

# Part 5.

## Risk characterization: response to Codex questions

### 5.1 INTRODUCTION

This section addresses the three risk questions posed by CCFH in 2001 in relation to the risk from *L. monocytogenes* in RTE foods. The specific question addressed is given in each case.

### 5.2 QUESTION 1

*Estimate the risk from L. monocytogenes in food when the number of organisms range from absence in 25 grams to 1000 colony forming units per gram, or millilitre or does not exceed specified levels at the point of consumption.*

The question posed by the CCFH primarily requires a consideration of how the relative risk of acquiring listeriosis is affected by the level of *L. monocytogenes* present in a serving of food at the time of consumption. The ability to answer this question is dependent on the ability to articulate and interpret dose-response relationships for *L. monocytogenes*. However, there are a number of potentially confounding factors that could influence the approach taken and the complexity of the answer provided. In view of the generic nature of the CCFH question and the fact that this is one of the first microbial risk assessments requested by CCFH, it was decided that the response to this question should focus on communicating the key risk assessment concepts. It is also important to note that this question implies a series of comparisons based on relative risks and does not require the much more daunting task of calculating absolute risk. Accordingly, consideration of potential confounding factors was limited and a detailed consideration of uncertainty and variability was not undertaken in addressing this question. An introduction to issues related to the uncertainty and variability associated with dose-response models is provided in the hazard characterization section of this document. In addition to not explicitly addressing uncertainty and variability, a number of simplifying assumptions were made in developing the examples used to answer the question posed by CCFH. For instance, to calculate the ingested dose, knowledge of the size of the serving is needed. A fixed serving size of 31.6 g was assumed for convenience to simplify the calculations because it approximates a typical serving size and because dose levels were estimated in 0.5 log<sub>10</sub> increments ( $10^{0.5} = 3.16$ ). To calculate the concentrations for other serving sizes in the tables that follow, the dose levels would have to be divided by the serving size.

As discussed in the hazard characterization, the exponential model was selected to describe the relationship between the dose of *L. monocytogenes* ingested and the probability of developing systemic listeriosis. Dose-response curves were developed for both the healthy

population and the susceptible population and include the entire range of ingested doses (i.e. not restricted to 1000 CFU/g food). These curves are population based and describe the average dose-response relationship. A specific outbreak that involves a strain with high virulence or an unusually susceptible population may still result in a significant number of cases from food containing comparatively low numbers of *L. monocytogenes*. For the purposes of this example, only the dose-response curve for the susceptible population was used, and it was assumed that all cases of listeriosis were restricted to that population. The specific dose-response curve selected was the one where the maximum level to which *L. monocytogenes* could grow in a food was assumed to be  $10^{7.5}$  CFU/serving. The end result of these assumptions is that the most “conservative” dose-response model was used, i.e. the maximum virulence of *L. monocytogenes* was assumed. The r-value for this relationship was  $5.85 \times 10^{-12}$  (Table 2.18). The dose ingested is a function of the level of the microorganism in the food (CFU/g) multiplied by the size of the serving. Thus, the equation for calculating the probability of listeriosis was:

$$P = 1 - e^{-(5.85 \times 10^{-12})(31.6g \times n)}$$

where  $n$  is the number of *L. monocytogenes* per gram. By substituting different values for  $n$ , the likelihood of listeriosis at levels between 0.04 (1 CFU/25 g) and 1000 CFU/g was calculated.

The overall affect on the number of cases of listeriosis was estimated by multiplying the likelihood of listeriosis per serving by the total number of servings. For this calculation, the total number of RTE servings was assumed to be  $6.41 \times 10^{10}$  servings, i.e. the estimated total number of servings per year consumed in the United States of America for the 20 classes of RTE food considered in FDA/FSIS (2001). The corresponding number of listeriosis cases for the susceptible population was considered to be 2130 (FDA/FSIS, 2001), and will be used to represent the current incidence of listeriosis when comparing the effect of changes to incidence under different theoretical scenarios.

As a simple, worst-case scenario, the predicted risk per serving and predicted number of annual listeriosis cases were estimated by assuming that all  $6.41 \times 10^{10}$  servings had the maximum contamination level being considered. The effects on the incidence of listeriosis of six levels of pathogen were evaluated (0.04, 0.1, 1, 10, 100 and 1000 CFU/g) (Table 5.1).

