and potential increase in risk.

Grau (1993) traced the flow of *L. monocytogenes* through meat processing plants and found it to be transferred to many sites, such as trolleys, door handles and the surface packaging of finished products by contact contamination and cross-contamination. In these cases, paucity of nutrients and moisture may inhibit the growth of the organism, and limit the numbers of *L. monocytogenes* transferred to any product.

L. monocytogenes is known to colonize processing plants and, in particular, wet areas in plants. In these areas, if organic matter is present, growth can be expected to occur given sufficient time. Investigations in the United States of America suggest that listeriosis outbreaks often arise when virulent strains "colonize" a production line (Tompkin, 2002). Sites of colonization include hard-to-clean processing equipment. Hollow rollers on production lines are also known to deteriorate and crack, allowing water, nutrients and bacteria to colonize the interior. These niches are very difficult to clean, and provide a reservoir of pathogenic contaminants.

On equipment that is in direct contact with food and becomes fouled with food, growth would be expected to be occur. The amount of growth that could occur would be determined by the product composition, the temperature of that part of the plant, and the time before the contamination was removed by cleaning. As an example, if the processing line were operating at 15°C and slicing a processed meat product (e.g. pH 6.2, a_w 0.975, 100 ppm nitrite), *L. monocytogenes* growing in a residual material on contact surfaces could double in numbers approximately every 5 hours.

A3.10.3 Point in food chain at which contamination occurs

As stated above, there is no increase in risk as a consequence of cross-contamination unless there is increased potential for microbial growth as a result. The amount of increase will depend on the product, its storage conditions, and the time between the contamination event and consumption. If the integrity of the chill chain between the point of production and consumption were uniform, the potential consequences of contamination would be expected to be greater for contamination at the point of production than at the point of retail sale or in the consumer's home. This is because of the increased time available for growth to high numbers before consumption.

A3.10.4 Likelihood of transfer

Even if uncontaminated material comes into contact with contaminated material, the probability of cross-contamination is not absolute, but would be expected to depend on the concentration of pathogens, and their distribution on, or in, the contaminated material.

A3.11 RELATIVE RATE FUNCTIONS

Growth rate modelling can be simplified enormously by using relative rate functions, particularly when combined with square root-type models, or cardinal parameter models. The simple square root model (Ratkowsky et al., 1982) describes the effect of temperature on the growth rate of almost all bacteria. The square root model is:

$$\sqrt{\mu} = b(T - T_{min})$$

where μ is the rate of growth
T is temperature (°C)
b is a constant to be fitted related to the maximum growth rate of the organism
T_{min} is the temperature at which the growth rate is predicted to be zero.

It should be noted that T_{min} is a notional temperature, and is usually several degrees below the minimum temperature at which growth is observed to occur. It should also be noted that the simple square root model above applies only to the sub-optimal temperature region for growth, up to ~35–37°C for *L. monocytogenes.*

If:

- temperature is the only factor affecting the growth rate of a bacterium in a food that varies during the storage and distribution of the product (i.e. if pH, water activity, etc., are constant),
- T_{min} is known for the organism, and
- the growth rate in a product of interest is known at one temperature,

then the growth rate of the organism in that product at any other temperature can be derived using the following relationship, based on the simple square root model (McMeekin et al., 1993):

$$\mu_T = \mu_{REF} * \frac{(T - T_{\min})}{(T_{REF} - T_{\min})}$$

where μ_{REF} is the known growth rate at some temperature T_{REF},
μ_T is the unknown growth rate at some temperature T,
and the other parameters are as previously defined.

For example, FDA/FSIS (2001) collated data for the growth rate at 5°C of *L. monocytogenes* in many RTE food products. Growth at a temperature other than 5°C was calculated using the relationship $\sqrt{\mu_T} = \sqrt{\mu_5}(T - T_{\min})(5 - T_{\min})^{-1}$. This approach has been adopted in several of the exposure assessments in Part 4 of the main report.

The use of the relative rate function is a simplification. As conditions become less favourable for microbial growth, e.g. due to decreased water activity or increased acidity, the difference between T_{min} and the minimum temperature at which growth is possible increases. This was discussed above in relation to models for growth limits under multiple hurdles to growth (e.g. Tienungoon et al., 2000). Bajard et al. (1996) suggested that the simple square root model does not describe the growth rate response to temperature of *L. monocytogenes* as well as it does for other organisms. Nonetheless, in the context of the other sources of

uncertainty that arise in microbial risk assessments, these are considered to be relatively minor deficiencies.

A3.12 PREDICTIVE MICROBIOLOGY MODELS

A summary of some currently available predictive models for *L. monocytogenes* is presented in Table A3.1.

Table A3.1 Summary of predictive models available for the growth, survival and inactivation of *Listeria monocytogenes* in foods.

1.	2.	3. (°C)	4. (a_w or %)	5. pH	6.	7. (µg/ml)	8.	9.	10.
GROWTH									
Y	broth	5–37	0.5–4.5%	4.5–7.5	–	0–1000	aerobic/ anaerobic	N	[1]
Y	food	3–35	2–8%	4.5–7.5	–	–	aerobic	Y	[2]
Y	broth	5–35	0.5–8%	4.6–7.4	–	–	aerobic	Y	[3]
N	broth	4–20 1–20	–	4.5–7.0 4.3–7.2	acetic 0–10 000 lactic 0–20 000	–	aerobic	Y	[4]
Y	broth	9	1.0–4.0%	5.5–6.5	lactic 0–0.6%; acetic 0–0.6%	70	aerobic	Y	[5]
Y	broth	2–46	–	–	–	–	aerobic	N	[6]
Y	broth	4–20	0.5–8%	4.5–7.0	–	–	CO_2: 0–100%, balance N_2	Y	[7]
Y	food	3, 7, 11	0.5	–	–	–	Air: 0.03% CO_2, 78.03% N_2, 20.99% O_2; Modified atmosphere #1: 76% CO_2, 13.3% N_2, 10.7% O_2; Modified atmosphere #2: 80% CO_2, 20% N_2	N	[8]
Y	meat broth	4–30	0.992–0.960 (a_w)	5.4–7	–	–	aerobic	Y	[9]
N	broth	20–35	2–10%	4–8.5	–	–	–	N	[10]
N	broth	1.0–35	0.5–11.5%	4.0–7.2	–	0–200	–	N	[11]
Y	lean beef and fatty beef tissue	0–30.6	–	5.46–6.98	–	–	aerobic	Y	[12]
Y	lean beef fat beef	0–43 0–31	~0.99	5.6–6.7	–	–	aerobic	Y	[13]
Y	broth	3–37	0.5–13%	4.2–7.3	lactic 0–450 mM	–	aerobic	Y	[14]
Y	broth	4–37	0.5–13%	5.6–7	lactic 0, 200 mM		aerobic	Y	[15]
N	roast beef	-1.5 & 3	–	6.1	–	–	vac. pack and saturated CO_2	N	[16]
N	broth	5–30	0.5–8%	4.6–7.4	–	0–400	–	N	[17]
Y	broth	5–35	0.95–0.997	#1 4.6–6.7 #2 4.6–7.4	–	–		Y	[18]
Y	broth	5–37	0.5 & 4.5	6.0 & 7.5	–	0–1000	aerobic and anaerobic	N	[19]

1.	2.	3. (°C)	4. (a_w or %)	5. pH	6.	7. (µg/ml)	8.	9.	10.
Dynamic growth									
Y	fluid whole milk		4, 6, 8, 10, 15, 20, 25, 30, 35	–	–	–	–	Y	[20]
Repair of heat injury									
Y	broth	4–43	0.5–10.0	4.2–9.6	–	–	–	N	[21]
SURVIVAL/GROWTH LIMITS/GROWTH INITIATION									
Probability of growth initiation in defined period of time									
N	broth	4–30	0.5–12.5	>5.9	–	See Note (1)	–	N	[22]
Survival and ongrowth									
		5, 10, 30	0–18	4.19–4.83	–	–	aerobic	N	[23]
Growth limits									
	broth	3.1–35.8 3.1–36.4	0.5–13% 0.5–13%	3.9–7.3 3.9–7.7	lactic 0–500 lactic 0–450	–	aerobic	Y	[24]
Effect of heat stress									
Y	broth	53–60	–	–	–	–	Stationary phase cells	N	[25]
INACTIVATION									
Thermal									
Y	milk (bovine)	60.5–69.5 for 3–60 secs (HTST pasteurization process)	–	–	–	–	–	Y	[26]
Y	food	55, 60, 65	–	5, 6, 7	–	–	milkfat 0, 2.5, 5%	Y	[27]
Y	food	55–65	0–6%	4–8	–	–	sodiumpyro-phosphate 0–0.3%	Y	[28]
Y	food (infant formula)	55, 60, 65	0, 2, 4%	5, 6, 7	–	–	physiological states (lag, exponential, stationary) of test cultures	Y	[29] using data from [41]
Heating rate and thermal inactivation									
Y	broth	50–64	–	–	–	–	Sodiumpyro-phosphate 0–0.3%	N	[30]
Heat resistance									
Y	broth	50, 60, 65	–	–	–	–	Physiological state of cells (end of log phase cells; heat shocked cells; cells resistant to prolonged heat)	Y	[31]
Y	buffer	50, 55, 60	–	–	–	–	–	Y	[32]
Y	broth	30, 10, 5	0–18%	4.19–4.83	–	–	–	Y	[33]
Non-thermal									
N	broth	4 to 42	0.5–19%	3.3–7.3	lactic 0–2%	0–200		N	[34]

