$$I(a,x) = \frac{1}{\Gamma(a)} \int_0^x t^{a-1} e^{-x} dx \qquad\qquad \textbf{(A3.1.9)}$$

Then the loglikelihood for r observations of concentrations below a detection limit x_1, n–r observations of concentrations in the range from the detection limit to a maximum observed concentration of x_2, and no observations of any higher concentrations, is just

$$r \ln P(0, x_1) + (n-r) \ln P(x_1, x_2) \qquad\qquad \textbf{(A3.1.10)}$$

Taormina *et al*. (2003), in addition to reporting the range of concentrations, also reported the mean concentration of those detected. This allows an additional approximate term[59] to be added to the loglikelihood of the form

$$-\ln(\sigma) - 0.5\big((m-\mu)/\sigma\big)^2 \qquad\qquad \textbf{(A3.1.11)}$$

where m is the observed mean value of the detects, and μ and σ are respectively the expected value of that mean, and its expected standard error, given by

$$\mu = ab\big(I(a+1, x_2/b) - I(a+1, x_1/b)\big)\big/\big(I(a, x_2/b) - I(a, x_1/b)\big) \qquad \textbf{(A3.1.12)}$$

and

$$\sigma = \Big(\big(b^2 a(a+1)\big(I(a+2, x_2/b) - I(a+2, x_2/b)\big)\big/\big(I(a, x_2/b) - I(a, x_2/b)\big) - \mu^2\big)\big/(n-r)\Big)^{1/2} \textbf{(A3.1.13)}$$

Foster *et al*. (1977) reported numbers of samples within ranges of estimated CFU/g, but in such a way as to allow deduction of the corresponding ranges of observed colony counts. In addition, they reported the mean concentration observed. This allows use of the distribution given in equation (A3.1.3), giving likelihood contributions of the form

$$\left(\sum_r k_r\right) \ln\left(\sum_r P(r, m, a, b)\right) \qquad\qquad \textbf{(A3.1.14)}$$

for each range of colony counts, where the sums are over the specific colony counts within that range, and the terms have the same meaning as for equations (A3.1.3) and (A3.1.4) (so in this case only these sums of k_r are known, not the individual k_r). Finally, the mean may be used to give an additional approximate loglikelihood contribution of the form of equation (A3.1.11), where again m is the observed mean concentration, and μ and σ are respectively the expected value of that mean, and its expected standard error. For the distribution given in equation (A3.1.3), these are (assuming a total of N samples)

$$\mu = ab$$
$$\sigma = \sqrt{ab(b+1/m)/N} \qquad\qquad \textbf{(A3.1.15)}$$

Estimates for the parameters a and b were obtained by maximizing the likelihood (using the Solver in Excel[®]). If more than one experiment was fitted simultaneously (*e.g.* with a common parameter), all relevant parameters were estimated simultaneously to maximize the sum of the loglikelihoods, with constraints on the parameters, or relations between them, if necessary. Joint

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[59] The approximation is two-fold — a normal approximation for the distribution of the mean, and an approximation induced by the omission of any correlation between the mean estimate and the other information used in the likelihood estimate. Both approximations should be accurate here.

uncertainty distributions for the parameters were obtained by first finding transformations of the parameters such that the individual marginal profile likelihoods for the transformed parameters were approximately quadratic (so that the profile likelihood behaved approximately as a normal distribution). The object was to obtain a parameterization of the loglikelihood in which a (multi-dimensional) quadratic approximation about its maximum value was reasonably accurate over a range extending out several standard deviations, so that the uncertainty distribution approximated the likelihood reasonably closely over as large a range as possible. Empirical investigation of some of the loglikelihoods used in this risk assessment showed that the procedure adopted substantially improved the quadratic approximation (although further improvement was generally possible).

The variance-covariance matrix for the transformed parameter estimates was approximated numerically by inverting an approximation of the information matrix (the matrix of second derivatives with respect to the transformed parameters, evaluated at the maximum likelihood). The second derivative matrix at the maximum likelihood was approximated numerically by making small changes in the transformed parameter values away from the optimum, first one parameter at a time, then in pairs. The resulting changes in loglikelihood were fit in the same sequence as just described to the corresponding quadratic approximation in second derivatives. The sizes of the small changes were generally chosen to approximate the standard deviations of the transformed parameter estimates, so that correlations at relatively large deviations would not be inadvertently omitted. The uncertainty distribution for the transformed parameters was then taken to be a multinormal[60] distribution with the numerically estimated variance-covariance matrix.

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[60] The multinormal distribution has a density that is proportional to the exponential of minus a quadratic form in the vector of variates. This distinguishes it from the many other multivariate distributions with normal marginal distributions.

Appendix 3.2 Growth models for *C. perfringens*

A3.2.1 Some background mathematics

Modeling of growth for *C. perfringens* from spores following heat shock has mostly been based on empirical fits to growth curves, with only heuristic connections between the parameters of the models and biological phenomena. Usually what have been used are Gompertz or logistic curves fit to observed counts of CFU density, or more usually to the logarithm of the density, the density including both vegetative cells and any remaining spores that can germinate under the cultivation conditions used for CFU counting (generally different from the growth conditions under test). While such empirical fits to growth curves can provide a very useful summary of the growth to be expected under the conditions tested, extrapolation to other conditions is impeded by the lack of direct connection between model parameters and biological phenomena. The model parameters have to be interpreted in some biologically plausible way in order to make inferences about them under different conditions; and such plausibility arguments are difficult to test without a more rigorous basis for the models.

An approach that may allow more direct inferences of growth under alternative conditions is to explicitly model the biological phenomena involved. The choice of mathematical models is then generally governed by a combination of factors, including incorporation of plausible mathematical representations of the biological processes, and convenience, usually interpreted so that the resulting equations are exactly soluble, easily computed, or have simple structure.

Primary models[61] for bacterial growth at fixed temperature directly attempt to separate the processes of spore germination and vegetative growth. The spore is envisioned as going through some process or set of processes that result in it forming a vegetative cell capable of replication. Before such processes are complete, replication is impossible; after they are complete, replication proceeds at some rate that can be characterized by a growth rate. Replication continues until high vegetative cell densities, at which point some feedback mechanism slows down replication until it stops entirely at a limiting cell density.

The latest models to examine particular and distinguishable processes occurring are of the form (Juneja and Marks, 2002; Huang, 2004):

$$\frac{\partial C_s}{\partial t} = -kC_s$$

$$\frac{\partial C_v}{\partial t} = qkC_s + \mu C_v\left(1 - C_v/C_m\right)$$

(A3.2.1)

where the terms are

C_s number of viable spores
Cv number of dividing, vegetative, cells
C_m maximum number of dividing cells
k transformation rate of spores (possibly time-dependent)

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[61] "Primary" models relate cell density to time at fixed temperature. "Secondary" models then relate the parameters of the primary model to temperature.

μ growth rate for dividing cells (possibly time-dependent)

q the fraction of transformed spores that survive to divide.

Partial derivatives are used to indicate fixed temperature. The boundary condition examined here is that $C_v = 0$, $C_s = C_0$ at $t = 0$. In all cases discussed below, $q = 1$ is selected (Juneja *et al.* 2001 examined $q \neq 1$ to some extent; however, in most cases only those spores that are capable of transforming are ever enumerated, so that all experiments measure only such spores). The first equation represents the conversion of spores to vegetative cells, and the second the replication of vegetative cells.

Strictly speaking, such equations should be written as probabilistic equations (indicating the probabilities for cells to transform from spore to vegetative state, and then the probability for vegetative cells to divide), to account for the granularity of cell densities, especially at low cell densities. Currently, however, cell densities are treated as continuous quantities, with deterministic equations for them, and that is the approach taken here. For large cell densities, the uncertainties induced by such a treatment should be small. For small cell densities, especially during the early stages of growth where there may be only one or a few cells in any volume of interest, reality is likely to be more uncertain than suggested by the solutions of these equations.[62]

For short times (where $C_v << C_m$) the last term in Equation (A3.2.1) (the quadratic term) can be ignored. The first equation in (A3.2.1) is trivially integrated (at fixed temperature) with a single quadrature:

$$C_S = C_0 \exp(-K(t)) \qquad \textbf{(A3.2.2)}$$

where C_0 is the initial (at $t = 0$) number of spores, and

$$K(t) = \int_0^t k(s)ds \qquad \textbf{(A3.2.3)}$$

so the second equation in (A3.2.1) can be reduced to a Riccati equation:

$$\frac{\partial y}{\partial t} = P + \mu y(1 - y) \qquad \textbf{(A3.2.4)}$$

where

$$y = C_v/C_m$$
$$P = qk\, C_s/C_m \qquad \textbf{(A3.2.5)}$$

so that $P = P(t)$ and $\mu = \mu(t)$ are known functions of time, and $y = 0$ at $t = 0$.