A more realistic approach would be to use a distribution of *L. monocytogenes* levels in foods when consumed. To explore that more complex approach, the overall distribution of *L. monocytogenes* levels in 20 classes of RTE foods from the FDA/FSIS (2001) risk assessment was used (see Table 5.2) to calculate the probability of listeriosis and the predicted number of cases. At each maximum *L. monocytogenes* level considered, the number of servings from the distribution exceeding the designated contamination level was added to that maximum level. For example, for an upper limit of 1000 CFU/g, the number was  $1.18 \times 10^8$  servings, i.e.  $6.23 \times 10^7$  (servings originally predicted to be at 1000 CFU/g) +  $2.94 \times 10^7$  (servings originally predicted to be at 10 000 CFU/g) +  $1.39 \times 10^7$  (servings originally predicted to be at  $10^5$  CFU/g) +  $3.88 \times 10^6$  (servings originally predicted to be at  $10^{5.5}$  CFU/g) +  $8.55 \times 10^6$  (servings originally predicted to be at  $>10^6$  CFU/g). The predicted annual numbers of listeriosis cases were calculated and summed, and the predicted number of cases for each maximum level is given in Table 5.3.

**Table 5.1** Probability of illness per serving for the susceptible population estimated for different levels of *Listeria monocytogenes* at the time of consumption and the estimated number of cases per year in the United States of America if all RTE meals were contaminated at that level.

Level (CFU/g)	Dose <sup>(1)</sup> (CFU)	Log <sub>10</sub> dose (log <sub>10</sub> CFU/serving)	Probability of illness per serving	Relative risk <sup>(2)</sup>	Estimated annual number of cases <sup>(3)</sup>
<0.04	1	0	$7.39 \times 10^{-12}$	1	0.54
0.1	3	0.5	$1.85 \times 10^{-11}$	2.5	1
1	32	1.5	$1.85 \times 10^{-10}$	25	12
10	316	2.5	$1.85 \times 10^{-9}$	250	118
100	3 160	3.5	$1.85 \times 10^{-8}$	2500	1 185
1000	31 600	4.5	$1.85 \times 10^{-7}$	25000	11 850

NOTES: (1) Serving size of 31.6 g. (2) Using the risk from a dose of 1 CFU as reference. (3) A total of  $6.41 \times 10^{10}$  servings per year assumed.

**Table 5.2** Predicted distribution of levels of *Listeria monocytogenes* occurring in RTE foods.

Level of <i>L. monocytogenes</i> in a food at consumption (CFU/g)	Number of servings at the specified dose
<0.04	$6.18 \times 10^{10}$
0.1	$1.22 \times 10^9$
1	$5.84 \times 10^8$
10	$2.78 \times 10^8$
100	$1.32 \times 10^8$
1000	$6.23 \times 10^7$
10000	$2.94 \times 10^7$
100000	$1.39 \times 10^7$
316000	$3.88 \times 10^6$
>1000000	$8.55 \times 10^6$
<b>Total</b>	$6.41 \times 10^{10}$

SOURCE: FDA/FSIS, 2001.

**Table 5.3** Predicted annual number of listeriosis cases in the susceptible population when the level of *Listeria monocytogenes* was assumed not to exceed a specified maximum value and the levels of *L. monocytogenes* in the food are distributed as indicated in Table 5.2.

Level (CFU/g)	Maximum Dose <sup>(1)</sup> (CFU)	Percentage of servings when maximum level <sup>(2)</sup>	Estimated number of listeriosis cases per year <sup>(3)</sup>
0.04	1	100	0.5
0.1	3	3.6	0.5
1	32	1.7	0.7
10	316	0.8	1.6
100	3160	0.4	5.7
1000	31 600	0.2	25.4

NOTES: (1) Serving size of 31.6 g. (2) Number of servings in the highest *L. monocytogenes* level assumed divided by  $6.41 \times 10^{10}$  times 100. (3) Levels of *L. monocytogenes* per serving used to calculate predicted number of cases based on the overall distribution from the FDA/FSIS risk assessment (2001) (see Table 5.2). A total of  $6.41 \times 10^{10}$  servings per year was assumed.

Tables 5.1 and 5.3 show vast differences in the estimated number of cases for the worst-case answer to the question (Table 5.1) compared with that estimated when an attempt is made to consider the frequency and extent of contamination actually encountered in RTE foods (Table 5.3). While either set of predictions can be challenged on the basis of the assumptions used, such scenarios are useful in framing the extent of the risk likely to be encountered.

These two scenarios (Tables 5.1 and 5.3) demonstrate that when dealing with an infectious agent where a non-threshold model is assumed, where either the frequency of contamination (percentage of contaminated samples) or the extent of contamination (*L. monocytogenes* levels in a contaminated food) increases, then so does the risk and the predicted number of cases. Thus, if all RTE foods went from having 1 CFU/serving to 1000 CFU/serving, the risk of listeriosis would increase 1000-fold (assuming a fixed serving size). Conversely, the effect of introducing into the food supply 10 000 servings contaminated with *L. monocytogenes* at a level of 1000 CFU/g could theoretically be compensated by removing from the food supply a single serving contaminated at a level of  $10^7$  CFU/g.