1.	2.	3. (°C)	4. (a_w or %)	5. pH	6.	7. (µg/ml)	8.	9.	10.
N	broth	5 to 42	0.5–19%	3.3–7.4	lactic 0–2%	0–200	O_2 levels reduced, N_2 flushed vessels	N	[35]
Y	broth	28	–	4–7	lactic 0–18%; acetic 0–12%	–	–	Y	[36]
Y	broth	4, 19, 28	–	3–4.5	acetic: 0–2.0%; ascorbic: 0–2.0%	–	aerobic	N	[37]
Y	broth	4–42	0.5–19%	–	lactic 0–1%	0–200	–	Y	[38]
COMBINED									
Growth survival death									
Y	broth	4–12	2–4%	6.2	–	–	phenol: 5, 12.5, 20 ppm	Y	[39]
Biotic interactions									
Y	broth	10	2%	5–5.8	0–5 mM protonated lactic acid	–	*Lactococcus lactis* (non-nisin producing)	Y	[40]

KEY TO COLUMNS: (1) Model given? Y = Yes; N = No. (2) Medium. (3) Temperature (°C). (4) Water activity (a_w) or salt (NaCl) percentage. (5) pH. (6) Organic acids. (7) Nitrite, expressed as µg/ml (= ppm). (8) Other. (9) Validation data? Y = Yes; N = No. (10) Source (see below).

NOTES: (1) methyl paraben 0–2%; sodium propionate 0.3%; sodium benzoate 0.1%; potassium sorbate 0.3%; inoculum size 0.01–100 000 CFU/ml; *Listeria* spp. (*L. monocytogenes, L. innocua, L. seeligeri* and *L. ivanovii*).

SOURCES: [1] Buchanan and Phillips, 1990. [2] Murphy, Rae and Harrington, 1996. [3] Wijtzes et al., 1993. [4] George, Richardson and Peck, 1996. [5] Nerbrink et al., 1999. [6] Duh and Schaffner,1993. [7] Fernandez, George and Peck, 1997. [8] Zhao, Wells and Marshall,1992. [9] Lebert, Bégot and Lebert, 1998. [10] McClure, Roberts and Otto Oguru, 1989. [11] McClure et al., 1997. [12] Grau and Vanderlinde, 1993. [13] Grau and Vanderlinde, 1992. [14] Tienungoon, 1998. [15] Ross, 1993. [16] Hudson, Mott and Penny, 1994. [17] McClure, Kelly and Roberts, 1991. [18] McClure, Zwietering and Roberts, 1993. [19] Buchanan, Stahl and Whiting, 1989. [20] Alavi et al., 1999. [21] Chawla, Chen and Donnelly, 1996. [22] Razavilar and Genigeorgis, 1998. [23] Cole, Jones and Holyoak, 1990. [24] Tienungoon et al., 2000. [25] Breand et al., 1998. [26] Piyasena, Liou and McKellar, 1998. [27] Chabra et al., 1999. [28] Juneja and Eblen, 1999. [29] Xiong et al., 1999. [30] Stephens, Cole and Jones, 1994. [31] Augustin, Carlier and Rozier, 1998. [32] Linton et al., 1995. [33] Cole, Jones and Holyoak, 1990. [34] Buchanan and Golden, 1995. [35] Buchanan, Golden and Phillips, 1997. [36] Buchanan and Golden, 1995. [37] Golden, Buchanan and Whiting, 1995. [38] Buchanan et al., 1994. [39] Farber, Cai and Ross, 1996. [39] Membre, Thurette and Catteau, 1997. [40] Breidt and Fleming, 1998. [41] Linton et al., 1996.

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Appendix 4. Prevalence and incidence of *Listeria monocytogenes* in Fermented Meat Products

A4.1 REPORTED PREVALENCE AND INCIDENCE

The prevalence and incidence *of Listeria monocytogenes* in fermented meat products (FMPs) as reported in the literature is summarized in Table A4.1. It is to be noted, as mentioned in Section A4.2, below, that some products in the list in Table A4.1 might have a single name but represent very different products and processes in different countries. The authors have not attempted to distinguish these in the risk assessment modelling, but have instead treated all prevalence and concentration data as representative of all FMPs.

Table A4.1 Reported prevalence and incidence *of Listeria monocytogenes* in fermented meat products

Product Description	Positive (samples or proportion)	Samples	% positive	Conc.	Location of Survey	Ref.
Fermented sausages	up to 0.20	5	20.00%		Various countries	[1] [2]
Fermented sausages					Austria	[3]
Fermented sausages	4	21	19.05%		Yugoslavia	[4]
Raw sausage	16	20	80.00%		Brazil	[5]
Fermented sausage	0.22 to 0.83				Spain	[6]
Dry sausages	0.22 to 0.83	18	44.00%		Various countries	[7] [8] [9] [10] [11]
Fermented sausages	6	30	20.00%		Canada	[12]
Raw sausage	13	25	52.00%		UK	[13]
Mettwurst with onion, fresh	1	11	9.09%		Germany	[14]
Sausages	2	8	25.00%		Hungary	[15]
Mettwurst with onion	27	245	11.00%		Germany	[16]
Spreadable, fermented	43	381	11.30%		Germany	[16]
Sliceable, fermented	11	228	4.80%		Germany	[16]
Raw sausage	30	120	25.00%	<100 CFU/g	Germany	[17]
Mettwurst, coarse	6	30	20.00%	<1000 CFU/g	Germany	[18]
Mettwurst, fresh	18	30	60.00%	<1000 CFU/g	Germany	[18]
Raw sausage, salami type	5	30	16.67%	<100 CFU/g	Germany	[18]
Beef sausage	0	1	0.00%		UK	[19]
Sausage	0	3	0.00%		UK	[19]
Raw fresh sausages	4	98	4.08%		France	[20]
Raw sausage	12	68	17.65%		Germany	[21]
Mettwurst, fresh	22	132	16.67%		Germany	[22]

Product Description	Positive (samples or proportion)	Samples	% positive	Conc.	Location of Survey	Ref.
Raw sausage, sliced	2	126	1.59%		Germany	[22]
Salsiccia	6	52	11.54%		Italy	[23]
Fermented sausages, salami type	0	70	0.00%		Norway	[24]
Ground/minced muscle (dry fermented sausages)	36	308	11.69%		Belgium	[25]
Fermented sausages	up to 0.20	5	20.00%	less than in non-fermented RTE cooked meats		[26]
Salami		128	10.00%		UK	[27]
Salami		67	16.00%		UK	[28]
Salami		59	5.00%	20 CFU/g	Switzerland	[29]
Mettwurst		14	0.00%		Switzerland	[30]
Dry cured		136	10.00%		Hungary	[31]
Fermented		21	10.00%		Hungary	[31]
Smoked		23	13.00%		Hungary	[31]
Cervelat		44	0.00%		South Africa	[32]
Vacuum-packed salami		19	0.00%		Australia	[33]
Salami		132	40.00%		Australia	[34]
Uncooked, preserved meat products						
1994/5 data	77	328	23.50%	1.8% > 10 (& <100) CFU/g; 0.6% >100 CFU/g		
1997 data from retail-level processors	19	132	14.40%	13.6%>10 (& <100) CFU/g; 0.8% > 100 CFU/g	Denmark	[35]
1998 data from retail-level processors	37	225	16.50%	14.7%>10 (& <100) CFU/g; 1.8% > 100 CFU/g		

SOURCES: [1] Breer and Schopfer, 1989. [2] Farber, Sanders and Johnston, 1989. [3] Breuer and Prandl, 1988. [4] Buncic, 1991. [5] Destro, Serrano and Kabuki, 1991. [6] Encinas et al., 1999. [7] Farber, Sanders and Johnston, 1989. [8] Nicolas and Vidaud, 1985. [9] McClain and Lee, 1988. [10] Breuer and Prandl, 1988. [11] Schmidt et al., 1988. [12] Farber, Sanders and Johnston, 1989. [13] Gilbert, Hall and Taylor, 1989. [14] Karches and Teufel, 1988. [15] Kiss et al., 1996. [16] 1991–2 data supplied to FAO/WHO by BgVV, Germany, in response to a call for data, 2000. [17] Leistner and Schmidt, 1992. [18] Leistner, Schmidt and Kaya, 1989. [19] MacGowan et al., 1994. [20] Nicolas and Vidaud, 1985. [21] Noack and Jockel, 1993. [22] Ozari and Stolle, 1990. [23] Pacini et al., 1995. [24] Rørvik and Yndestad, 1991. [25] Uyttendaele, Troy and Debevere, 1999. [26] WHO, 1988. [27] Velani and Gilbert, 1990. [28] Gilbert, 1991. [29] Trüssel, 1989. [30] Trüssel, 1989. [31] Kovacs-Domjan, 1991. [32] Vorster, Greebe and Nortje, 1993. [33] Grau and Vanderlinde, 1992. [34] Varabioff, 1992. [35] Nørrung, Andersen and Schlundt, 1999.