There is no advantage in writing the first equation of (A3.2.1) in the particular form shown. Indeed, it turns out to be more convenient to write

$$\frac{\partial C_s}{\partial t} = -C_0 g(t) \quad \text{with} \quad \int_0^\infty g(s)ds = 1 \qquad \textbf{(A3.2.6)}$$

where $g(t)$ is some known function of time. Then

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[62] Some of the extra uncertainty induced by the integral number of cells may be captured to some extent by uncertainty analyses applied to experimental data, provided the number of cells used in those experiments is close to the numbers that are important in practice.

$$C_s = C_0 \left(1 - \int_0^t g(s)ds\right) = C_0 (1 - G(t))$$

$$\text{where} \quad G(t) = \int_0^t g(s)ds \quad \text{so} \quad G(\infty) = 1$$

(A3.2.7)

This is really equivalent to equation (A3.2.2) — writing $K(t) = -\ln(1-G(t))$ gives the exact equivalence— but it allows choosing the functional form of $g(t)$, hence of P, more easily. The definition of y is unaltered, but P is altered to give

$$y = C_v / C_m$$

$$P(t) = qg(t)C_0 / C_m$$

(A3.2.8)

The Riccati equation (A3.2.4) has no known analytic solution, so it is difficult to use. There are various assumptions that went into its derivation, including:

a.   The rate of transformation of spores to viable dividing cells is independent of the dividing cell density.

b.   The rate of division decreases as the limiting density decreases in a way that is adequately modeled by the term $(1 - y)$. [Replacing the term $(1 - y)$ with a function $F(y)$ that is monotonic increasing on [0,1] and tends to zero as y tends to 1 leads to a more generalized equation; for the homogeneous case ($P = 0$), for example, replacing $(1 - y)$ with $-\ln(y)$ gives a Gompertz curve in place of the logistic — see also Section A3.2.3 below.]

Replacing assumption a. with an equally plausible assumption, that the rate of transformation to vegetative cells is independent of cell density, but that the survival of those vegetative cells decreases quadratically to zero as $y \to 1$, leads to an equation with an analytic solution that is much easier to work with. Thus, replacing Equation (A3.2.4) with

$$\frac{\partial y}{\partial t} = P(1-y)^2 + \mu y(1-y)$$

(A3.2.9)

(which is also a Ricatti equation) gives the analytic (fixed temperature) solution

$$y = \frac{z}{1+z}$$

(A3.2.10)

where

$$z(t) = \exp(M(t)) \int_0^t P(s)\exp(-M(s))ds$$

(A3.2.11)

(which is also the small time approximate solution of (A3.2.4), equivalently the solution of the linearized version of that equation), and

$$M(t) = \int_0^t \mu(s)ds$$

(A3.2.12)

In practical applications, there is likely to be negligible difference between equations (A3.2.4) and (A3.2.9), since spore densities are likely to be substantially smaller than limiting densities for dividing cells. Moreover, equation (A3.2.9) is more convenient to work with, because of the availability of an expression for the analytic solution for all times.

A limited set of modifications to the quadratic in y multiplying P are possible, obtaining other equations that have the solution form (A3.2.10). Thus:

$$\frac{\partial y}{\partial t} = P\left(1+(\beta-2)\,y-(\beta-1)\,y^2\right)+\mu y\left(1-y\right)$$

$$= P\left(1-y\right)\left(1+(\beta-1)\,y\right)+\mu y\left(1-y\right) \tag{A3.2.13}$$

where β is a constant has a solution of the form (A3.2.10) with

$$z(t) = \exp\left(M(t)+\beta R(t)\right)\int_0^t P(s)\exp\left(-M(s)-\beta R(s)\right)ds \tag{A3.2.14}$$

where

$$R(t) = \int_0^t P(s)\,ds \tag{A3.2.15}$$

The value $\beta = 1$ gives a particularly simple form, and it is straightforward (although a little less convenient) to perform the analysis below with such a modification. However, the differences between all these equations are of order C_0/C_m, which is negligibly small in current applications.

A3.2.2 Application

Juneja *et al.* (2001) suggested using the linearized version of equations (A3.2.1) (that is, omitting the quadratic term on the right hand side in the second equation) with

$$k(t) = \lambda t^{\alpha-1} \tag{A3.2.16}$$

but then specialized to $\alpha = 1$, corresponding to an exponential for P, and $\mu = $ constant. This specialization results in easily computed analytic solutions for z in equation (A3.2.11), and over the exponential growth phase z was used in place of y as an approximate solution. Juneja and Marks (2002) used essentially the same approach. Huang (2004) suggests using equations (A3.2.1), but again with $k(t)$ and μ constant (that is, with $\alpha = 1$), obtaining the solution using a numerical integrator to cover the full range of growth, including the saturation at large times.

The following discussion is more general, and uses equation (A3.2.9) to allow analytic solutions over the full growth range; and such solutions are negligibly different from those of equation (A3.2.4) for C_0/Cm small. Also, since $\mu = $ constant (*i.e.* a constant cell division rate or growth rate at constant temperature) appears to fit all available data, that is also assumed in what follows.

A3.2.2.1 Model 1

A simple generalization of $k = $ constant that also allows analytic solutions for z is

$$k(t) = a+bt \tag{A3.2.17}$$

since then

$$z(t) = \frac{C_0}{C_m}\left(e^{\mu t} - e^{-at-bt^2/2} - \mu\sqrt{\frac{2\pi}{b}}\,e^{\mu t+(a+\mu)^2/2b}\left[\Phi\left(\sqrt{b}\left(t+\frac{a+\mu}{b}\right)\right)-\quad\left(\frac{a+\mu}{\sqrt{b}}\right)\right]\right) \tag{A3.2.18}$$

where Φ is the standard normal integral

$$\Phi(x) = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{x} e^{-x^2/2} dx \tag{A3.2.19}$$

(this is g_model_1 in the accompanying workbook; evaluation of z is straightforward except for small values of b).

Applying this model to the data of Huang (2003)[63] leads to strong selection for $a = 0$, matching with the expected biological behavior of germinating spores — that they go through some process that takes non-zero time during germination to the vegetative state in which they can start dividing. Indeed, consideration of this behavior suggests selecting for $k(t)$ a function that allows for a very low or zero initial rate of transformation from spore to vegetative cell. The total number of cells transforming should then increase to a maximum and decrease.[64]

A3.2.2.2 Model 2

To test for such behavior, the model given by equation (A3.2.16) was implemented in the form[65]

$$k(t) = \frac{a}{t_m} \left(\frac{t}{t_m} \right)^a \tag{A3.2.20}$$

so that

$$P(t) = \frac{C_0}{C_m} \frac{a}{t_m} \left(\frac{t}{t_m} \right)^a \exp\left(-\frac{a}{a+1} \left(\frac{t}{t_m} \right)^{a+1} \right) \tag{A3.2.21}$$

The form of $k(t)$ is here chosen so that $P(t)$ has a maximum at $t = t_m$, and this maximum has a relative width approximately proportional to $1/a$ for large a. This parameterization was chosen to give some physical meaning to the parameters — t_m is roughly the time it takes for a spore to germinate, and a measures the spread of such times. This physical interpretation also allows an easy modification to account for varying temperatures — see Section A3.2.5 below.[66]

Applying (A3.2.21) in (A3.2.4) to the data of Huang (2003) strongly suggests that a is large. This may be due to either a lack of discrimination in the experimental measurements (quite likely) or because spores germinate almost simultaneously (also possible). Direct testing would require some direct observation of germination of the spores that was not interfered with by the vegetative cells; this may be possible optically.

A3.2.2.3 Model 3

Using the model (A3.2.21) is inconvenient because of the lack of analytic solutions. However, initial efforts indicate that a functional form for $P(t)$ that is similar — with a negligible initial

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[63] These models have been applied to other experimental data also, but the discussion here is limited. Practical implementations of the models are available in the workbook CP_fixed_temp.xls accompanying this Risk Characterization.

[64] The transformation rate may keep increasing, but with a finite density of initial cells, the number transforming will decrease again after some time.

[65] There is no connection between the a parameter in this paragraph and that in the last. The symbol is just being re-used.