In interpreting these results and in attempting to predict the actual effect of a change in the regulatory limits for *L. monocytogenes* in RTE foods, one also has to take into account the extent to which deviations from established limits occur. The current example is based on data from the United States of America, where the current allowable limit for *L. monocytogenes* in RTE foods is effectively 0.04 CFU/g (1 CFU/25 g), a level that if consistently achieved would be expected to result in less than one case of listeriosis per year in the United States of America. However, the baseline level for the United States of America population was 2130 cases (Mead et al., 1999). Both the current risk assessment and the United States of America FDA/FSIS draft risk assessment (2001) indicate that a portion of RTE food contain a substantially greater number of the pathogen than the stated limit and that the public health impact of *L. monocytogenes* is, most probably, almost exclusively a function of the foods that greatly exceed the current limit. Thus, in addressing the question posed by CCFH, the current risk assessment indicates that increasing the level of *L. monocytogenes* in RTE foods from 0.04 to 1000 CFU/g would increase the risk of foodborne listeriosis, provided that the current rate of deviations above the established limit remained proportionally the same. However, it could also be asked whether public health could be improved if a less stringent microbiological limit for RTE foods resulted in a substantial decrease in the number of servings that greatly exceeded the established limit, e.g. if the change encouraged manufacturers to routinely screen for *L. monocytogenes* in the plant environment and to take appropriate remedial actions. Models developed during the current risk assessment could be used estimate the extent of control over deviations from established limits that would be needed to improve public health if regulatory limits were relaxed, provided that sufficient data on the rate and extent of deviations were available for individual RTE foods.

As a means of further examining this concept, a simple hypothetical “what-if” scenario was developed based on the information provided in Tables 5.2 and 5.3. It examines the impact that compliance with a microbiological limit (i.e. defect rates) has on public health. Two potential limits, 0.04 CFU/g and 100 CFU/g, were examined in conjunction with different defect rates, i.e. the percentage of servings that exceed the specified limit. As a means of simplifying the what-if scenario and dramatizing the impact of compliance, a single

level of *L. monocytogenes*,  $10^6$  CFU/g, was assumed for all “defective” servings. Thus, if a serving of food was in compliance, it had a level of *L. monocytogenes* at or below the specified microbiological limit based on the distribution of *L. monocytogenes* levels (Table 5.2) used to calculate the 100% compliance values depicted in Table 5.3. Conversely, if a serving of food was out of compliance, it was assumed to have a set level of *L. monocytogenes* of  $10^6$  CFU/g, or since the assumed serving size was 31.6 g, a consumed dose of  $3.16 \times 10^8$  CFU. The predicted number of cases as a function of the percentage of defective servings is provided in Table 5.4.

As noted in Table 5.3, at 100% compliance the number of predicted cases for both limits is low, with an approximate 10-fold differential between the two microbiological limits. As expected, the number of predicted cases increases with an increasing frequency of defective servings. At defect rates  $>0.0001\%$  a 10-fold increase in the defect rate results in an approximate 10-fold increase in the number of predicted cases, regardless of the microbiological limits (i.e. 0.04 CFU/g versus 100 CFU/g). It is interesting to note that based on the conditions and assumptions of this simple what-if scenario, the defect rate that yielded a value approximately equivalent to the baseline value of 2130 cases used in the FDA/FSIS draft risk assessment (2001) was 0.018%.

A more detailed consideration of compliance could be achieved by incorporation of distributions reflecting the levels of *L. monocytogenes* observed in variety of foods. However, such a detailed consideration of compliance rates was beyond the scope of the current risk assessment. Furthermore, the simple hypothetical what-if scenario presented adequately demonstrates key concepts related to how compliance rates can strongly influence the actual risk associated with a microbiological criterion. In fact, it could be argued that the rate of compliance is a more significant risk factor than the numeric value of the criterion within the range that CCFH asked the risk assessment team to consider. The what-if scenario also demonstrates the concept that a less stringent microbiological limit could lead to an improvement in public health if new criteria lead to a substantive decrease in defect rates. For example, the model (Table 5.4) predicts that if a microbiological limit of 0.04 CFU/g with a 0.018% defect rate (2133 cases) was replaced with a 100 CFU/g limit and a 0.001% defect rate (124 cases), the predicted result based on the scenario is an approximate 95% reduction in foodborne listeriosis.

**Table 5.4** Hypothetical “what-if” scenario demonstrating the effect of “defect” rate on the number of predicted cases of foodborne listeriosis.

Assumed percentage of “Defective” servings <sup>(1)</sup>	Predicted number of listeriosis cases <sup>(2)</sup>	
	Initial standard of 0.04 CFU/g	Initial standard of 100 CFU/g
0	0.5	5.7
0.00001	1.7	6.9
0.0001	12.3	17.4
0.001	119	124
0.01	1185	1191
0.018	2133	2133
0.1	11837	11848
1	117300	117363

NOTES: (1) For the purposes of this scenario, all defective servings were assumed to contain  $10^6$  CFU/g.