A4.2 PRODUCTION METHODS AND STYLES OF FERMENTED MEATS

A4.2.1 Introduction to fermented meat products

Fermented meats, including salami, have been manufactured for centuries (Lücke, 1985; Leistner, 1995; Ricke and Keeton, 1997). European sausages have been produced since the Middle Ages and per capita production and consumption of fermented meat products (FMPs) is still greatest in Europe. European migrants to North America, and elsewhere, took their FMPs methods and styles with them to their new homelands, where new variations evolved, i.e. these traditional products in some cases were changed to suit conditions in the New World. It is important, then, to recognize that FMPs products from the Old and New Worlds that have the same name may often differ in composition and processing. For example, all "Mettwurst' and "Teewurst" in the United States of America is cooked, and NaCl levels in United States of America products are normally higher than their European counterparts, due to regulations for the control of the parasite, *Trichinella*. United States of America producers typically use nitrite only, with no nitrate added (B. Tompkin, pers. comm., 2001).

Dry sausages include chorizo (Spanish, smoked, highly spiced), frizzes (similar to pepperoni, but not smoked), pepperoni (not cooked, air dried), Lola or Lolita and Lyons sausage (mildly seasoned pork with garlic), and Genoa salami (Italian, usually made from pork but may have a small amount of beef; in the preparation process it is moistened with wine or grape juice and seasoned with garlic).

Chinese-style fermented sausages, with pork as the main ingredient, are also common in Asia and date back thousands of years (Leistner, 1995; Yu and Chou, 1997). The Thai fermented sausage Nham is also receiving attention in the scientific literature (ASCA, 1986; Petchsing and Woodburn, 1990).

Most FMPs products have long shelf lives due to the combination of acidification (through fermentation), removal of oxygen, addition of compounds that favour the growth of some microbes while retarding the growth of others, and, ultimately, the removal of water.

Semi-dry sausages are usually heated in a smokehouse to fully cook the product and partially dry it. Semi-dry sausages are semi-soft sausages with good keeping qualities due to their lactic acid fermentation. "Summer Sausage" (another word for cervelat) is the general classification for mildly seasoned, smoked, semi-dry sausages like mortadella and Lebanon bologna.

Unless otherwise noted the information in these sections is drawn from Lücke (1985), Leistner (1995), Lücke (1995) and Ricke and Keeton (1997).

A4.2.1 Processing

The fundamental steps involved in the production of FMPs are:

- chopping and mixing of ingredients, and filling into casing;
- fermentation; and
- drying (or maturation).

A4.2.3 Ingredients

Meat and Fat

From a product quality perspective, the type of meat used in FMPs is important. It is less important for the microbiological safety of the product, unless some types of meat are more highly contaminated with pathogens than others. The proportion of meat to fat, and the type of fat, is not important microbiologically except in the sense that the proportion of fat affects the amount of free water in the product. Of the lean muscle in the mix, about 70–75% by weight is water. It is the *concentration* of the additives in the aqueous (water) phase of the food that is important for understanding the microbiology of the product. More fat in the mixture means that there is less lean meat, which in turn means less water. As a guide, for a product containing 30% (by weight) fat, water makes up only about 53% of the weight of the batch. Thus, the effective concentration of any water-soluble additives is about twice that predicted simply on the basis of its weight compared to the overall weight of the batch. During maturing of FMPs, weight losses of 20–30% occur in "semi-dry" FMPs, and even more for "dry"-style products. This is due to loss of water only, and further increases the effective concentration of the water-soluble components, so that the final concentration can be up to four times the apparent level added to the mixture expressed on a weight-for-weight basis.

Table A4.2 Typical physico-chemical properties of styles of finished FMPs products.

Category	Final pH	Lactic acid (%)	Moisture : protein ratio	Moisture loss	Moisture[(1)]	Comments
Dry sausages	5.0–5.3 (<5.3)	0.5–1.0	<2.3:1	25–50	<35	See Note (2)
Cervelat			1.9:1		32–38	Shelf-stable
Cappicola			1.3:1		23–29	Shelf-stable
German Dauerwurst	4.7–4.8		1.1:1		25–27	Shelf-stable
German salami	4.7–4.8		1.6:1	1	34–35	Shelf-stable
Peperoni	4.5–4.8	0.8–1.2	1.6:1	35	25–32	Shelf-stable
Italian salami, hard or dry			1.9:1	30	32–38	Shelf-stable
Genoa salami	4.9	0.79	2.3:1	28	33–39	Shelf-stable
Thüringer, dry	4.9	1.0	2.3:1	28	46–50	Shelf-stable
Semi-dry sausages	4.7–5.1 (<5.3)	0.5–1.3	>2.3<3.7:1	8–15	45–50	See Note (3)
Lebanon bologna	4.7	1.0–1.3	2.5:1	10–15	56–62	Refrigerate
Cervelat, soft			2.6:1	10–15		Refrigerate
Salami, soft			2.3–3.7:1	10–15	41–51	Refrigerate
Summer sausage	<5.0	1.0	3.1:1	10–15	41–52	Refrigerate
Thüringer, soft			3.7:1		46–50	Refrigerate
For comparison						
Dried beef			2.04:1	29		
Beef jerky			0.75:1	>50	28–30	
Air-dried sausage			2.1:1			

NOTES: (1) Water activity ranges for dry and semi-dry sausages are <0.85 to 0.91, and 0.90 to 0.94, respectively. European Council Directive 77/99/EEC (Health problems affecting the production and marketing of meat products and certain other products of animal origin) requests a_w of <0.91 or pH <4.5 for dry sausages to be shelf-stable, or a combined a_w and pH of 0.95 and <5.2, respectively. (2) Heat processed (optional, but see note (4)); dried or aged after fermentation for moisture loss; smoked.(3) Heat processed (but see Note (4)); typically smoked; packaged after processing and chilling.(4) USDA/FSIS Title 9 CFR may be amended to require specified time and temperature heating combinations after fermentation, or verification that processing conditions destroy all pathogenic micro-organisms.SOURCES: Various authors, cited in Ricke and Keeton, 1997.

Salt

Typically, 2.5–3.3% NaCl (w/w) is added to FMPs mixes. The water activity (a_w) of the product decreases during processing as the product loses water. This leads to effective concentrations in the typical finished semi-dry product of about 7.5–12% salt, corresponding to water activities in the range 0.95–0.92. Lower water activities (~0.85) are achieved in southern European style dry sausages (Calicioglu et al., 1997; Ricke and Keeton, 1997). Water activity values can be translated to aqueous phase NaCl concentration by reference to calibration tables or curves, e.g. Chirife and Resnik (1984).

Sugar, pH and organic acids

Sugars (0.4–0.8%) are added to the mixture as a carbon source for the fermentative bacteria. These bacteria, usually lactic acid bacteria, metabolize the sugars, producing lactic acid in the process, which is released into the FMPs. In a review of lactic acid bacterial fermentation and the principal antimicrobial factors produced by lactic acid bacteria, Adams and Nicolaides (1997) concluded that the principal antimicrobial factor is the ability of all lactic acid bacteria to produce organic acids and decrease the pH of foods in which they grow.

The biochemistry of conversion of simple sugars (e.g. glucose) results in almost twice as much lactic acid being produced as the concentration of simple sugars added. For more complex sugars, a smaller ratio of lactic acid to sugar results due to incomplete utilization of the carbohydrate. Other organic acids are also produced, but at much lower levels. The presence of lactic acid reduces the pH of the product during fermentation, typically to the range 4.6–5.0. The range of lactic acid concentrations in the final product is shown in Table A4.2. The range corresponds to total effective lactic acid concentrations (i.e. in the water phase) of from about 100 (e.g. semi-dry) to 500 mM (pepperoni).

Other additives

Other ingredients of FMPs may include a variety of spices, and nitrite or nitrate. Spices, including pepper, paprika, garlic, mace, pimento and cardamom, may be added, but their primary role is sensory. The redox potential (E_h) of FMPs is low. After mixing, the unfermented product is stuffed into casings. This effectively removes some oxygen. The predominant spoilage organisms of raw, aerobically stored meat, will be included in the mix and quickly consume residual oxygen. The presence of ascorbate and sugars also contributes to the creation of a low redox potential in the sausage.

A4.2.4 Production of “safe” FMPs

Production of safe FMPs requires prevention of the growth of pathogens during the fermentation step and maximizing death of surviving pathogens during maturation and storage. Some processors (especially in North America) include a heating step after fermentation that is intended to inactivate pathogens, including *Salmonella*, pathogenic *E. coli* and *Trichinella spiralis*. The initial stages of the fermentation process can permit growth of enteric pathogens such as *Salmonella*, *E. coli* and *Staphylococcus aureus*. The rapid acidification of the medium by the starter culture is considered a critical control point for minimization or prevention of pathogen growth (Bacus, 1997).