[66] This model is g_model_2 in the accompanying workbook CP_fixed_temp.xls; there is no analytic solution in terms of well known function, so it is implemented using a 5th order adaptive-step-size Runge-Kutta integrator, which works fairly well.

rate and a peaked shape — should be adequate. The effect of different functional forms for $k(t)$ is easiest to implement using the alternative formulation given at equation (A3.2.6). Further work therefore used equation (A3.2.9), with[67]

$$P(t) = \frac{C_0}{C_m} \frac{1}{t_m \Gamma(a)} \left(\frac{at}{t_m}\right)^a \exp\left(-\frac{at}{t_m}\right) \qquad \text{(A3.2.22)}$$

which again has a maximum at $t = t_m$, but the relative width is now about $1/\sqrt{a}$. The advantage of this functional form is that equation (A3.2.11) may then be analytically integrated in terms of standard functions:

$$z(t) = \frac{C_0}{C_m} e^{\mu t} \left(\frac{a}{a + \mu t_m}\right)^{a+1} I\left(a+1, t\left(\mu + a/t_m\right)\right) \qquad \text{(A3.2.23)}$$

where I is the incomplete gamma integral

$$I(\alpha, x) = \frac{1}{\Gamma(\alpha)} \int_0^x w^{\alpha-1} e^{-w} dw \qquad \text{(A3.2.24)}$$

Provided a is reasonably large, a and t_m have natural interpretations; the latter as an average time to germination of a spore, the former measuring the variation in this time to germination. Using the previous definitions (equations (A3.2.7), (A3.2.8), and (A3.2.10)) gives

$$C_s(t) = C_0\left(1 - I\left(a+1, at/t_m\right)\right)$$
$$C_v(t) = C_m \frac{z(t)}{1 + z(t)} \qquad \text{(A3.2.25)}$$

Fitting this model to the data of Huang (2003, and personal communication) gave MLE values for a that ranged from 55 to (effectively) infinity for individual temperatures, and that were not significantly different for any temperature (p=0.99, likelihood ratio test). The MLE for the joint value was effectively infinity ($>10^5$). With this model also, the product μt_m is temperature independent in these data (p=0.16, likelihood ratio test), as are the initial concentrations (p=0.99, likelihood ratio test), and the maximum concentrations (p=0.49, likelihood ratio test) except at 50°C (where the maximum concentration is substantially lower).

A3.2.3 Connection with usual growth curve fitting techniques

It is interesting to observe that the limit $a \to \infty$ in (A3.2.22) (or in (A3.2.21)) gives a simple connection to the usual *ad hoc* fitting of logistic curves to growth data, and suggests a way of modifying those approaches to give parameters that (may) have biological significance. Taking this limit reduces $P(t)$ to a delta function at t_m

$$P(t) = \frac{C_0}{C_m} \delta(t - t_m) \qquad \text{(A3.2.26)}$$

Equations (A3.2.4) or (A3.2.9) may then be analytically integrated. For the usually measured[68] (and usually fitted) quantity $C_s + C_v$, the former gives

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[67] There no mathematical connection between the parameters in this paragraph and those in the last, although they have been given the same symbols and represent the same physical quantities.
[68] This assumes that the measurement technique will measure all spores that have started to germinate, and all vegetative cells. It is possible that some of the spores that transform to vegetative cells during measurement would not have so transformed in the original mix — if there is any feedback, for example, as implied by (A3.2.9).

$$C_s + C_v \;\; = \;\; C_0 \qquad\qquad\qquad\qquad \text{for} \quad t < t_m$$

$$= \;\; \frac{C_m}{1 + (C_m/C_0 - 1)\exp(-\mu(t - _m))} \qquad \text{for} \quad t > t_m \qquad \textbf{(A3.2.27)}$$

$$= \;\; \frac{C_m}{1 + \exp(-\mu(t - t_m) + \ln(C_m/_0 - 1))}$$

Equation (A3.2.9) gives a minor modification:

$$C_s + C_v \;\; = \;\; C_0 \qquad\qquad\qquad\qquad \text{for} \quad t < t_m$$

$$= \;\; \frac{C_m}{1 + (C_m/C_0)\exp(-\mu(t - _m))} \qquad \text{for} \quad t > t_m \qquad \textbf{(A3.2.28)}$$

$$= \;\; \frac{C_m}{1 + \exp(-\mu(t - t_m) + \ln(C_m/C_0))}$$

(There is a slight mismatch at $t = t_m$ in the second equation, corresponding to some spores not germinating to viable vegetative cells in the presence of other vegetative cells, as implied by equation (A3.2.9) — but they might germinate under the conditions used to measure concentrations, for example if diluted).

The same sort of analysis can give a Gompertz growth curve[69] with a slight modification of equation (A3.2.4). If the growth curve is instead given by

$$\frac{\partial y}{\partial t} = P - \mu y \ln y \qquad\qquad\qquad\qquad \textbf{(A3.2.29)}$$

(which has the same generic shape as equation (A3.2.4)), then the solution with a delta function at $t = t_m$ is

$$C_s + C_v \;\; = \;\; C_0 \qquad\qquad\qquad\qquad \text{for} \quad t < t_m$$

$$= \;\; C_m \exp\left[\ln\left(\frac{C_0}{C_m}\right)\exp(-\mu(t - _m)) \right] \qquad \text{for} \quad t > t_m \qquad \textbf{(A3.2.30)}$$

$$= \;\; C_m \exp\left(-\exp(-\mu(t - t_m) + \ln(\ln(C_m/ _m)))\right)$$

Equation (A3.2.29) appears less plausible as a representation of biological processes, in that it presumes that the replication rate of cells at very low cell densities is substantially higher than at the intermediate cell densities where replication rates are generally considered maximal.

A3.2.4 Variation of parameter values with temperature

The growth curves discussed so far are for fixed temperatures. As that fixed temperature is changed, the parameter values also change in a regular way. The variation in values is typically fitted by a secondary model of Ratkowsky form, and that approach is adopted here. Thus the variation of growth rate μ with temperature would usually be given by a model of the form

$$\mu = \mu(T) = a(T - T_{\min})^2 \left(1 - \exp(b(T - T_{\max}))\right) \qquad\qquad \textbf{(A3.2.31)}$$

where the symbols represent:

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[69] This Gompertz curve is for the cell density. However, one usual empirical fitting procedure is to use a Gompertz curve to fit the logarithm of cell density.

T temperature,
T_{min} the minimum temperature below which growth does not occur,
T_{max} the maximum temperature above which growth does not occur,
a a parameter of the model, and
b the second parameter of the model.

This model form is entirely heuristic, designed to represent the shape of the growth-rate versus temperature curve (and the shape of other temperature-dependent functions, such as $1/t_m$) observed empirically for various organisms. However, the $(a, b, T_{min}, T_{max}, T)$ parameterization has several disadvantages:

- The parameters a, b do not relate to any obvious feature of the curve — widely varying combinations of these parameters can give curves that are only slightly different. As a result, estimates of a and b based on data are highly correlated.
- The parameters a, b are implicitly positive. However, imposing positivity on them restricts the range of shapes of the curve — in particular, its maximum cannot be any closer to the minimum temperature T_{min} than 2/3 of the way between T_{min} and T_{max}. Allowing a, b to be simultaneously negative removes this restriction, but the connection between the two possibilities is not smooth (a and b tend to positive infinity, then back from negative infinity, as the maximum temperature goes through the point 2/3 of the way between T_{min} and T_{max}). As a result, estimation procedures for a and b can easily obtain unintended results.

To overcome these disadvantages, but retain the standard shape function, the curve was re-parameterized in terms of x_m, the fractional distance downwards between T_{max} and T_{min} of the maximum of the curve, and A, the maximum value of the curve, in the form:

$$\mu = \mu(T) = A\frac{(1-x)^2(1-\exp(-\theta x))}{N} \tag{A3.2.32}$$

where

$$x = \frac{T_{max} - T}{T_{max} - T_{min}} \quad \text{and} \quad N = N(x_m) = (1-x_m)^2(1-\exp(-\theta(x_m)x_m)) \tag{A3.2.33}$$

and $\theta = \theta(x_m)$ is the unique solution of

$$\exp(\theta x_m) = 1 + \theta(1-x_m)/2 \quad \text{for} \quad 0 \le x_m \le 1 \tag{A3.2.34}$$

(this choice of θ ensures that x_m is the location of the maximum of the curve). With this parameterization, the location of x_m can be varied from 0 to 1 while retaining the form (A3.2.32) for the curve (strictly speaking, at $x_m = 1/3$, the equation takes on a limiting form since both θ and N vanish at that point, but their ratio is well-defined).

A3.2.5 Extension to varying temperature[70]

Juneja *et al*. (2001) have pointed out the likely necessity of taking account of memory effects — that is, that current rates of biological processes may depend on the past history of the cells involved — when modeling the effect of varying temperatures on growth. They suggested one approach that requires an empirical choice of a temperature function to act as a "pivot point." The approach discussed here can provide a natural approach to the problem of varying temperature.

The growth rate μ is generally expected to depend on temperature, but to be practically independent of the temperature history of the cell culture. On the other hand the time to germination, t_m in the current parameterization, is likely to depend strongly on temperature history. This parameter provides a natural time-scale against which to measure the passage (of a spore, following heat shock) towards germination at fixed temperature, and the following discussion extends this idea to varying temperatures.

At fixed temperature, the equations of motion for model 3 above can be written:

$$\frac{\partial C_s}{\partial t} = -\frac{C_0}{t_m} h\left(a, t/t_m\right)$$

$$\frac{\partial C_v}{\partial t} = \frac{C_0}{t_m} h\left(a, t/t_m\right)\left(1 - C_v/C_m\right)^2 + \mu C_v\left(1 - C_v/C_m\right)$$

(A3.2.35)

where

$$h(a, w) = (aw)^a \exp(-aw)/\Gamma(a)$$

(A3.2.36)

Both t_m and μ are temperature dependent, but a does not appear to be (insofar as it is identifiable in the available data). One natural extension of these equations to variable temperature is then

$$\frac{dw}{dt} = \frac{1}{t_m\left(T(t)\right)}$$

$$\frac{dC_s}{dt} = -\frac{C_0}{t_m} h(a, w)$$

(A3.2.37)

$$\frac{dC_v}{dt} = \frac{C_0}{t_m} h(a, w)\left(1 - C_v/C_m\right)^2 + \mu C_v\left(1 - C_v/C_m\right)$$

where the temperature T is time-dependent, $T=T(t)$, and the temperature, hence time, dependence of t_m has been written in full in the first equation (in these equations, the other parameters may also be temperature dependent, hence also time dependent). In this formulation, w may be interpreted as a dimensionless parameter that measures the fraction of the process of germination that has occurred at any time, with $w = 1$ corresponding to an average time of germination (with a relative variability of $1/\sqrt{a}$).