(2) For the purposes of this scenario, an  $r$ -value of  $5.85 \times 10^{-12}$  was employed and a standard serving size of 31.6 g was assumed. In the case of the 100 CFU/g calculations, the defective servings were assumed to be proportionally distributed according to the number of servings within each cell concentration bin.

### 5.3 QUESTION 2

*Estimate the risk for consumers in different susceptible population groups.*

As noted in Section 5.2, listeriosis is primarily a disease of certain subpopulations with impaired or altered immune function (e.g. pregnant women and their fetuses, the elderly, individuals with chronic diseases, AIDS patients, individuals taking immunosuppressive drugs). Susceptibility varies within the broadly defined susceptible group (e.g. the risk of listeriosis appears to be less for pregnant women than transplant recipients). It has been estimated that various subpopulations may have a 20- to 2500-fold increased risk of acquiring listeriosis (FDA/FSIS, 2001; Marchetti, 1996). CCFH requested that the risk assessment team attempt to estimate the differences in the dose-response relations for the different subpopulations with increased susceptibility. While previous risk assessments had considered the relative susceptibility of the entire population at increased risk, versus the general population, these risk assessments did not develop the type of detailed comparisons of subpopulations with increased susceptibility requested by CCFH. Thus, the current risk assessment had to develop *de novo* a means for addressing the request.

The basic approach taken to developing the requested dose-response relations was to take advantage of epidemiological estimates of the relative rates of listeriosis for different subpopulations. These “relative susceptibility” values were generated by taking the total number of listeriosis cases for a subpopulation and dividing it by the estimated number of people in the total population that have that condition. This value is then divided by a similar value for the general population. While there is a substantial uncertainty associated with these values (i.e. a relative susceptibility value is the ratio of two uncertain estimates and the exposures (diets) of the different subpopulations are assumed to be equivalent), it does provide a useful estimate of the differences in the susceptibility among the different subpopulations and the role that immune status has in determining an individual’s risk from *L. monocytogenes* (Table 5.5).

Relating the relative susceptibility values to the dose-response relations for the different subpopulations requires a means of converting these point estimates to a dose-response curve. The unique characteristics of the exponential model allowed this to be done. Being a single parameter model, the exponential model allows the entire dose-response curve to be generated once any point on the curve is known. Thus, the r-value for an exponential dose-response curve can be estimated for a subpopulation using a relative susceptibility ratio and a reference r-value for the general population. Using the relative susceptibility value for cancer patients as an example (Table 5.5), the equation for the relative susceptibility is:

$$\text{Relative susceptibility} = \text{RS} = P_{\text{cancer}}/P_{\text{healthy}} = [1 - \exp(-r_{\text{cancer}}*N)]/[1 - \exp(-r_{\text{healthy}}*N)]$$

where  $P_{\text{cancer}}$  and  $P_{\text{healthy}}$  denote the probability of systemic listeriosis for a cancer patient and a healthy adult, respectively, when exposed to a dose  $N$  of *L. monocytogenes*, and where  $r_{\text{cancer}}$  and  $r_{\text{healthy}}$  are the r-values of exponential dose-response relationships specific for those population sub-groups.

This equation can be rearranged to:

$$r_{\text{cancer}} = - \ln [\text{RS} * \exp(-r_{\text{healthy}}*N) - (\text{RS} - 1)]/N$$

As long as the value for  $N$ , the number of *L. monocytogenes* consumed, is much smaller than the maximum assumed dose, the above relationship can be used to estimate the  $r_{\text{subpopulation}}$  value. Using the above equation, the  $r$ -values for different classes of patients were estimated based on epidemiological data from France (Tables 5.5) and the United States of America (Table 5.6).

**Table 5.5**  $r$ -values (exponential dose-response model) for different susceptible populations calculated using relative susceptibility information from France. Relative susceptibilities for the different subpopulations are based on the incidence of listeriosis cases (outbreak and sporadic) in these groups in 1992.

Condition	Relative susceptibility	Calculated $r$ -value <sup>(1)</sup>	Comparable outbreak $r$ -value
Transplant	2 584	$1.41 \times 10^{-10}$	Finland butter $3 \times 10^{-7}$
Cancer – Blood	1 364	$7.37 \times 10^{-11}$	
AIDS	865	$4.65 \times 10^{-11}$	
Dialysis	476	$2.55 \times 10^{-11}$	
Cancer – Pulmonary	229	$1.23 \times 10^{-11}$	
Cancer – Gastrointestinal and liver	211	$1.13 \times 10^{-11}$	
Non-cancer liver disease	143	$7.65 \times 10^{-12}$	
Cancer – Bladder and prostate	112	$5.99 \times 10^{-12}$	
Cancer – Gynaecological	66	$3.53 \times 10^{-12}$	
Diabetes, insulin dependent	30	$1.60 \times 10^{-12}$	
Diabetes, non-insulin dependent	25	$1.34 \times 10^{-12}$	
Alcoholism	18	$9.60 \times 10^{-13}$	
Over 65 years old	7.5	$4.01 \times 10^{-13}$	
Less than 65 years, no other condition (reference population)	1	$5.34 \times 10^{-14}$	

NOTES: (1) The  $r$ -value assumed for the reference population – “Less than 65 years, no other medical condition” – was  $5.34 \times 10^{-14}$ , which is the median of the  $r$ -value calculated assuming a maximum level of  $8.5 \log_{10}$  CFU per serving.