A4.3 PHYSICO-CHEMICAL PARAMETERS OF FMPs

See Table A4.3 and Figure A 4.1

Table A4.3 Typical composition and processing parameters for various FMPs styles

	Semi-dry sausage	Dry sausage	
		Northern European type	Southern European type
Examples	Summer sausages German cervelat Bologna sausages	German salamis Danish salamis	Italian salamis (Milanese; Calabrese) Saucissons secs Spanish chorizos
Raw mixture			
Meat : fat	Pork or beef, lean and fat	Lean pork : lean beef : fat pork (1 : 1 : 1)	Lean pork : fat pork (2 : 1)
Sugars (e.g. glucose, lactose, sucrose)	0.3–1.5%	0.3–0.8%	0–0.4%
Nitrate	–	–	<300 ppm
Nitrite	0–150 ppm	20–200 ppm	20–200 ppm
NaCl	2–2.5%	2–2.5%	2–2.5%
Seasoning (e.g. pepper, garlic, cardamom)	++	++	++
Starter cultures (10^6 CFU/g)	Yes	Yes	Yes
Lactobacillus sakei, L. curvatus, L. plantarum	+	++	+++
Pediococcus acidilactici, P. pentosaceus	+++	+++	+
Staphylococcus carnosus, S. xylosus	–	++ or –	++
Kocuria varians	–	++ or –	+
Penicillium chrysogenum, P. nalviogense	+	–	++
Debaryomyces hansenii, Candida lipolytica	+	–	++
Fermentation period (time/temperature/ relative humidity)	15–20 h/27–41°C/90% (USA) 18–48 h/20–32°C/85–95% (Germany)	18–48 h/20–30°C/58–95%	Day 1 – 22–24°C/94–96% Day 2 – 20–22°C/90–92% Day 3 – 18–20°C/85–88% or 2–3 d at 22–25°C/90–95%
Drying period (time/temperature/relative humidity)	2–3 d/10°C/68–72% (USA) 10–25 days (Germany)	1–3 weeks/12–15°C/75–80%	4–6 weeks/12–15°C/75–78%, or 8–14 weeks (traditional)
Method of production			
Smoking	Yes	Yes	No
Cooking	Yes	No	No
Product caracteristics			
Final pH	4.4–5	4.6–5.1	5.1–5.5
Final a_w	0.93–0.98	0.92–0.94	0.85–0.86
Water content	40–50%	30–40%	20–30%
Moisture : protein ratio (w/w)	2.3–3.7	2–2.3	1.6–1.9

KEY: + = occasionally used; ++ = frequently used; +++ = regullarly used; – = not used.

SOURCE: Reprinted from Montel, M.C. Fermented meat products. pp. 745–753, *in:* R.K. Robinson, C.A. Batt and P.D. Patel (eds). *Encyclopaedia of Food Microbiology.*

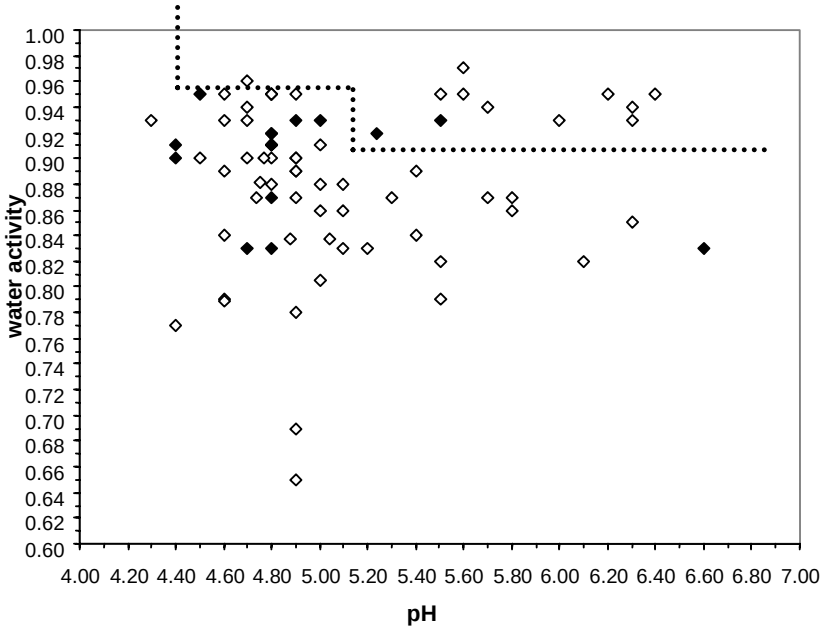

Figure A4.1 Collation of final pH and water activity of fermented meat products available in North America (open diamonds) and Australia (closed diamonds). pH and water activity limits below which the product is considered safe, in the absence of other inhibitors of microbial growth, are also shown (dotted lines)

SOURCE: After Ross et al., in press.

A4.4 ESTIMATION OF LACTIC ACID CONCENTRATION IN FMPs

The primary determinant of pH in meat and fermented meat is lactic acid. Natural levels in post-mortem meat are up to 125 mM (Gill, 1982) for meat in the pH range 5.5–6.5.

pH values, lactic acid and moisture content levels presented in Table A4.1 were tabulated. The lactic acid level was converted to lactic acid (using the Henderson-Haselbalch equation) in the aqueous phase and pH plotted against lactic acid concentration. Data for pH and lactic acid concentration in meat were also included in the tabulation, and the data plotted (*see* Figure A4.2).

The simple regression through the data is described by the line:

$$\text{lactic acid (ppm)} = 50 + (((6.6\text{-pH})/2.3) \times 300)$$

The model was generated based on the assumption that the lactic acid concentration in meat at pH 6.6 (highest pH reported in salami in Figure A4.1) is 50 mM, and that the lactic acid concentration in the salami with the lowest pH (4.3) is ~350 mM (*see* Section A4.2). It was further assumed that pH was directly related to lactic acid concentration in the range pH 6.6–4.3.

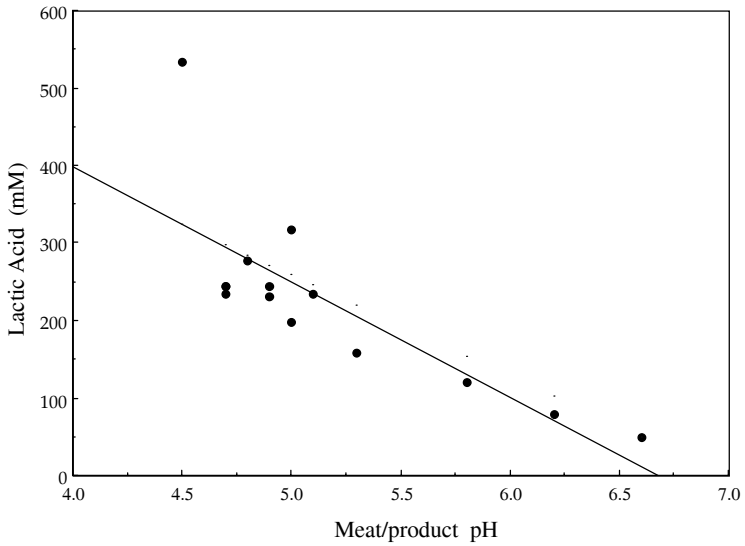

Figure A4.2 Relationship between the pH of FMPs and the aqueous phase lactic acid concentraton.

A4.5 DATA ILLUSTRATING THE REMAINING SHELF-LIFE OF AUSTRALIAN FMPs AT THE TIME OF PURCHASE BY DOMESTIC CONSUMERS

An ad hoc survey was conducted of retail outlets in Hobart, Australia, including supermarkets and small stores and delicatessens, of nominal remaining shelf-life of fermented meats on retail display. The survey involved 28 samples of 13 different products from 3 Australian producers. The survey was part of a larger survey of all processed meats, involving >700 samples.

By comparing the "use-by" (or "expiration") date with the survey data, it is possible to infer the nominal shelf-life remaining. From the "use-by" date and manufacture date it is possible to infer the total shelf-life. The survey revealed that the mean stated shelf-life of Australian FMPs is 140 (±70) days, but samples included only three producers.

It is assumed that the survey represents a snapshot of the remaining shelf-life that a purchaser would have available to them. Full details of the survey are presented in Ross et al. (in press).

A summary is shown in Figure A4.3.

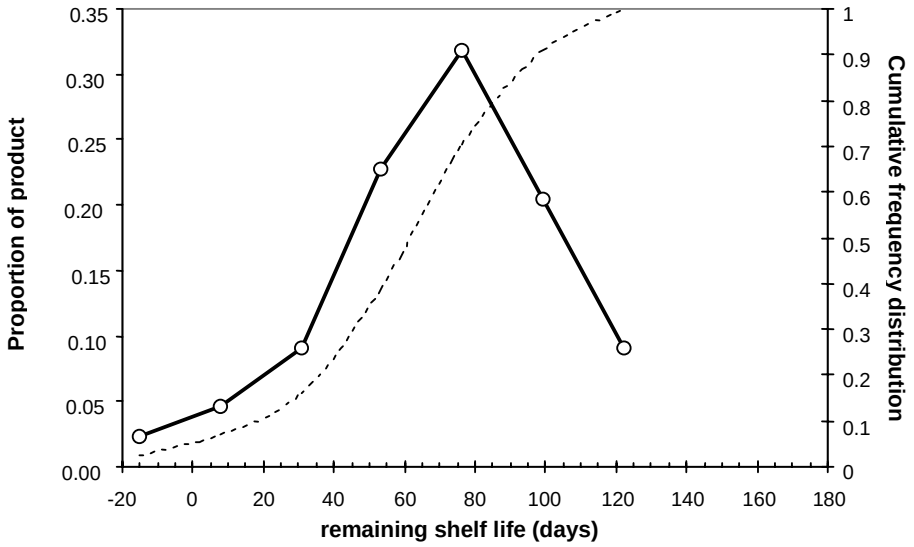

Figure A4.3 A histogram of nominal remaining shelf-life of Australian fermented meats on retail display (heavy lines) and the cumulative frequency curve derived from that data. The sample size was 24, comprising 13 different products from three producers.