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[70] It is not necessary to model growth from spores under varying temperature conditions in this Risk Characterizaton.

Equations (A3.2.37) have simple analytic solutions analogous to those of model 3, and these solutions are especially simple if μt_m is constant. The analytic solutions are obtained by treating w as the fundamental variable — multiply the second and third equation by t_m, and use the first to obtain

$$\frac{dw}{dt} = \frac{1}{t_m\left(T(t)\right)}$$

$$\frac{dC_s}{dw} = -C_0 h(a, w) \qquad \text{(A3.2.38)}$$

$$\frac{dC_v}{dw} = C_0 h(a, w)\left(1 - C_v/C_m\right)^2 + \mu t_m C_v\left(1 - C_v/C_m\right)$$

The first of these allows computation of w, and the second two are entirely analogous to the equations of model 3. If μt_m is constant (*i.e.* independent of temperature, hence of time when the temperature is varying), we obtain

$$C_s(w) = C_0\left(1 - I(a+1, aw)\right)$$

$$C_v(w) = C_m \frac{u(w)}{1 + u(w)} \qquad \text{(A3.2.39)}$$

where

$$u(w) = \frac{C_0}{C_m} e^{\mu t_m w}\left(\frac{a}{a + \mu t_m}\right)^{a+1} I\left(a+1, w\left(a + \mu t_m\right)\right)$$

4. Limitations of the Exposure Model

4.1. Representativeness assumptions

The major limitation of the exposure modeling used here lies in the representativeness of the data used and the implied assumptions of the analysis methods. The following list identifies the principal places where such representative assumptions are made.

- The selected 26,548 food servings are representative of RTE and partially cooked food servings in the U.S.
- The four categories adequately represent and distinguish differences in handling of the food servings.
- The Taormina *et al*. (2003), Kalinowski *et al*. (2003), and FSIS (2003) studies provide representative spore concentrations for all meat products entering the system.
- The Strong *et al*. (1963), Foster *et al*. (1977), and Taormina *et al*. (2003) studies provide representative vegetative cell concentrations for meat products entering the system.
- Distinct meat products (*e.g.* beef, pork, chicken, ground or whole meat) have the same distribution of spore and vegetative cell concentrations.
- The Powers *et al*. (1975), Rodriguez-Romo *et al*. (1998), and Candlish *et al*. (2001) studies provide representative spore concentrations for spices entering the system.
- Combination of spices into the groups selected here adequately represents the spice concentrations in diverse spices.
- The selected data from the studies of Daube *et al*. (1996), Kokai-Kun *et al*. (1994), and Skjelkvale *et al*. (1979) for raw meat; and from the study of Rodriguez-Romo *et al*. (1998) for spices, provide representative information on the fraction of *C. perfringens* present in meat and spices that are type A, CPE-positive.
- There is no external contamination of foods with *C. perfringens* during serving manufacture and distribution.
- Reported laboratory experimental measurements of the growth rate of *C. perfringens* and *C. botulinum* from spores in simulated food matrices under anaerobic conditions provide representative estimates for the growth rates of vegetative cells expected in RTE and partially cooked foods in normal food production and distribution.
- The studies selected in Section 3.13.2.1 adequately represent death rates of vegetative cells in cold conditions.
- The times and temperatures of storage selected from non-random surveys and discussed in Section 3.13.3 are representative of times and temperatures of storage for all RTE and partially cooked foods.
- The use of two storage times and temperatures adequately represents the time-temperature history of RTE and partially cooked foods between manufacture and consumption.
- Cooking time-temperature conditions are adequately represented by the adopted dichotomy in heating times (characterized as by microwave ovens and other ovens).
- The experimental data of Section 3.14.1, and its analysis as being with and without heat shock, are representative for all type A, CPE-positive *C. perfringens* during re-heating of RTE and partially cooked foods in microwave ovens and other ovens respectively.
- Re-heating temperatures are adequately represented by the selected cooking temperatures from those collected by Audits International/FDA (1999).

- Hot holding temperatures are adequately represented by the incidental data collected by FDA (FDA, 2000).

4.2. Other assumptions consistent with but not proved by available data

The model is simplified by making assumptions that are consistent with available data, but such data could also be open to other interpretations, usually because of lack of defining experiments. The principal such assumptions are listed here. Cases where data are available, but too sparse to analyze fully, have been separately considered in the sensitivity analyses, although there may be some overlap.

- The gamma distribution adequately represents the variability of spore and vegetative cell concentrations in meat products and spices entering the system.
- Partial cooking has no effect on vegetative cell or spore concentrations in meat products.
- *C. perfringens* in spices is entirely present as spores.
- Partial cooking converts spores in spices to vegetative cells at the same efficiency as the methods used to measure spores in spices (with no heat step).
- The fraction of type A and non-type A *C. perfringens* in spices is the same, within each *cpe* category, as in meat and other foods.
- The growth and toxicological properties of *C. perfringens* spores are independent of their source.
- The minimum and maximum temperatures for growth of *C. perfringens* are identical to the minimum and maximum temperatures for spore germination.
- Selection of a value of 100 for the *a* parameter used in the growth model adequately represents the transition from the germination, outgrowth, and lag phase to the exponentially growing phase.
- Spore germination (particularly during heat treatment) is not substantially affected by the salt concentrations present in the RTE and partially cooked foods evaluated here.
- Spore germination is not substantially affected by the pH of the foods evaluated here.
- Spore germination is not significantly delayed by nitrite concentrations to be found in RTE and partially cooked foods.
- Suppression of *C. perfringens* vegetative cell growth by nitrite is by the same factor over the entire temperature range permitting *C. perfringens* growth in the absence of nitrite, and that factor is independent of salt content of the food.
- The water activity of all the selected food servings is sufficiently high to have no effect on germination or growth of C. perfringens.
- Vegetative cells present in RTE and partially cooked foods are ready to begin exponential growth, and start such exponential growth as soon as temperature conditions are favorable.
- Spontaneous germination of spores during storage of RTE and partially cooked foods is adequately represented by assuming all such germination occurs at the beginning of storage.
- Cold shock has negligible effect on the concentration of vegetative cells in practical situations for cooling RTE and partially cooked foods, and similarly for freeze/thaw cycles during storage.

- Storage below some minimum temperature leads to cell death for *C. perfringens* vegetative cells, with a probability per unit time that is independent of time; whereas storage above that minimum temperature leads to growth.
- Re-heating prior to hot holding is always sufficient to kill all vegetative cells.
- The effect of re-heating on ungerminated spores is equivalent to an initial heating.
- In the meat dishes examined, once cell densities have increased to stationary phase, they do not substantially decline.
- Maximum cell densities are independent of the food for the selected food servings.

4.3.   Limitations introduced by the methods used in modeling

In addition to the limitations already listed, there are also limitations introduced by the methods used to analyse data inputs to the risk assessment. These include:

- The variability incorporated in the growth modeling is adequate to represent the stochastic processes that probably occur at low cell densities (particularly the likely stochastic variation in delay times).
- The statistical methodologies used to evaluate data; in particular, the use of likelihood techniques and the use of approximations to the likelihood function to represent uncertainty.
- The use of the Ratkowsky equation as the secondary model to correlate growth rates of C. perfringens vegetative cells with temperature.
- The distribution shapes for variability or uncertainty are adequately represented by the choices made.

4.4.   Other limitations

Once the modeling had been completed and the results obtained, it became apparent in hindsight that other assumptions had been implicitly made in the modeling. Two that are examined in Section 6.5 are

- At low temperatures (but above the minimum temperature for *C. perfringens* growth), overgrowth and suppression of *C. perfringens* by other organisms does not occur.
- Consumers would not notice *C. perfringens* even at cell densities corresponding to stationary phase in purchased foods or food servings, or in such food servings taken out of their own refrigerators.

5. Hazard Characterization

5.1. Data for Dose-response relationship

The purpose of a dose-response relationship is to provide an estimate of probability of illness following ingestion of a specified number of pathogenic organisms. The dose-response model described in this chapter was developed to express the relationship between the dose of the pathogen *C. perfringens* and the likelihood of diarrheal illness in humans. The following outlines the rationale behind defining illness as diarrhea:

- Diarrhea is a representative symptom caused by *C. perfringens* food poisoning (McClane, 2001). Moreover, it is the end-point addressed by this risk assessment.
- Criteria for determining whether an infected individual has experienced diarrhea is objective, as compared to other, more subjective criteria (*e.g.*, 'feelings of lightheadedness').
- Diarrhea was one of the symptoms assayed in each of the *C. perfringens* human feeding trials discussed below.