SOURCE: Marchetti, 1996.

**Table 5.6** Dose-response curves for different susceptible populations calculated using relative susceptibility information from the United States of America. Relative susceptibilities for the different sub-populations are based on the incidences of listeriosis cases (outbreak and sporadic) in these groups.

Condition	Relative susceptibility	Calculated $r$ -value <sup>(1)</sup>	Comparable outbreak $r$ -value
Perinatal	14	$4.51 \times 10^{-11}$	Los Angeles cheese $3 \times 10^{-11}$
Elderly (60 years and older)	2.6	$8.39 \times 10^{-12}$	
Intermediate-age population (reference population)	1	$5.34 \times 10^{-14}$	

NOTES: (1) The  $r$ -value assumed for the reference population – “Intermediate-age population” – was  $5.34 \times 10^{-14}$ , which is the median of the  $r$ -values calculated under the assumption of a maximum level of  $8.5 \log_{10}$  CFU per serving.

SOURCE: FDA/FSIS, 2001.

Comparison of the relative susceptibility values and corresponding r-values are consistent with the physiological observation that as an individual's immune system is increasingly compromised, the risk of listeriosis at any given dose increases and this is reflected in a corresponding increase in the r-value of the dose-response curve. The most compromised group in the French data, transplant patients, has an r-value approximately 4 orders of magnitude greater than the reference population (i.e. individuals less than 65 years old with no other medical conditions). The relative susceptibility values for the elderly population showed close agreement, 7.5 and 2.6 for the French and United States of America data, respectively. The differences reflect, in part, the different definitions of the age corresponding to the category "elderly" and the reference population. The United States of America intermediate-age population includes the patients that are separated out from the less-than-65-years-of-age group in the French data and the two reference populations are not expected, therefore, to have the same r-values. Nevertheless, the two tables indicate the magnitude of the impact that the impairment of the immune system by the specific conditions and disease states has on susceptibility to listeriosis.

The two outbreak r-values provide an indication of the validity of the models. The r-value for the Los Angeles outbreak in pregnant women from consumption of Hispanic cheese was very close to that estimated (Table 5.6). The r-value for the Finland outbreak from butter in hospitalized transplant patients differed from the values based on transplant patients by 1000-fold (Table 5.5). This may have resulted from the smaller number of individuals exposed, the extremely compromised and highly variable immunological status of the population, or the involvement of a highly virulent strain of *L. monocytogenes*. There is a clear need in future outbreaks for exposure levels, immune status of the patients and strain characteristics to all be investigated so that these dose-response models can be further refined and validated.

## 5.4 QUESTION 3

*Estimate the risk from L. monocytogenes in foods that support growth and foods that do not support growth at specific storage and shelf-life conditions.*

*L. monocytogenes* growth on foods is not the only determinant of risk of listeriosis. Additional factors that affect the risk associated with any food, regardless of whether it does or does not support *L. monocytogenes* growth, include:

- frequency of contamination;
- level of contamination;
- frequency of consumption; and
- susceptibility of consuming population.

This question suggests a number of alternative approaches to a simple growth/no-growth evaluation, such as a consideration of the effect on consumer risk of limiting the storage temperature and shelf-life of a product that supports the growth of *L. monocytogenes*. The risk assessment team has attempted to also consider these approaches while formulating its answer to the question.

As was discussed in the response to Question 1 (Section 5.2), it is possible that a food that does not permit the growth of *L. monocytogenes* but that is frequently contaminated at moderate levels could pose a greater risk than a food infrequently contaminated, or contaminated at low levels, but that does support growth of *L. monocytogenes*. Also, as noted previously, it is clear that an increase in the *total* numbers of *L. monocytogenes* in a food (whether through growth or increased frequency of contamination) will lead to increased consumer risk because, for *L. monocytogenes*, the dose-response model used indicates that public health risk is proportional to total number of *L. monocytogenes* in the food when consumed. Furthermore, as bacterial growth is exponential, the risk might be expected to increase exponentially with storage time.

Three approaches for answering this question are provided:

- (i) general consideration of the impact of the ingested dose on the risk of listeriosis;
- (ii) comparison of four foods that were selected, in part, to evaluate the effect of growth on risk; and
- (iii) comparison of what-if scenarios for the foods evaluated that do support *L. monocytogenes* growth if they did *not* support *L. monocytogenes* growth. Each of the approaches is discussed below.