A4.6 SERVING SIZE DISTRIBUTION

See Figure A4.4.

A4.7 NATIONAL CONSUMPTION OF FMPs

Differences in consumption between nations is expected and reported indirectly (Holdsworth et al., 2000), but practically no data quantifying national FMPs production were found. Typically, FMPs statistics are included in total processed meat statistics. However, some data were extracted from supermarket sales records and nutritional surveys, as described below.

United States of America

FDA/FSIS (2001) estimated an average of 6.41 servings of FMPs per annum for a population of 271 000 000 (NGS, 1999). This consumption is relatively low compared to other developed nations, and expert opinion (B. Tompkin, pers. comm., 2001) also suggests that this estimate of consumption is unrealistically low. The 50th percentile serving size is 46 g. This equates to 295 g/person-year, or a total national consumption of 82 265 tonne/year. It is noted that total consumption of "deli meats" in the United States of America is 12 times higher than FMPs consumption.

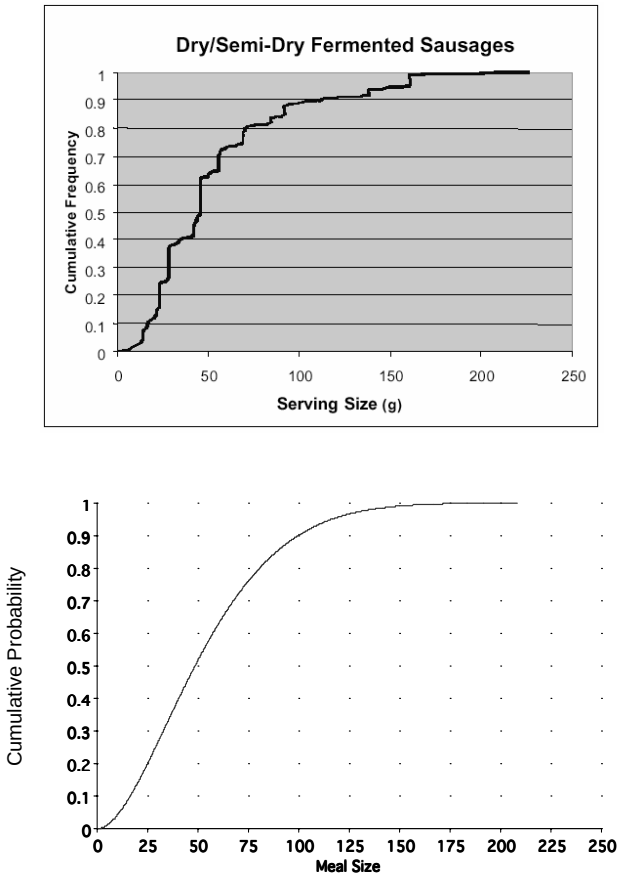

Figure A4.4 Comparison of serving size distribution from United States of America data (FDA/FSIS, 2001) (top) and that generated by the model used in this study (bottom).

Australia

National survey data (ABS, 1995) suggests that annual per capita consumption of processed meat is between 5 and 19 kg, but this could include sausages and other meat products intended to be cooked prior to consumption. From diverse Australian production and sales data reviewed by Ross et al. (in press), it was estimated that total production of FMPs in Australia was 7795–32 379 tonne/year, equivalent to an annual per capita consumption of 400–1680 g. It is noted that this also suggests that in Australia the total FMPs consumption is one tenth to one twelfth of total processed meats, as reported for the United States of America. The Australian population is about 19 800 000 (ABS, 2002).

Canada

National consumption of FMPs in Canada is estimated as 912 g/person/year, based on 5% of consumers daily eating approximately 50 g. Canada has a population of about 31 000 000 (NGS, 1999).

Germany

van Schothorst (1997) suggests that per capita annual average national consumption of processed meats in Germany is 28.5 kg. Assuming that 10% of this consumption is FMPs, (analogous to Australian and United States of America estimates), an estimate of 2.85 kg FMPs/person/year is made. However, German survey data from 1986 (G. Klein, pers. comm., 2000) indicate that per capita annual consumption of semi-dry fermented salami averaged only 723 g in West Germany, lower than that estimated by comparison with other nations. The reason for this large difference in estimates is currently unresolved. The population of Germany is about 81 000 000 (NGS, 1999).

Finland

FFDIF (2000) reported that in 1998 and 1999 national annual per capita consumption of processed meat averaged 32 kg. National consumption included about 7000 tonnes dry sausage, 120 000 tonnes of other sausage, and 38 000 tonnes of hams and other processed meats. The population of Finland is approximately 5 170 000 (NGS, 1999). If it is assumed that dry sausage refers only to FMPs, then consumption is estimated at 1.35 kg/person/year. If the assumption from other nation's data is used, i.e. that 10% of processed meat consumption is FMPs, estimated annual per capita consumption is about 3.2 kg, and for consistency this was the estimated value used in the present risk assessment.

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Appendix 5. Background for the cold-smoked fish assessment

A5.1 ESTIMATE OF GLOBAL PRODUCTION AND NATIONAL AND INDIVIDUAL CONSUMPTION OF COLD-SMOKED FISH

A5.1.1 Scope

The most abundant type of cold-smoked fish is cold-smoked salmon. Due to a paucity of data, cold-smoked fish consumption estimates were based on data describing global production of cold-smoked salmon.

A5.1.2 National and global consumption characteristics

National consumption was initially estimated from data in Globefish (1996)[3] detailing national production and imports/exports of cold-smoked salmon, as shown in Table A5.1. Various other sources of consumption estimates are also shown, and it is noted that the estimates from different sources are not completely consistent, which leads to uncertainty in the estimates.

National population data (NGS, 1999) were combined with the national consumption (calculated as "disappearance" data), to determine per capita consumption. Those data are also shown in Table A5.1.

In Germany and Denmark, hot-smoked product constitutes only a negligible or very small proportion of smoked salmon consumption (P.K. Ben Embarek, pers. comm., 2000; G. Klein, pers. comm., 2000). Similarly, in Australia, hot-smoked salmon products constitute ~10% of production and consumption (Walsh, 1999). Conversely, the contribution of other types of cold-smoked fish is not included in the estimates. Recognizing this limitation, the data are nonetheless used as proxy values for total cold-smoked fish consumption.

From that data, there are various approaches available to calculate the annual per-person consumption of cold-smoked fish and its variability and uncertainty. If the total population of the nations is considered against the total production, the average consumption is 90.2 g/person/year. Per capita consumption in individual nations appears to vary between 8 and 1000 g/person/year, with a median value of 138 g/consumer/year. The average of the estimates of national per-person annual consumption is, however, 231 g. This estimate

[3] Globefish have published an updated report on Salmon - A Study of Global Supply and Demand (Globefish, 2003). This provides more recent data on national production and imports/exports of cold-smoked salmon. However, due to limited time and resources it was not possible to incorporate the more recent data into this risk assessment.

differs from the global average obtained if each national data set has equal weight in the average global consumption estimate. If each national consumption estimate is weighted according to the population size, the global average is calculated to be 146 g. The difference between this and the original global estimates arises because data for Canada, Chile, Germany and West Germany, and Norway could not be used because one element of the needed data was missing; see Table A5.1. The median of the remaining national estimates is 144 g/person/year.

Table A5.1 Production, import and export of cold-smoked salmon, and estimated per capita consumption.

	Production (*P*)	**Import (*I*)**	**Export (*E*)**	**Consumption (inferred from *P+I - E*)**	**Population**	**Consumption per person-year**	**Note**
	(tonne)	(tonne)	(tonne)	(tonne)	(million)	(gram)	
Australia	980	700	–	1 167	18.53	90.7	[1]
Austria	0	848	0	848	8.09	104.9	
Belgium	2 775	2 324	297	4 802	10.23	469.6	
Canada	501	–	501	0	30.59	–	
Chile	1 074	–	1 074	0	15.02	–	
Denmark	15 786	1 406	13 102	4 090	5.24	781.9	[2]
Denmark	–	–	–	–	–	202	[3]
Faeroe islands	407	–	407	0	–	–	
France	11 059	2 941	1 059	12 941	59.07	219.1	
Germany	5 063	7 279	693	11 649	81.95	142.1	
W. Germany	–	–	–	–	–	47.8	[4]
Germany	–	–	–	–	–	1 000	[5]
Ireland	357	–	357	0	3.73	–	
Italy	2 500	5 100	0	7 600	57.72	131.7	
Italy (1998)	–	–	–	9 000	57.72	145.5	[6]
Japan	7 853	765	0	8 618	126.75	68.0	
Netherlands	3 668	0	0	3 668	15.80	232.2	
Norway	2 446	0	2 446	0	4.46	–	
Others	3 954	5615	2 916	6 653	–	–	
Spain	0	313	0	313	39.42	7.9	
Sweden	–	–	–	2 400	8.86	271.0	[7]
Switzerland	0	656	0	656	7.12	92.1	
UK	11 000	146	3 247	7 899	59.36	133.1	
USA	5 116	1133	164	6 085	270.93	22.5	
Total or average	**73 562**	**28 526**	**26 263**	**79 389**	**880.56**	**90.2**	

SOURCES: Data from Globefish (1996) unless otherwise noted.