Generally speaking, when determining dose-response relationships, data from human feeding studies are considered better than those from animal model studies, which in turn are considered better than those from surrogate model studies (*e.g.*, the rabbit ileum loop model). Thus, we sought to evaluate data from *C. perfringens* human feeding studies to develop a dose-response relationship for the ingestion of *C. perfringens*.

The *PubMed* (www.ncbi.nlm.nih.gov) and *AGRICOLA* (www.nal.usda.gov) databases were searched for relevant papers. The references cited in these papers were similarly searched for additional human feeding studies, which may not have been retrieved, by the searches. All articles were obtained through the *National Agricultural Library's Document Delivery Service*.

Studies in which purified enterotoxin (CPE) was fed to human volunteers were found but not employed in this risk assessment (Skjelkvale and Uemura, 1977a; 1977b). Using data from such studies to establish a dose-response relationship would require assumptions that ultimately result in greater uncertainty than studies in which cells were fed to hosts. For example, the quantity of enterotoxin produced per vegetative *C. perfringens* cell, referred to as CPE, would have to be characterized before a model could incorporate this evidence. Additionally, toxic substances such as CPE isolated from the filtrate may be destroyed by the gastric juice, but the whole organism, particularly if enclosed in meat, may survive passage through the stomach, allowing it to produce toxin in the intestine (Hobbs *et al*., 1953). For these reasons and due to the strength of the human feeding trial data described in this chapter, such studies were not used to develop a dose-response relationship.

Six *C. perfringens* human feeding studies were identified and are summarized in the following two sections. In none of these was the number of doses per strain or people per dose sufficient to adequately define a dose-response curve. Most data represent single strain and matrix challenges. In these human feeding studies, all the administered doses were higher than 10^8 cells, so the effect of smaller doses must be conjectural. Some clinical data obtained from administering strains in four of the studies described below were included in the dose-response

modeling. Other data from the same studies were not used, and no data from two other studies were included for reasons that will be discussed.

5.2. Data Summary
5.2.1. Data included in dose-response modeling

The data described in this section were included in deriving a dose-response relationship. Only those portions of the data that were used are described here. Omitted data on human volunteers are discussed in the following section, and no mention is generally made of any control experiments, since they generally confirmed that the background rate of diarrheal illness can be ignored in such studies. Table 5.1 summarizes the evidence, in the strains included in the dose-response modeling, for production of the CPE toxin; in addition, most of these strains were originally isolated in association with outbreaks of human food poisoning.

- **Dische and Elek (1957):** This paper described human volunteer studies conducted with three strains of heat-resistant type-A *C. perfringens* (*C. welchii*[71]). This study used the following bacterial strains:

    i. *C. perfringens* strain NCTC 8797: Symptoms were observed in 16 of 18 people fed cells in Robertson's cooked-meat culture medium (mean 1.3×10^9 cells, ranging from 5.1×10^8 to 3×10^9 cells) and 5 of 6 people fed the supernatant broth portion for Robertson's medium (mean of 9.8×10^8 cells, range of 7.4×10^8 to 1.3×10^9 cells). Symptoms included diarrhea, abdominal pain and discomfort, vomiting, headache, and pyrexia. Among the total of 24 volunteers, 17 reported diarrhea (mean dose of 1.2×10^9 *C. perfringens* cells).

    ii. *C perfringens* strain NCTC 8797: Five volunteers were fed cell suspensions[72] containing a mean 1.2×10^9 cells (range 9.6×10^8 to 1.9×10^9). Three developed diarrhea. One of seven volunteers subsequently developed diarrhea after being fed lower doses (mean 1.9×10^8, range 3×10^7 to 4.2×10^8) of cell suspensions.

    iii. *C. perfringens* strain NCTC 8238: Two volunteers were fed cells in Robertson's cooked-meat culture medium (8.5×10^8 cells) and one person had 2 loose stools 11 hours post-ingestion.

- **Strong *et al*. (1971):** The authors examined the effect of feeding human volunteers individual strains or culture filtrates of rabbit-positive *C. perfringens* strains (those that produce fluid accumulation in the ligated ileum of young rabbits or overt diarrhea following intra-ileal injection of the non-ligated gut). Strains were administered to the volunteers in chocolate-flavored dairy drink (100 mL containing an average of 3.3×10^{10} total viable cells

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[71] *C. welchii* is an early name used in place of *C. perfringens*; however, for the sake of consistency, the term *C. perfringens* is used throughout this document.

[72] Bacterial cell suspension were prepared from the "broth fraction of Robertson's cultures, decanted from the meat or, in a few cases, from nutrient broth or 2% glucose-broth cultures, by centrifuging and resuspending the deposit in distilled water"

and 2.5×10^8 spores) or in canned beef stew (213 g containing an average of 2.5×10^{10} total viable cells and 7.8×10^7 spores). As spores are not expected to germinate into vegetative cells within a human being, it was assumed administered spores did not affect the outcome of these clinical trials. Of 92 volunteers tested, a total of 27 (29%) experienced diarrhea in various trials of different strains and doses.

For dose-response modeling, human trial data obtained from administering *C. perfringens* strains NCTC 10240, NCTC 8798, NCTC 8239, NCTC 10239, 68900, 79394, E13, and 027 were used (Table 5.2).

- **Hauschild and Thatcher (1967):** This study used *C. perfringens* strain S-79 (Table 5.2), previously isolated from roast beef. Six human volunteers ingested between 4 and 6×10^9 vegetative cells of this strain in cooked milk. Five of 6 volunteers experienced diarrhea and abdominal pain.

- **Dack *et al*. (1954):** Veal infusion broth cultures of *C. perfringens* strains ("isolated from suspected foods") identified as 683, 689, 690, and 692 were administered in milk to 5 volunteers each, and chicken broth cultures of strains 690 and 692 were administered to 6 volunteers each (Table 5.2). The volunteers were male or female physicians, nurses, students and other reliable hospital personnel who ranged in age from 21 to 45 years old. None of the volunteers experienced diarrhea following the dosages administered (between 4.62×10^8 and 5.56×10^9 viable *C. perfringens* cells).

Table 5.1   Evidence for toxin production and consequent inclusion of human clinical data in dose-response modeling.

Strain	Direct evidence of enterotoxin		Indirect evidence of enterotoxin			Strain reference
	PCR analysis *cpe* gene[b]	CPE protein[a]	No. of monkeys with diarrhea/ no. tested[e]	Fluid accumulation[c]	Spore heat-resistance ($\geq$30 mins at 100 C)[d]	
683, 689, 690, 692	ND[73]	ND	ND	ND	ND	Dack *et al.*, 1954
NCTC 8238	+	+	ND	+	+	Dische and Elek, 1957
NCTC 8797	ND	ND	ND	ND	+	
NCTC 8239	+	+	3/5	+	+	Dische and Elek, 1957; Strong *et al.*, 1971
S-79	ND	+	ND	+	-	Hauschild and Thatcher, 1967
NCTC 8798	+	+	2/5	+	+	Strong *et al.*, 1971
NCTC 10240	ND	+	3/5	+	+	
68900	ND	ND	ND	+	ND	
NCTC 10239	+	+	4/5	+	+	
79394	ND	ND	5/5	+	ND	
027	ND	ND	3/5	+	ND	
E13	+	+	0/5	+	+	

a.   Immunoblotting, erythema test or ELISA. Sarker *et al.*, 2000; Niilo, 1973; McClane and Strouse, 1984.
b.   Kokai-Kun *et al.*, 1994; van Damme-Jongsten *et al.*, 1990.
c.   Rabbit or lamb ligated intestinal loop experiments. Duncan and Strong, 1969a, 1969b; Strong *et al.*, 1971; Niilo, 1973.
d.   Hall *et al.*, 1963; Sarker *et al.*, 2000.
e.   Duncan and Strong, 1971.

The data that were used for dose-response modeling from these studies are summarized in Table 5.2. The dose-response relationships between total cells and attack rate (all included studies) are plotted in Figure 5-1. In this figure, points joined by lines indicate multiple-dose experiments for a single *C. perfringens* strain, while isolated points are for single dose experiments (with multiple *C. perfringens* strains)[74].

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[73] ND: Not Determined

[74] The two cases where observed rates decreased with increasing dose are ascribed here to the randomness of invidual responses and the very small numbers of people tested. In the case with two doses, the response rate declined from 2/4 to 0/4; in the second, the response rate at three increasing doses was 1/4, 0/5, 2/4. The graph is somewhat misleading without uncertainty estimates on the proportions plotted, but becomes confusing with them because all such uncertainty estimates for individual points are relatively large. The analysis takes correct account of the small numbers and resultant uncertainties.

Table 5.2   Data used to model the *C. perfringens* dose-response relationship.