### 5.4.1 Growth rates in foods

*L. monocytogenes* is able to grow in many RTE foods, even if stored under appropriate refrigeration conditions. Factors affecting the growth of *L. monocytogenes* in foods are discussed in detail in Section 3.5. These include product formulation, storage time and temperature, and interactions with other microorganisms present in the product. In vacuum-packed foods, lactic acid bacteria can reach stationary phase without causing product spoilage. This can slow, or even prevent, the subsequent growth of *L. monocytogenes*. Table 5.7 presents representative generation times for different products as a function of product type and storage temperature. For every three generations of growth, there is

approximately a 10-fold increase in the bacterial population. As discussed in Section 5.2, a 10-fold increase in the levels of *L. monocytogenes* ingested produces a corresponding 10-fold increase in risk to human health. Thus, the risk from a food that supports the growth of *L. monocytogenes* increases with increasing storage time. However, the degree that the risk increases is dependent on the extent of growth in the food, which, in turn, is largely a function of *L. monocytogenes*' growth rate in the food and the storage duration and conditions.

*L. monocytogenes* has been reported to grow in foods at temperatures as low as 0°C, water activities as low as 0.91–0.93 and pH as low as 4.2 (see Table 3.1). Combinations of suboptimal levels reduce the growth rate and can prevent growth at less extreme conditions than any of these factors acting alone. This principle, often referred to as hurdle technology or combination treatment, is exploited in food processing to prevent or limit the growth of bacteria in RTE foods.

The potential extent of growth varies among different foods, depending on the pathogen's growth rate in a specific food, which is a function of the product's composition and storage conditions, and on shelf-life of the product. From Table 5.7 it is evident that the growth of *L. monocytogenes* within the normal shelf-life of products could be substantial. For example, fresh cut vegetables have a relatively short shelf-life and do not support as rapid growth of *L. monocytogenes* as some other foods, such as milk or deli-meats. Thus, it would be expected that extent of growth in fresh cut vegetables would not be as great as those in other foods, resulting in a lower risk for given initial contamination rates and levels.

The example of the effect of storage time and temperature on the growth of *L. monocytogenes* and the subsequent risk of listeriosis can be considered a worst-case scenario in that it only considers the effect of temperature on generation times. Additional factors that act to delay the initiation of growth of *L. monocytogenes* (e.g. consideration of the lag phase), reduce the rate of growth (e.g. modified-atmosphere packaging), or suppress the maximum level reached by *L. monocytogenes* (e.g. growth of lactic acid bacteria) would decrease the extent of growth within a specified period of a product's shelf-life, with a corresponding decrease in risk. The actual calculation of risk would also have to consider that different servings would be consumed at various times within the total product shelf-life, as typically only a small fraction of a product is consumed near the end of its declared shelf-life.

#### 5.4.2 Comparison of four foods

As discussed above, the four foods evaluated in the risk assessment (milk, ice cream, cold-smoked fish, and fermented meat products) were selected, in part, to compare the effect of various product characteristics on growth. This included specific consideration of the ability of foods to support growth. Thus, milk and ice cream were compared because they have similar compositions, servings sizes, frequencies of consumption, and rates and extents of initial contamination. However, milk supports *L. monocytogenes* growth while ice cream does not. Similarly, cold-smoked fish and fermented meat products have similar rates of initial contamination, serving sizes and frequencies of consumption, but the former supports the growth of *L. monocytogenes* while the latter does not.

**Table 5.7** Representative generation times (hours) and growth potential of *Listeria monocytogenes* at different temperatures and shelf lives at 5°C in various RTE foods.

Temperature (°C)	Generation time (hours)			
	Milk	Vacuum-packed cold-smoked fish	Vacuum-packed processed meats	Sliced vegetables
5 <sup>(1)</sup>	27.6	46.6	29.6	111
(95% confidence interval)	(14–226)	(20–infinite)	(14–infinite)	(28–infinite)
5 <sup>(2)</sup>	25–30	40–49	16–48	–
10 <sup>(2)</sup>	5–7	8–11	7–10	–
25 <sup>(2)</sup>	0.7–1.0	1.2–1.7	1–1.6	–
	<b>Growth potential<sup>(3)</sup></b>			
5	~2–3	~4–5	~8–9	~0.3
	<b>Advisory shelf-life (weeks)</b>			
5	1–2	4–6	6–8	1

NOTES: (1) Values based on data collated in FDA/FSIS, 2001.

(2) Representative predictions and ranges from several published predictive models developed for growth rate of *L. monocytogenes*. No predictions were possible for vegetables because none of the published models were developed, or validated, for use with sliced vegetables.

(3) Log increase ignoring lag phase or suppression of growth by lactic acid bacteria.