NOTES: [1] Estimated by Ross and Sanderson, 2000. [2] P.K. Ben Embarek, pers. comm., 2000. [3] Danish food Authority *via* P.K. Ben Embarek, pers. comm., 2000, [4] 1986 data, G. Klein, pers. comm., 2000, based on 1998 population, including East Germany. [5] Buchanan et al., 1997. [6] AC Nielsen data supplied to FAO, 2000. [7] Lindqvist and Westöö, 2000.

A5.1.3 Serving size estimates

Individual consumption frequency by nation

There is limited data available on the number of consumers who eat cold-smoked salmon products. West German data from 1986 (G. Klein, pers. comm., 2000,) reports that 311 of 23 131 interviewees (1.34%) consumed cold-smoked salmon on the survey day and that the mean serving size among eaters was 9.77 g/day, but with an upper 95th percentile of 28.60 g. From the same data source, differences among population sub-groups were revealed but are not used explicitly in this assessment. Using data for consumption of all smoked seafoods, there was no significant difference in serving size by geographical region (north or south Germany) or age group (more or less than 60 years).

In Australia, cold-smoked salmon is considered a luxury food. National consumption was estimated by Ross and Sanderson (2000) at approximately 0.15–0.20 kg/person/year, roughly equivalent to 1% of consumers per day eating a 60 g serving of cold-smoked salmon, or all members of the population eating 3 to 4 servings per year.

Canadian data (CFPNS, 1992–1995) shows that cold-smoked salmon is consumed infrequently in that country. Approximately 5% of consumers on the survey day ate cold-smoked fish products, which included kippered Atlantic herring; cold-smoked Chinook (spring) salmon; smoked haddock; Chinook (lox) salmon; and smoked cod. About half of these were smoked salmonid products. It should be noted that smoked cod is normally cooked before consumption, but represents 8% of eating occasions in the data.

Smoked fish meal size data were estimated by FDA/FSIS (2001) using data from CSFII and NHANES. The data were modified for this case study by removal of data (mostly for smoked oysters) that did not relate to smoked fish products. The average serving size based on age and gender is shown in Figure A5.1. It should be noted that there were few data available – the number of data represented by each bar in the figure varies from 1 to 8.

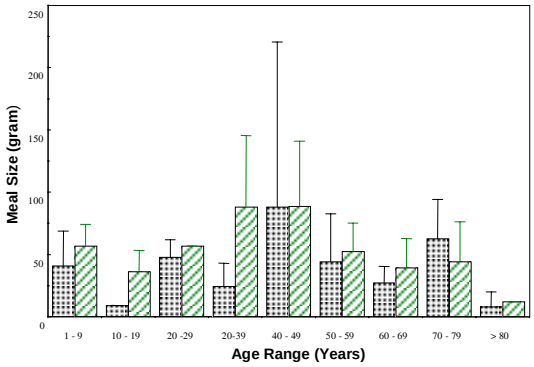

Figure A5.1 Cold-smoked fish serving size as a function of age and gender based on United States of America data (FDA/FSIS, 2001).

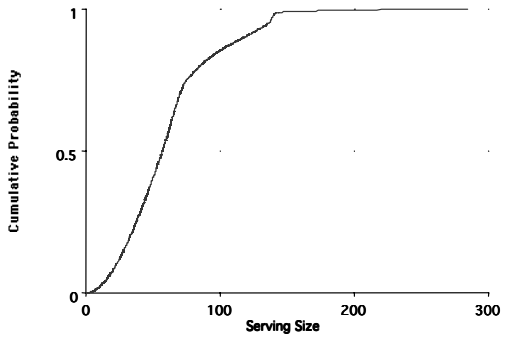

Figure A5.2 Modelled cumulative probability distribution of serving sizes.

Those data indicate that serving size for cold-smoked fish products varies between 1 and 357 g with a median value of ~50 g per serving, and an average value of 58 g/serving. Similarly, using Canadian data (CFPNS, 1992–1995), consumption amounts were aggregated over the smoked fish foods considered, up to the amount consumed on all occasions in a day. The observations are skewed, with median value at 61 g, and long upper tail extending to approximately 225 g, representing approximately the 97.5th percentile.

The above serving size data was used to estimate per capita frequency of consumption from the annual per person consumption estimates in Table A5.1. Those data were used as the basis for the meal size distribution that was used in the model, which is shown in Figure A5.2 and was described in the model using a CumDist function based on the values shown in Table A5.2. The median value of the distribution is 57 g, and the mean value is 63 g.

For the nations considered, the consumption frequency is in the range 0.15–18 servings per person per year, with most in the range 2–5 servings per person per year. The distribution of the number of meals per consumer per year in the model is described empirically by Beta(0.5, 2.5, 0, 18), as shown in Figure A5.3.

Table A5.2 Values used to generate the distribution of serving sizes of cold-smoked fish used in the simulation model.

Serving size (g)	**Cumulative probability**
0	0.00
57	0.50
75	0.75
136	0.95
142	0.99
284	1.00

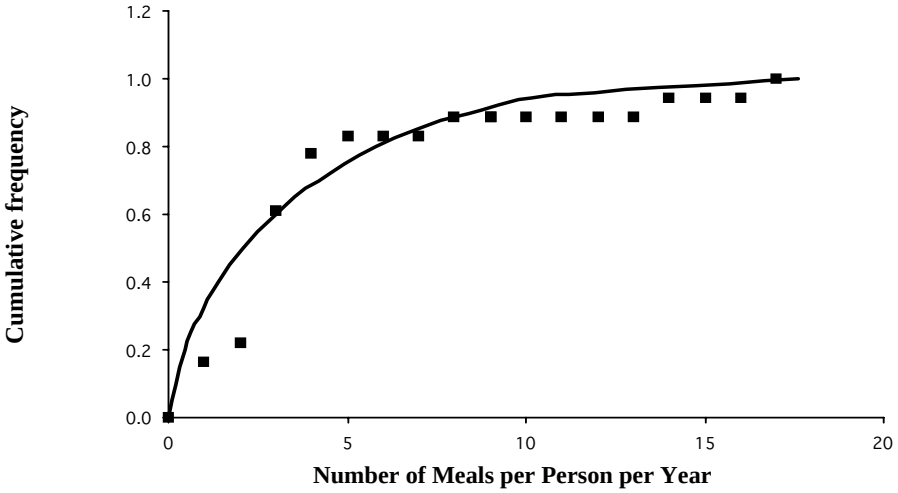

Figure A5.3 Modelled cumulative probability distribution of number of cold-smoked fish servings compared to the observed data.

A5.1.4 Reality check

From the values described above, global total annual consumption in the nations considered can be estimated from the simulation model from the distribution of the product of serving frequency estimate and serving size estimate. This is a useful check on the performance of the simulation model. The median modelled global consumption is 62 300 tonnes and the mean modelled global consumption is 118 000 tonnes. The latter value is ~50% higher than the consumption of cold-smoked salmon estimated from the data in Table A5.1. The basis of this difference is not known with certainty, but may derive from the fact that serving size estimates in the model are derived from all types of smoked fish whereas consumption is based on smoked salmon data only.

A5.2 DESCRIPTION OF STORAGE TEMPERATURES

The storage temperature distribution (Section 4.5.3.4) is derived from Audits International (2000) data for refrigerated cabinets used for storage of cold-smoked fish at retail.

The data used is tabulated below (Table A5.3) and was fitted empirically to a Beta distribution. Comparison of the original data and the fitted distribution is shown in Figure A5.4.

Table A5.3 Data used to simulate storage temperature.

Temperature (°C)	Observed % Frequency	Cumulative % Frequency
-1.67	5	5
0.00	5	10
1.67	8	18
3.33	21	39
5.00	26	65
6.67	16	81
8.33	8	89
10.00	7	96
11.67	2	98
13.33	0.4	98.4
15.00	0.5	98.9
16.67	0.5	99.4
18.33	0.2	99.6
20.00	0.2	99.8
21.67	0.2	100

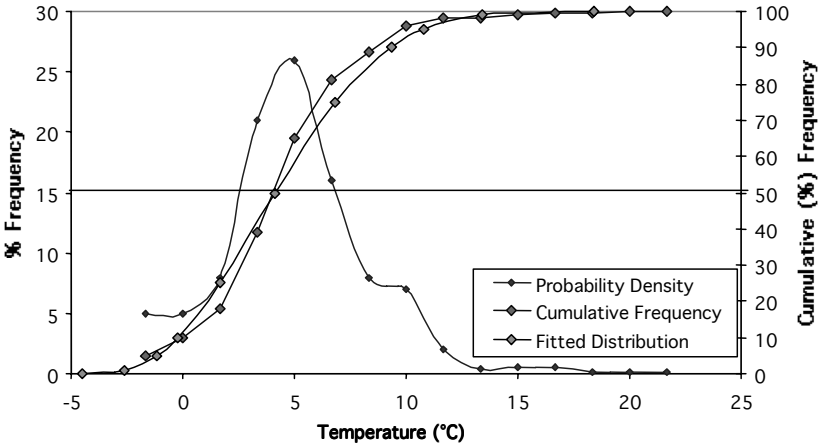

Figure A5.4 Comparison of observed and fitted temperature distribution data.