Strain	No. of cells fed		Human subjects no. diarrhea / no. tested	Reference
	Total count	Spores		
027	3.20E+11	3.20E+08	2 / 4	4
683	2.90E+09	NM	0 / 5	1
689	2.12E+09	NM	0 / 5	1
690	4.62E+08	NM	0 / 6	1
690	1.29E+09	NM	0 / 5	1
690	1.03E+09	NM	0 / 6	1
692	5.56E+09	NM	0 / 5	1
68900	3.00E+10	3.20E+07	2 / 4	4
79394	7.90E+10	5.20E+05	4 / 4	4
E13	4.50E+12	1.60E+08	3 / 4	4
NCTC 10239	3.60E+10	6.40E+08	1 / 4	4
NCTC 10239	4.70E+10	5.40E+06	1 / 4	4
NCTC 10239	1.60E+11	4.20E+07	3 / 5	4
NCTC 10240	1.80E+09	2.70E+06	2 / 4	4
NCTC 10240	1.30E+10	3.40E+07	0 / 4	4
NCTC 8238	8.50E+08	NM	1 / 2	2
NCTC 8239	2.30E+09	NM	0 / 6	2
NCTC 8239	6.60E+09	7.80E+08	2 / 5	4
NCTC 8239	5.80E+10	1.60E+10	3 / 3	4
NCTC 8797	1.90E+08	NM	1 / 7	2
NCTC 8797	1.20E+09	NM	3 / 5	2
NCTC 8797	1.20E+09	NM	17 / 24	2
NCTC 8798	3.20E+09	1.50E+08	1 / 4	4
NCTC 8798	1.10E+10	1.50E+10	0 / 5	4
NCTC 8798	4.10E+10	2.10E+08	2 / 4	4
S-79	5.00E+09	NM	5 / 6	3

NM: not measured (*i.e.*, No attempts were made to measure from these studies)
1. Dack *et al.*, 1954.
2. Dische and Elek, 1957.
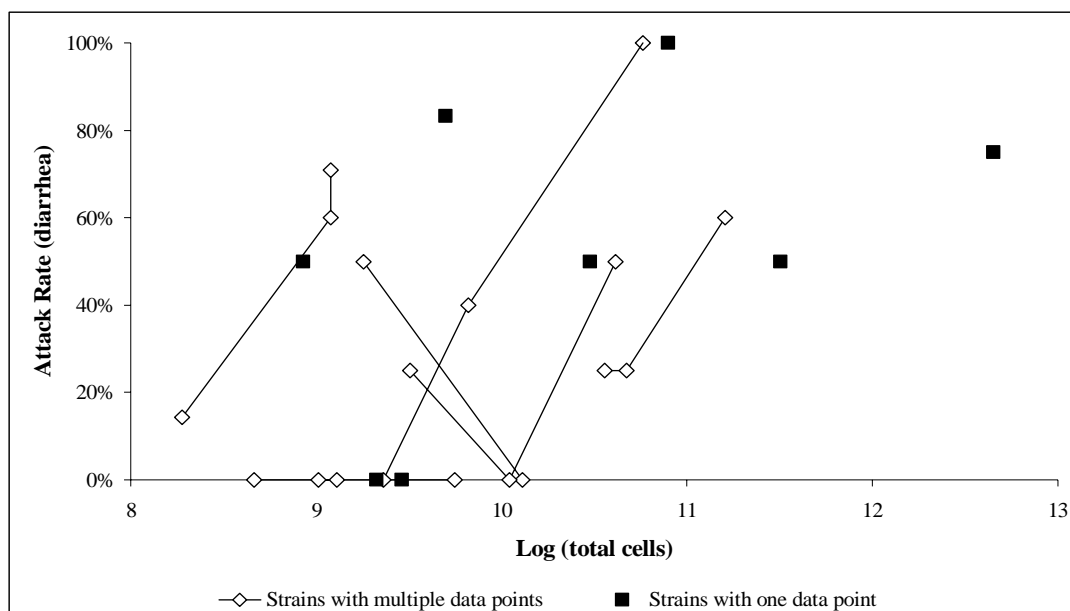3. Hauschild and Thatcher, 1967.
4. Strong *et al.*, 1971.

Figure 5-1 Dose-response relationship for *C. perfringens* (total cells).

5.2.2. Data not included in dose-response modeling

As mentioned in Section 5.2.1, data from four studies were included in dose-response modeling. However, some of the six studies identified also included data acquired by administering strains of *C. perfringens* which are not expected to cause disease, or that were otherwise unusable in dose-response modeling. The reasons for excluding human feeding data from such studies are discussed in the following paragraphs.

- **Strong *et al*. (1971):** Clinical data from *C. perfringens* strains 215b, F42, and FD1 were not used for the dose-response analysis. These strains (215b, F42, and FD1) were known to be rabbit-negative (do not produce fluid accumulation or overt diarrhea) and have subsequently been shown to lack the *cpe* gene (by PCR analysis) and/or to not produce the CPE protein (Table 5.3). In the absence of this gene, these *C. perfringens* strains would not be expected to cause *C. perfringens* food poisoning.

  Additionally, Strong *et al*. tested *C. perfringens* strain NCTC 8247 in human volunteers at two doses, 1.2×10^7 and 2.2×10^{10} cells. At the lower dose, one of five volunteers experienced diarrhea some 31 hours after ingestion (whereas all other symptoms observed in these experiments occurred within 24 hours), and three of five experienced some symptom. At a dose almost 2000 times higher in the same experimental series, no volunteers (of four tested) experienced symptoms of any kind. At least the former inconsistency was noted by Strong *et al*. (1971), who suggested the possibility that this case was not associated with the

experimental procedure.[75] Although spores of this strain are heat resistant ($\geq$30 mins. at 100°C: Hall *et al.*, 1963; Sarker *et al.*, 2000), other characteristics of this strain do not correlate with diarrheal activity in humans. Enterotoxin was not observed to be produced by this strain *in vitro* by an erythema test (Niilo, 1973), although extrapolation of such experimental conditions to *in vivo* toxin production is problematic. Feeding of NCTC 8247 to five monkeys did not induce illness at a dose of 9.5×10^9 cells (Duncan and Strong, 1971). Finally, *C. perfringens* strains that did not produce consistent fluid accumulation in rabbit ligated intestinal loop experiments, of which NCTC 8247 was one, were positively correlated with lack of diarrhea from monkey and human volunteers (Duncan and Strong, 1969a, 1969b; Strong *et al.*, 1971).

We regard these inconsistencies in the observed single case of human diarrhea associated with NCTC 8247 (Strong *et al.*, 1971) to be sufficient to demonstrate it does not correspond to the *C. perfringens*-caused diarrhea examined in this risk assessment. Consequently, the human data on NCTC 8247 are omitted from consideration.

Table 5.3   Evidence for exclusion of clinical data obtained from use of various *C. perfringens* strains.

Strain	Direct evidence of enterotoxin		Indirect evidence of enterotoxin	Strain reference
	PCR analysis *cpe* gene[b]	CPE protein[a]	Fluid accumulation[c]	
F42	-	ND	-	Strong *et al.*, 1971
215b	-	ND	-	
FD1	-	-	-	

a.   ELISA analysis. McClane and Strouse, 1984; Wnek *et al.*, 1985.
b.   Kokai-Kun *et al.*, 1994.
c.   Rabbit ligated intestinal loop experiments. Strong *et al.*, 1971.
ND: not determined.

The following two studies putatively addressed the dose-response relationship for *C. perfringens*; however, these studies could not be used to quantify a functional relationship.

- **Hobbs *et al.* (1953):** This study included feeding experiments with *C. perfringens* 3702 in monkeys and humans. However, the human feeding component of the study included neither the number of *C. perfringens* cells nor the quantity of *C. perfringens* toxin administered, but instead stated that the volunteers were fed "18-20 hour cultures in cooked meat (10-15 mL)." Due to the inadequate measure of dose in these experiments, these data were not useful for modeling a dose-response relationship and were thus excluded from further analyses.

- **Cravitz and Gillmore (1946):** This study reported results of *C. perfringens* feeding trials conducted with humans and animals (rabbits, dogs, and cats). *C. perfringens* strains 683, 685, 686, 689, 690, 691, 692, 694, and ATCC 846, 3624, 3626, 3628, 3629, 3609, 9081,

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[75]   The diarrhea and other symptoms could also have been associated with the experimental procedure if this particular culture, or the chocolate dairy drink in which it was administered, was contaminated with something other than *C. perfringens* strain NCTC 8247.

9856 were used in the study. For human volunteer studies, only strains 685, 686, and 690 were administered as live cultures. The doses administered in this study were not specified; therefore, data from this study were not used for modeling the dose-response relationship.

5.3. Dose-response modeling

5.3.1. Dose-response model employed

Infection and illness are considered to be the result of a host ingesting one or more pathogenic organisms and some fraction of the organisms surviving host defenses until infection or intoxication is sufficiently established to result in illness. Dose-response modeling is generally based on a probabilistic description of the number of pathogens actually ingested given a nominal dose, as well as a probabilistic description of the survival of ingested pathogens.