Comparisons of the predicted risk per million servings (Table 4.34) between milk and ice cream, and cold-smoked fish and fermented meat products, indicate that the ability of a product to support growth within its shelf-life can increase substantially the risk of that product being a vehicle for foodborne listeriosis. Thus, the predicted risk per million servings of milk was approximately 100-fold greater than that for ice cream, and the risk for cold-smoked fish was approximately 10 000-fold greater than the corresponding risk for fermented meat products.

### 5.4.3 What-if scenarios

One of the useful features of a quantitative risk assessment is that the underlying mathematical models can be modified to allow various what-if scenarios to be run to evaluate the likely impact of different risk management options. Accordingly, a limited number of what-if scenarios were evaluated for milk and cold-smoked seafood, the two foods that supported the growth of *L. monocytogenes* and considered in the risk assessment. The results of these analyses were then compared to the predicted baseline risks to determine the impact of the intervention.

#### 5.4.3.1 Milk

The initial assessment of risk associated with recontaminated pasteurized milk considered the likely growth of *L. monocytogenes* during the shelf-life of the product (see Section 4.2), using Canadian consumption characteristics as an example. To help answer CCFH Question 3, the model was re-executed after being modified so that the effect of growth was ignored, i.e. no growth during storage was modelled. The results of the two calculations were then compared to estimate the effect of growth on risk (Table 5.8).

The results suggest that an approximately 1000-fold increase in risk can be attributed to the predicted growth of *L. monocytogenes* in pasteurized milk by either measure of risk, i.e. risk per 1 million meals or risk per 100 000 population. The uncertainty measures associated

with the comparison suggested that the predicted increase in risk attributable to growth could be as little as 100-fold, or as much as >10 000-fold.

Several what-if scenarios were calculated for milk to illustrate the interactions of the various factors in determining the risks (Table 5.9). In one scenario, if all milk was consumed immediately after purchase at retail, the risks per serving and cases per population in both susceptible and healthy populations would decrease approximately 1000-fold. In contrast, if the contamination levels of milk were truncated at 100 CFU/g at retail but with growth still allowed, the incidence of listeriosis is predicted to be reduced by only about 70%. Two scenarios examined the impact of storage temperatures and times. When the temperature distribution was shifted so the median increased from 3.4 to 6.2°C, the mean number of illnesses increased over 10-fold for both populations. When the storage time distribution was shifted from a median of 5.3 days to 6.7 days, the mean rate of illnesses increased 4.5-fold and 1.2-fold for the healthy and susceptible populations, respectively.

#### 5.4.3.2 Smoked Fish

The assumptions used with the cold-smoked fish model differ slightly from those used with the pasteurized milk example. The cold-smoked fish model also considers the effect of the growth of indigenous lactic acid bacteria in the product, which, when they grow to high numbers, suppress the growth of *L. monocytogenes* (see Section 4.5). The extent of that growth suppression is not known with certainty. In the baseline model, two assumptions concerning the growth rate suppression by lactic acid bacteria were tested. In the what-if scenario the growth rate inhibition of *L. monocytogenes* by the lactic acid bacteria was set to zero. Table 5.10 compares the risk estimates when growth was modelled to occur or not, including the effect of different assumptions about the magnitude of the inhibition of *L. monocytogenes* growth rate due to the growth of lactic acid bacteria.

**Table 5.8** Estimates of the increase in risk of listeriosis from growth during storage of pasteurized milk between purchase and consumption.

	Normal-risk population		High-risk population		Mixed population	
	Mean	(s.e.) <sup>a</sup>	Mean	(s.e.)	Mean	(s.e.)
<b>With growth (baseline model)</b>						
Cases per 100 000 population	$1.6 \times 10^{-2}$	$(5.0 \times 10^{-4})$	$5.2 \times 10^{-1}$	$(3.1 \times 10^{-2})$	$9.1 \times 10^{-2}$	$(4.7 \times 10^{-3})$
Cases per 1 000 000 servings	$1.0 \times 10^{-3}$	$(1.0 \times 10^{-4})$	$2.2 \times 10^{-2}$	$(9.0 \times 10^{-4})$	$5.0 \times 10^{-3}$	$(2.0 \times 10^{-4})$
<b>Without growth</b>						
Cases per 100 000 population	$1.3 \times 10^{-5}$	$(6.7 \times 10^{-8})$	$3.8 \times 10^{-4}$	$(1.6 \times 10^{-6})$	$6.7 \times 10^{-5}$	$(2.4 \times 10^{-7})$
Cases per 1 000 000 servings	$5.9 \times 10^{-7}$	$(3.1 \times 10^{-9})$	$1.7 \times 10^{-5}$	$(7.5 \times 10^{-8})$	$3.6 \times 10^{-5}$	$(1.4 \times 10^{-8})$
<b>Increased risk with growth relative to that without growth (n-fold increase)</b>						
Cases per 100 000 population	1 231		1 366		1 358	
Cases per 1 000 000 servings	1 695		1 294		139	

KEY: s.e. = Standard error of the mean.