A5.3 DETAILS OF GROWTH MODELLING

A5.3.1 Physico-chemical parameters

Ranges of physico-chemical parameters of cold-smoked fish that could affect growth of *L. monocytogenes* were presented in Section 4.5.3.5. In the simulation model, these features were described by the following distributions:

- water activity Normal(0.98, 0.0027)
- pH Normal(6.2, 0.05)
- lactic acid concentration (mM) was estimated from pH by the empirical relationship: $1105.0 - 162.50 \times \text{pH}$.

Other growth inhibiting factors (e.g. phenol, spices, etc.) can be included in the model predictions by a simple multiplicative constant.

A5.3.2 *L. monocytogenes* growth rate model

The *L. monocytogenes* growth rate model is derived from Tienungoon (1998). It is a square root type model. The model was further developed, described and evaluated against independent literature data by Ross (1999) and found to have B_f = 0.88 and A_f = 1.94 (measures of predictive model performance – see Ross, 1996, and Baranyi, Pin and Ross, 1999), which was as good as or better than other published models for *L. monocytogenes* growth rate. In the modelling, the growth rate prediction of the model was multiplied by 0.9 to compensate for the bias of the model. This correction was implemented using the "Other growth inhibiting factors" input in the simulation model.

To calculate growth, physico-chemical parameters sampled from the distributions described above are first "filtered" through the growth/no-growth model of Tienungoon et al. (2000) to determine whether the scenario sampled represents a product that will allow growth of *L. monocytogenes.* If growth is predicted to be possible, the extent of growth is modelled using the sampled storage time and the growth rate model, including a correction for lag time.

The prediction of *L. monocytogenes* growth is further filtered by applying an upper limit to the population density (CFU/g product) predicted to be achievable. Including the effects of lactic acid bacteria in the model is expected to preclude this being necessary in most model iterations, but in those (rare) scenarios where *L. monocytogenes* growth is modelled not to be constrained by any other factor, it will eventually limit its own growth, i.e. achieve its maximum population density (MPD). FDA/FSIS (2001) reviewed the available literature and noted that *L. monocytogenes* rarely achieves levels in cold-smoked salmon as high as it does in pure culture in laboratory broth. It is probable, however, that many of those observations are due to the effects of other bacteria in the foods, which are modelled in this assessment. MPD was therefore set at 3×10^9 CFU/g, a level representative of otherwise ideal conditions for those scenarios in which no other factors constrain *L. monocytogenes* growth.

A5.3.3 Lag time

Lag time data specific for *L. monocytogenes* in cold-smoked fish were not found in the literature. Ross (1999) collated data for lag times from the published literature and expressed

these as relative lag times (see Section 3.5.3.3). *L. monocytogenes* relative lag times in foods were in the range 0–40, with a peak value near 2.5. Lag times in laboratory broths had a similar range, but the peak value was nearer to 4.5. Figure A5.5 presents this data.

Dalgaard and Jørgensen (1998) state that *L. monocytogenes* cells that contaminate cold-smoked fish are likely to be damaged due to the effects of processing. Other studies (Rørvik and Yndestad, 1991; Rørvik et al., 1997, 2000), however, suggest that most contamination of cold-smoked fish arises after smoking, from contamination sources in processing plants. Because of this ambiguity, two distributions were assessed to gauge the importance of assumptions about lag time distributions. These were termed "short" (Beta(3, 30, 0, 35)) and "long" (Beta(6, 35, 0, 35)) relative lag times, and produce the distributions shown in Figure A5.6. The overall growth model and the interaction of factors governing the extent of growth are depicted in Figure A5.7.

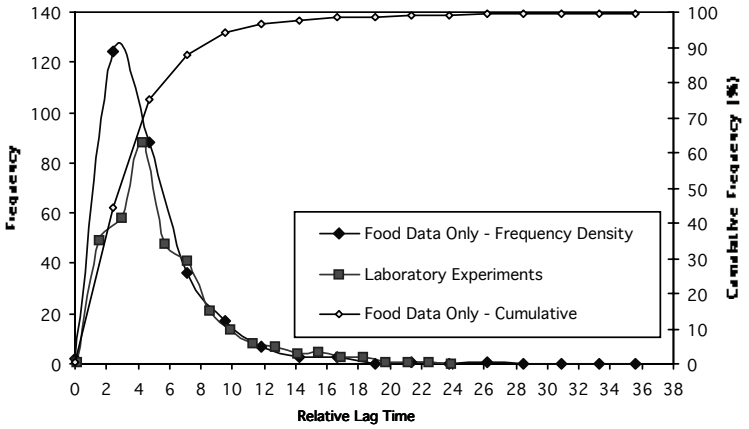

Figure A5.5 Relative lag time data for *Listeria monocytogenes* reported in published literature.
SOURCE: After Ross, 1999.

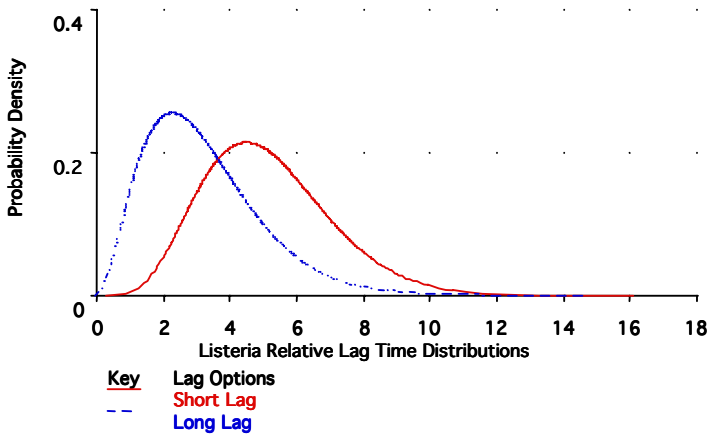

Figure A5.6. Outputs from the simulation model showing the two relative lag time distributions used to model the effects of lag time on risk of listeriosis from cold-smoked fish.

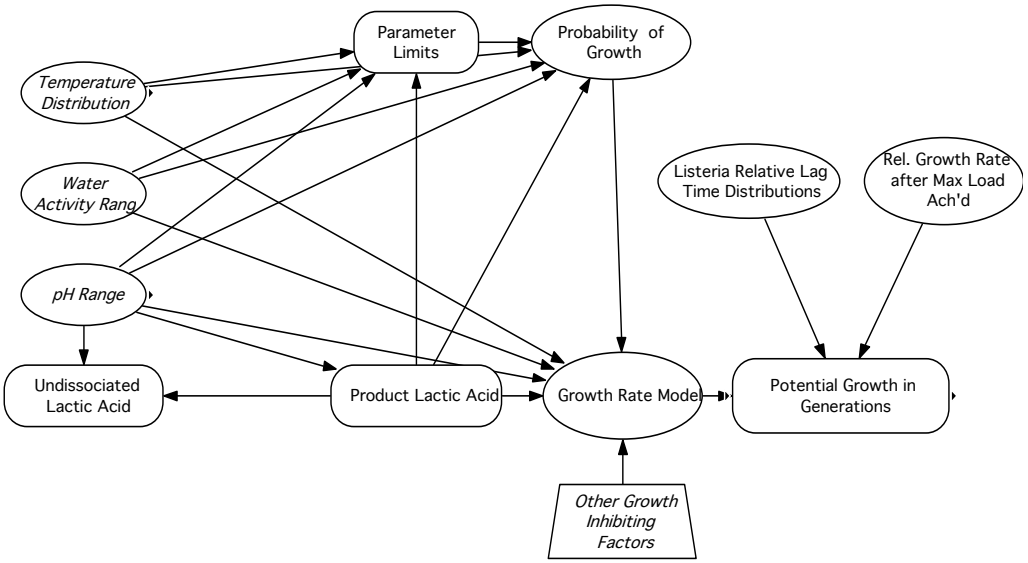

Figure A5.7 Influence diagram taken from the simulation model used in this risk assessment and showing the interaction of factors used to estimate growth of *Listeria monocytogenes* during storage of cold-smoked fish.

A5.3.4 Effect of lactic acid bacteria on shelf life and *L. monocytogenes* growth potential

The nominal shelf lives for vacuum-packed cold-smoked fish are in the range of 3 to 6 weeks at a storage temperature of 4–5°C. Several studies have assessed the sensory acceptability of cold-smoked salmon (Truelstrup Hansen, Drewes Røntved and Huss, 1998; Jørgensen, Dalgaard and Huss, 2000; Leroi et al., 2001) and found that at 5°C the sensory shelf-life of cold-smoked salmon is highly variable (3–9 weeks) and that there is no single indicator of the onset of spoilage. Attributes of cold-smoked salmon associated with spoilage are a softening of the texture and development of "stickiness" or "pastiness", and the presence of "sour", "bitter", "rancid", "ammoniacal", "cabbage" and faecal odours. The lack of a clear relationship between microorganisms present and spoilage is illustrated by data in Figures A5.8 and A5.9, derived from two independent research groups, one working with a Danish product, the other with a French product. Thus, spoilage per se is difficult to model mechanistically.