An important assumption in most microbial dose-response modeling is that a single pathogen cell is capable of infecting an individual who ingests it. Furthermore, if infection is possible then illness is also possible. The alternatives to this assumption include the possibility that more than one pathogen is needed to result in infection and illness, or that multi-cellular aggregation or other behaviors of clustered bacterial cells enhance pathogenicity and virulence. Such a requirement exists for some parasitic pathogens that require union of male and female forms inside the host to cause infection and illness. Nevertheless, bacterial pathogens are assumed to only require a single organism to infect. That a single organism could be capable of infecting a human host is important because that characteristic would constrain the mathematical dose-response function to be (essentially) non-threshold.

The simplest biologically plausible dose-response function is the exponential (Haas, 1983). One possible set of assumptions resulting in such a dose-response function is that the probability for a particular number of organisms in a given dose is poisson distributed about the mean estimate for that dose, and that each ingested pathogen is independent and has the same probability (within each host, and for different hosts) to survive and cause disease within the host. Then the probability of disease given a mean dose of d, $P(d;k)$, is expressible as:

$$P(d;k) = 1 - \exp(-kd) \tag{5.1}$$

where k may be interpreted as the probability that any individual organism survives and causes disease. This same dose-response function may be obtained with alternative assumptions, so that such an interpretation of k is neither necessary nor unique. No more complicated dose-response functions are considered here, since the available data cannot justify their use. The parameter k is interpreted heuristically as a measure of the relation between mean estimate of dose administered to a group of individuals, and the probability for any individual to suffer diarrhea as a consequence; in what follows it will be referred to as a potency to cause human diarrhea. Moreover, the shape of the dose-response function that is used turns out to be fairly unimportant, as explained below. Implicit in the use of this dose-response curve is that diarrheas caused by *C. perfringens* in the experimental studies can be uniquely identified, and that there was no background rate of diarrhea caused by *C. perfringens* among the volunteers in the studies evaluated here.

5.3.2. Evaluation of within-isolate dose-response

The data in Section 5.1 correspond to dose-response tests performed on particular isolates of *C. perfringens*. Each experiment is identified by a strain name for the isolate used, but it is possible that other isolates of the same strain, or the same isolate after serial passage through various hosts or cultivation conditions, might have a different potency for causing human diarrhea. In the following discussions, the experimental data are identified by reference to a strain, but it must be understood the reference is strictly to a particular isolate of that strain.

Examination of the data outlined in Section 5.1 suggests that there are large differences between the tested isolates of *C. perfringens* in their ability to cause diarrhea in humans. There are experiments listed on a total of 15 isolates of *C. perfringens* that were identified by strain. For five of these (NCTC strains 8239, 8797, 8798, 10239, and 10240) there are data at multiple doses that allow a (non-zero) estimate of the parameter k and a test of whether the data are consistent with the chosen dose-response curve.[76] For five further strains (strains 27, 68900, E13, NCTC 8238, and S-79), a non-zero, finite, point estimate of k may be obtained, while the final five strains give a zero (683, 689, 690, 692) and infinite (79394) point estimate for k respectively.

The dose-response function was fitted to the individual strain data using the maximum likelihood technique in order to estimate the potency parameter k for each strain. It was assumed that the doses used for each dose group within each experiment could be adequately represented by the mean dose reported for that dose group, and that the results in a group of individuals would be binomially distributed with probability given by the exponential dose-response function using that mean dose. In such circumstances, the loglikelihood (J) for a given set of observations is (up to an additive constant):

$$J = \sum_{i=1}^{N}\left[r_i \ln\left(p_i n_i / r_i \right) + \left(n_i - r_i \right) \ln\left(n_i \left(1 - p_i \right) / \left(n_i - r_i \right) \right) \right] \tag{5.2}$$

where the terms are:
- N number of independent dose groups,
- n_i number of people tested in dose group i,
- r_i number of people responding in dose group i, and
- p_i $= p(d_i;k)$, the probability for illness at dose d_i, given by the dose-response function:

$$p\left(d_i; k \right) = 1 - \exp\left(-k d_i \right) \tag{5.3}$$

This loglikelihood has been normalized so that it would disappear if each p_i matched the empirical observation (r_i/n_i) exactly (for $r_i = 0$ or $r_i = n_i$, the corresponding term of the loglikelihood disappears). With this normalization, an approximate goodness-of-fit test is available (Haas, 1983) using the statistic $-2J$, which will be approximately χ^2 distributed with a number of degrees of freedom equal to the number of dose groups minus the number of parameters estimated (one, in this case).

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[76] Some of the multiple dose experiments also reflect multiple matrices, so there is an additional implicit assumption in the analysis that the matrix has a relatively small effect.

Fitting the dose-response curve[77] for each of the fifteen strains gave estimates of k shown in Table 5.4 (for strains with only one dose, or with no responses at any dose, the maximum likelihood estimate corresponds to exactly fitting the dose-response curve to the observed fraction of volunteers who suffered diarrhea). The fit of the dose-response curve to the available multi-dose experiments was acceptable in all cases ($p > 0.01$).[78] A likelihood test for equality of the values of potencies k showed that a single value for all the strains tested was highly unlikely ($p \sim 10^{-35}$).

Table 5.4   Potency estimates for each of the fifteen strains of *C. perfringens*

Strain	Potency (k) estimate (per CFU)	p-value
027	2.17E-12	NA
683	0.00E+00	NA
689	0.00E+00	NA
690	0.00E+00	NA
692	0.00E+00	NA
68900	2.31E-11	NA
79394	∞	NA
E13	3.08E-13	NA
NCTC 10239	6.17E-12	0.98
NCTC 10240	3.49E-11	0.03
NCTC 8238	8.15E-10	NA
NCTC 8239	5.90E-11	0.61
NCTC 8797	9.65E-10	0.94
NCTC 8798	1.62E-11	0.41
S-79	3.58E-10	NA

NA indicates that no p-value was calculated because there was only one dose for the strain or because there was no response at any tested dose.

5.3.3. Evaluation of between-isolate variability of dose-response

As already noted, there is good reason to regard the measurements of potency as applying solely to the isolate tested in the particular experiments, under the particular conditions applied to that isolate after it was originally obtained. Thus what have been obtained are fifteen measurements on fifteen isolates that are probably serologically distinct (Strong *et al.*, 1971; Niilo, 1973; Hall

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[77] The calculations are performed in the workbook CP_dose_response.xls accompanying this risk assessment.
[78] This adequate fit suggests that no significant matrix effect could be obtained by analysis of these experiments, probably because of the very small number of people tested in each experiment.

et al., 1963). The following arguments suggest that these particular isolates were not selected in any way that is correlated with their potency.

- Most (perhaps all) of the isolates were associated with human diarrheal illness or foods implicated in *C. perfringens* food poisoning outbreaks, implying selection for Type A, CPE positive strains. This selection is required since we are concerned with human disease and evaluating only disease-causing *C. perfringens*, but does not imply selection for potency.
- There is no known indication that any attempt was made to obtain isolates from those most or least exposed, or from outbreaks in which CFU counts were particularly high or low, or from outbreaks in which food preparation methods or the foods themselves were more or less likely to result in low or high CFU counts in the food actually eaten, or from outbreaks that affected particularly the young or the elderly or any other potentially more susceptible or less susceptible population, or from outbreaks in which the case attack rate was considered high or low. Again, the selection for illness does not imply selection for potency.

Thus the fifteen estimates of potency are assumed to represent a random sample from the distribution of potencies of all Type A, CPE positive, *C. perfringens* affecting humans; and they will be treated here as a random sample from *C. perfringens* affecting RTE foods consumed by humans.[79]

The distribution of the ten finite, non-zero maximum likelihood estimates for *k* obtained for the individual isolates was examined and found to be entirely consistent (p=0.79, Shapiro-Wilk test) with lognormal.[80] Figure 5-2 shows the distribution of these ten estimates on a standard normal plot (Cunnane, 1978). The variation of potency for causing human diarrhea between isolates of *C. perfringens* potency was therefore modeled as a lognormal distribution. The fifteen *C. perfringens* isolates tested were thus assumed to provide an unbiased random sample (from the point of view of their potency to cause human diarrhea) of the *C. perfringens* organisms that might be present in RTE or partially cooked food. The fifteen isolates were not randomly sampled in any defined way, but, as argued above, their selection is unlikely to have been substantially correlated with their potency, justifying their treatment as an unbiased random sample.

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[79] If there is a bias towards more potent strains in this selection, this risk assessment will overestimate the rates and numbers of illness.