**Table 5.9** Three what-if scenarios that illustrate the impact of contamination and storage on the estimated risks of listeriosis per 100 000 population and per 1 000 000 servings for milk under typical conditions of storage and use.

Food	Estimated mean cases of listeriosis per 100 000 people	Estimated mean cases of listeriosis per 1 000 000 servings
Milk baseline	$9.1 \times 10^{-2}$	$4.6 \times 10^{-3}$
No growth	$6.7 \times 10^{-5}$	
With contamination truncated at 100 CFU/g	$2.8 \times 10^{-2}$	
Increase storage temperature (from 3.4 to 6.2°C)	$1.2 \times 10^0$	
Increase storage time (from 5.3 to 6.7 days)	$2.0 \times 10^{-1}$	

With either assumption concerning the effect of lactic acid bacteria on *L. monocytogenes* growth potential, growth greatly increased the risk of listeriosis. Assuming that 80 to 100% suppression occurred, it allowed more growth than the assumption of 95% growth rate suppression, a result of the faster overall growth rate after lactic acid bacteria have achieved maximum population growth. The risk per serving and cases per 100 000 population increased 700- to 1000-fold in the first assumption (80–100% growth rate suppression) and 67- to 85-fold under the latter assumption (95%) from the “no *L. monocytogenes* growth” to the baseline (growth) scenarios.

For the cold-smoked fish model, between 15 and 20% of the population were assumed to be in the high-risk category, but the cases attributable to the normal and high-risk categories were not estimated discretely. Rather, as in the previous example, the predicted number of cases is a weighted mean of the normal and high-risk populations. It is known that the population with increased susceptibility to listeriosis experiences between 80 and 98% of total reported cases of listeriosis. Also, in this example, no attempt to differentiate consumption between these two susceptibility classes was made, unlike that undertaken in the assessment of milk (Section 4.2). These differences do not affect the interpretation of the results with a food but some caution must be exercised in comparing the impact of growth on the risk *between* the foods. However, the differences in the modelling are relatively minor and the predicted increase in risk due to growth in the two examples is roughly comparable. For example, in the case of pasteurized milk (Table 5.9), the modelling also suggests that the increase in risk due to the growth of *L. monocytogenes* within the normal shelf-life of the product is between approximately 100- and 1000-fold, similar to the risk increase predicted for cold-smoked fish due to *L. monocytogenes* growth during storage.

A further what-if scenario was performed to estimate the effect on risk of reducing the shelf-life of smoked fish by 50%. This was tested by replacing the original shelf-life distribution of 1–28 days, with a most likely value of 14 days, by a shelf-life distribution of 1–14 days, with a most likely value of 7 days. The effect of this change resulted in an 80% reduction in the predicted increase in risk due to growth. The fact that the change was not greater is probably due to the effect of lactic acid bacteria, which is modelled to begin to suppress *L. monocytogenes* growth after approximately 3 weeks of storage at 5°C (see Section 4.5.3.7).

**Table 5.10** Impact of the growth of *Listeria monocytogenes* during storage of cold-smoked fish between purchase and consumption on the risk of listeriosis under typical conditions of storage and use.

Growth rate inhibition due to growth of lactic acid bacteria	Cases per 1 000 000 meals		Cases per 100 000 population	
	No Growth	Growth Modelled	No Growth	Growth Modelled
80–100%	$4.51 \times 10^{-4}$ ( $3.09 \times 10^{-5}$ ) <sup>(1)</sup>	$4.59 \times 10^{-1}$ ( $3.29 \times 10^{-1}$ )	$9.60 \times 10^{-5}$ ( $1.07 \times 10^{-5}$ )	$6.57 \times 10^{-2}$ ( $3.78 \times 10^{-2}$ )
Difference <sup>(2)</sup>		1020-fold		684-fold
95%		$3.82 \times 10^{-2}$ ( $1.96 \times 10^{-2}$ )		$6.48 \times 10^{-3}$ ( $2.26 \times 10^{-3}$ )
Difference <sup>(2)</sup>		85-fold		67-fold

NOTE: (1) Values in parentheses are standard deviations. (2) Increase in risk of listeriosis in the growth versus the no-growth scenarios

#### 5.4.4 Summary

Three different approaches were taken to demonstrate the effect of growth of *L. monocytogenes* on the risk of listeriosis associated with RTE foods. It is apparent that the potential for growth strongly influences risk, though the extent of that increase is dependent on the characteristics of the food and the conditions and duration of refrigerated storage. However, using the examples provided in the risk assessment, the ability of these RTE foods to support the growth of *L. monocytogenes* appears to increase the risk of listeriosis on a per-serving basis by 100- to 1000-fold over what the risk would have been if the foods did not support growth. While it is not possible to present a single value for the increased risk for all RTE foods because of the different properties of the various foods, the range of values here provide some insight into the magnitude of the increase in risk that may be associated with the ability of a food to support the growth of *L. monocytogenes*.