In raw and processed meats and fish chilled and stored under vacuum, lactic acid bacteria become the dominant population and preserve the product with a "hidden" fermentation (Stiles, 1996). Thus, of particular note in Figures A5.8 and A5.9 is the cessation of growth of any component of the population when the total psychrotrophic count appears to achieve a stationary phase at a level of 10^7–10^8 CFU/g. This behaviour is consistent with the Jameson effect (Stephens et al., 1997; see also Section 3.5.3.1).

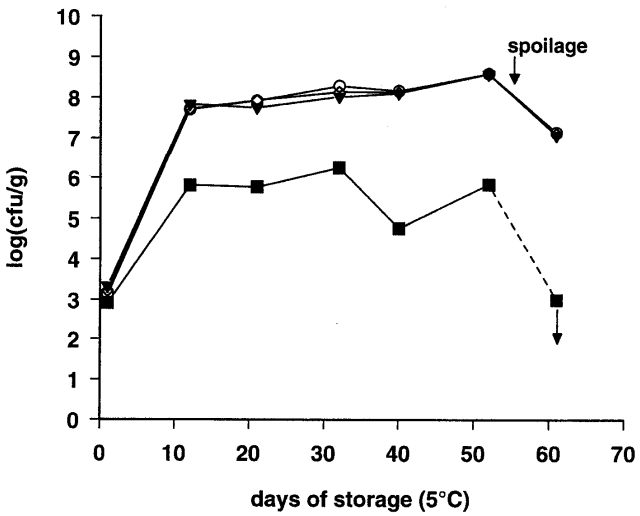

Figure A5.8 Microbiological changes in Danish-produced vacuum-packed cold-smoked salmon (4.6% water phase salt (WPS)) during storage at 5°C. Total count (○); total psychrotrophic count (▼); lactic acid bacteria (◊) and Enterobacteriaceae (■). Arrow indicates the time of sensory rejection. (Data of Truelstrup Hansen, Gill and Huss, 1995, reproduced from Gram and Huss, 1996).

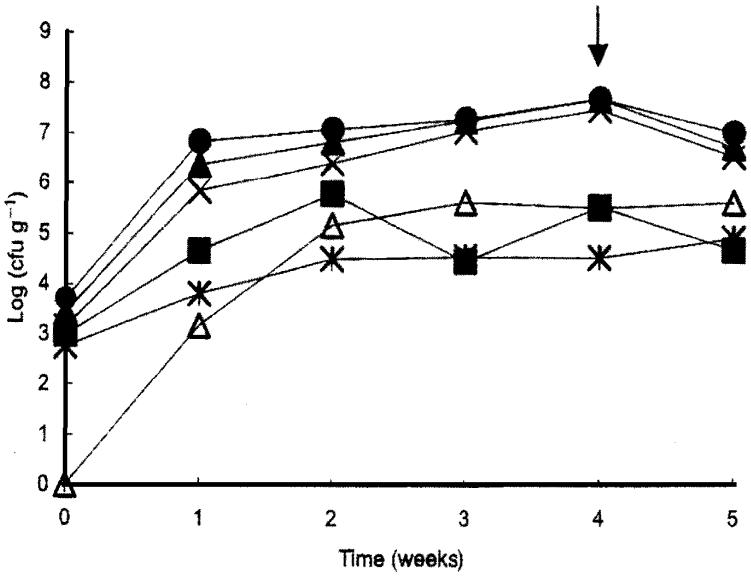

Figure A5.9 Microbiological changes in French-produced vacuum-packed cold-smoked salmon during storage at 5°C. Total psychrotrophic count (●); total lactic acid bacteria (▲); lactobacilli (△); Enterobacteriaceae (■); *Brocothrix thermosphacta* (X) and yeast (✳). The arrow indicates the time of sensory rejection (Reproduced from Leroi et al., 2001).

The Jameson effect can be likened to a race to reach stationary phase. The winner is that sub-group within the total microbial population that first achieves stationary phase.

When that happens, the race is over and all other contestants finish the race (i.e. they also enter stationary phase) at that point in time, although unpublished data (L.A. Mellefont, B. Davidson and T. Ross, Univ. of Tasmania, pers. comm., 2002) indicate that in some cases growth is not completely inhibited, but is nevertheless greatly reduced.

The Jameson effect has relevance for estimation of the risk from microbiological hazards in cold-smoked fish products. As Figures A5.8 and A5.9 show, and as has been reported also for vacuum-packed meats (Mol et al., 1971; Egan, Ford and Shay, 1980; Korkeala et al., 1989), spoilage of vacuum-packed meat and fish does not usually occur until well after the total count has reached stationary phase. In cold-smoked salmon, that occurs after one to two weeks under recommended storage conditions. Thus, growth of pathogens in the product may only be possible for 25–50% of the full shelf-life (use-by period) of the product.

A5.3.5 Modelling the effect of lactic acid bacteria

The mechanism of the Jameson effect is not yet fully understood. It may be due to competition for nutrients, production of toxic end products, or production of specific antibiotics against other bacteria. Under some circumstances, a pathogen may be numerically dominant at the time of production and, under improper storage, may grow fast enough to

reach stationary phase before any other element of the population on the food (e.g. see Nilsson, Huss and Gram, 1997). This possibility is explicitly recognized in the Seafood HACCP Alliance's recommendations (SHA, 1997):

> "In cold-smoked fish, it is important that the product does not receive so much heat that the number of spoilage organisms are significantly reduced. This is true because spoilage organisms must be present to inhibit the growth and toxin formation of *C. botulinum* type E and nonproteolytic types B and F."

Based on Figures A5.8 and A5.9, time to stationary phase of the lactic acid bacteria (i.e. the onset of the Jameson effect) at 5°C was described by Normal(14, 2) days. The variation in the time to reach stationary phase is assumed to be due to initial numbers of bacteria and specific product composition.

Because the time to stationary phase is expected to depend strongly on the temperature of storage, it is adjusted according to the temperatures selected using a relative rate function with a T_{min} of 0°C. This value was used as a first approximation, which was adjusted based on temperature–growth rate responses of lactic acid bacteria associated with vacuum-packed processed meat products, which have a similar microbial ecology (Mol et al., 1971).

The time to reach the stationary phase is deducted from the total storage time sampled in an iteration to determine the duration of the second, constrained, phase of growth. During the first phase of *L. monocytogenes* growth, the growth rate is predicted to be unconstrained and predicted by the growth rate model for the temperature and product characteristics sampled during that iteration. After that time, however, growth is predicted to be reduced by some factor. It could be complete inhibition (as described in Figures A5.8 and A5.9), but other data (L.A. Mellefont, B. Davidson and T. Ross, Univ. of Tasmania, pers. comm., 2002, unpublished data) that suggest that *L. monocytogenes* might continue to grow slowly. Thus, the specific growth behaviour is uncertain and has been left as an assumption whose influence can be tested (see Section 4.5.5).

Nominal storage life also has to be adjusted for storage conditions because higher temperatures will cause premature spoilage. Conversely, there are reports (Ben Embarek, pers. comm., 2001) that in some countries 2–3-month shelf lives are realized, apparently without consumer rejection. Accordingly, several scenarios have been modelled. However, in each case a filter is applied so that if the storage time at 5°C exceeds 10 weeks (or its equivalent calculated at other temperatures) the product is considered completely spoiled and no further growth occurs. This is achieved by truncating the predictions of growth based on shelf-life at the growth levels that could have occurred at the equivalent of 70 days at 5°C. These predictions are not, however, removed from the simulation.

These interactions are shown as an influence diagram in Figure A5.10.

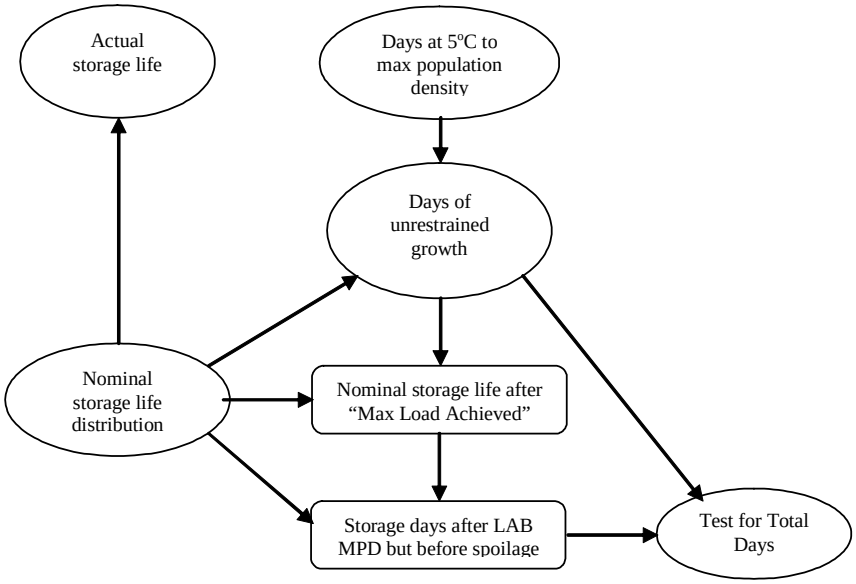

Figure A5.10 Influence diagram derived from the simulation model, showing the interaction of nominal storage life and spoilage due to bacterial growth, as well as the estimation of the time required for lactic acid bacteria to reach the stationary phase and for the Jameson effect to constrain *Listeria monocytogenes* growth.

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