[80] This does not rule out the possibility of other distributional forms; but we are biased towards the lognormal in view of its ubiquity in natural phenomena, and the usual explanation for that ubiquity in terms of random variations in multiplicative effects.
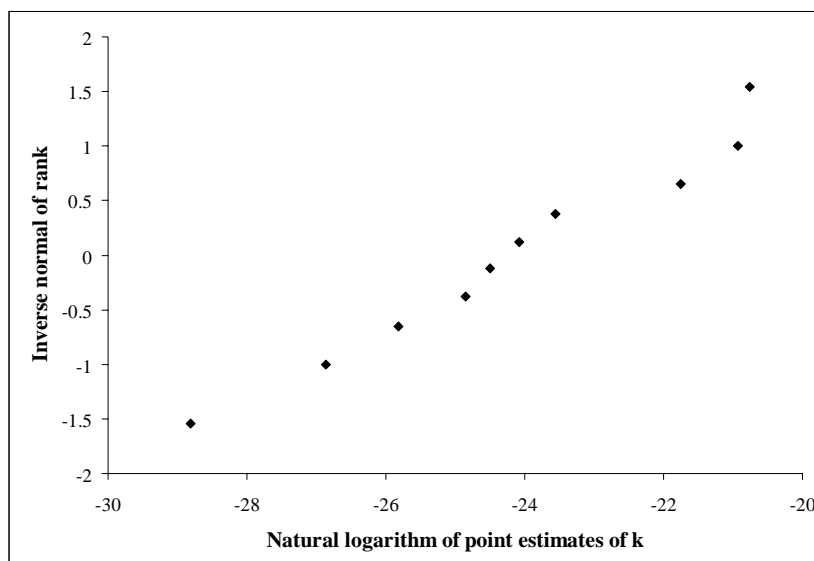
Figure 5-2   Distribution of maximum likelihood estimates for potency (*k*).

The lognormal distribution of potencies is parameterized by two values — its mean ($\hat{z}$) and standard deviation (σ) on a logarithmic scale. Estimates of these parameters and the uncertainties of those estimates in the form of a variance-covariance matrix were obtained using likelihood methods applied to all fifteen human tests of *C. perfringens* isolates. For a particular experiment, the likelihood for the observations is proportional to:

$$J = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} dz \exp\left(\frac{(z-\hat{z})^2}{2\sigma^2}\right) \prod_{i=1}^{N} \left(\frac{p_i n_i}{r_i}\right)^{r_i} \left(\frac{(1-p_i)n_i}{n_i-r_i}\right)^{n_i-r_i} \tag{5.4}$$

where the terms are:
- N number of independent dose groups,
- n_i number of people tested in dose group *i*,
- r_i number of people responding in dose group *i*, and
- p_i the probability for illness at dose d_i for a potency of e^z, given by the dose-response function:

$$p_i = 1 - \exp\left(-e^z d_i\right) \tag{5.5}$$

The normalization adopted here for the likelihood is the same as adopted for examination of individual experiments (terms in the product in the integrand with $r_i = 0$ or $r_i = n_i$ are interpreted as unity).

The maximum likelihood estimates[81] obtained for $\hat{z}$ and σ, together with an estimate of their uncertainty (standard deviations and correlation coefficient), are shown in Table 5.5. The

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[81] The integral for *J* was coded as a Visual Basic for Applications function in the workbook CP_dose_response.xls accompanying this risk assessment (the function returns the natural logarithm of *J* to ensure wide dynamic range) using a modification of a published technique (Crouch and Spiegelman, 1990). The information matrix was

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median potency estimate is estimated to be $\exp(\hat{z}) = 1.8 \times 10^{-11}$ per CFU, with a variation between isolates of a factor of $\exp(\sigma) = 10.2$ at one standard deviation.

Figure 5-3 illustrates the effective strain-averaged[82] dose-response curve (solid red line) obtained using the parameters of Table 5.5, together with individual strain dose-response curves at the median and 95% confidence limits for individual-strain potencies (dotted pink lines), and some percentage points of the strain-averaged dose-response curve are shown in Table 5.6.   The variation between strains is sufficiently large that, for the purposes of this risk assessment, identification of the exact shape of the individual-strain dose-response curve is much less important than accounting for the variability in potency between different isolates of *C. perfringens*. The effective dose-response curve (probability for diarrhea versus number of ingested cells) for arbitrary *C. perfringens* cells corresponds to the convolution of the within-isolate (exponential) dose-response and the between-isolate (lognormal) variation, so the assumed shape for the within-isolate dose-response is effectively smeared out.

Table 5.5   Parameters characterizing the lognormal distribution of potencies.

Parameter	Value
Mean of lognormal distribution ($\hat{z}$)	-24.7
Standard deviation of lognormal distribution (σ)	2.32
Median potency estimate (per CFU)	1.82E-11
Variation between isolates at one standard deviation	10.2
Standard deviations (main diagonal) and correlation coefficient (off-diagonal)	

	$\hat{z}$	σ
$\hat{z}$	0.684	0.078
σ	0.078	0.664

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obtained numerically by making changes in the parameters from optimum, and inverted to give the variance-covariance matrix. The changes were chosen approximately equal to the estimated standard deviations, to ensure that between-parameter correlations for relatively large deviations would not be omitted.

[82] The expected proportion of a human population falling ill if each member of that population ingested the same quantity but a different strain of illness-causing *C. perfringens*, each strain being selected at random for each member of the population.
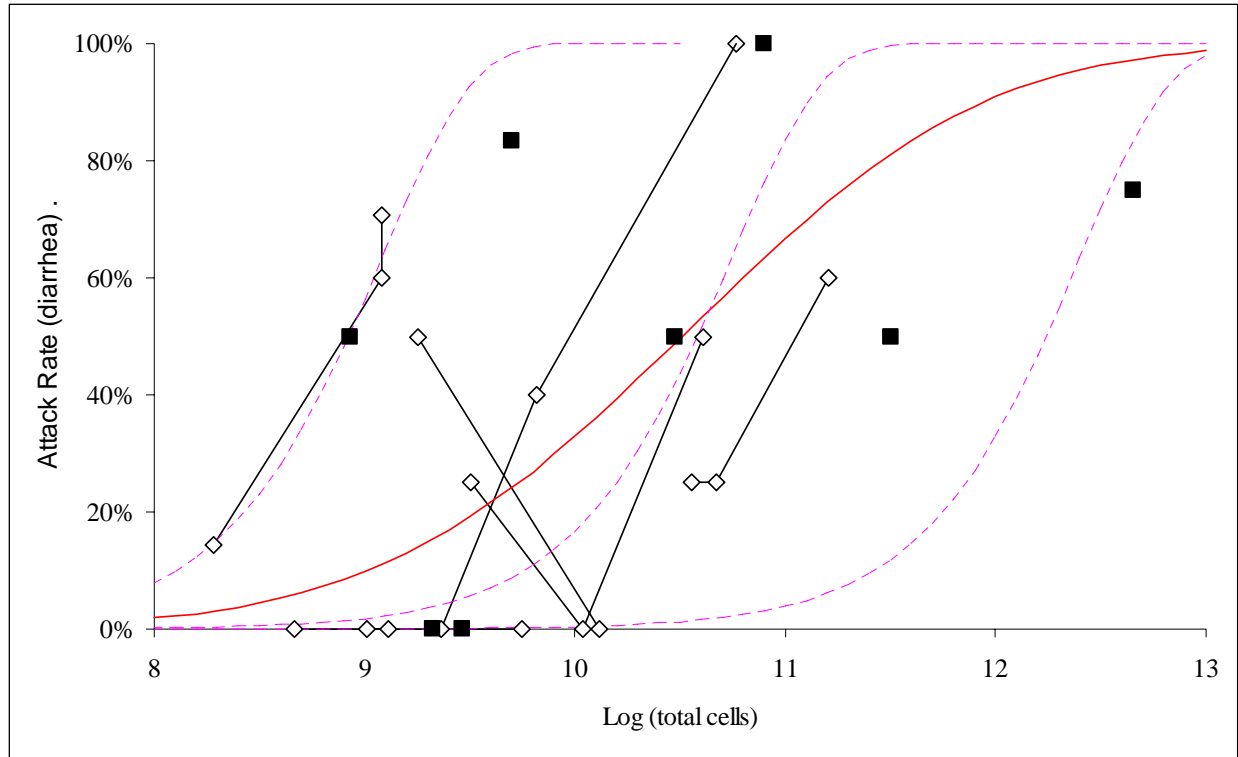
Figure 5-3   Individual strain dose-response curves (dotted, pink) at the median and 95 % confidence limits on the distribution for strains, and the strain-averaged dose-response curve (solid, red), superposed on experimental data.

Table 5.6   Percentage points of the strain-averaged dose-response curve shown in Figure 5-3

Percentage point (probability of illness)	Number of cells ingested
1%	4.8E+07
5%	3.7E+08
10%	1.0E+09
25%	5.4E+09
50%	3.2E+10
75%	1.9E+11
90%	8.8E+11
95%	2.2E+12
99%	1.2E+13

5.4. Uncertainties in dose-response modeling

Various assumptions have been made in the dose-response modeling and in the application of this dose-response modeling to the risk assessment, as are typically necessary. The uncertainty about such assumptions introduces a set of uncertainties of unknown size in addition to those that are evaluated in the risk assessment. Among the assumptions introducing such unknown uncertainties are:

- the dose-response is non-threshold
- diarrheas caused by *C. perfringens* in the experimental studies can be identified as being caused by the organism, and the background rate of diarrhea caused by *C. perfringens* is sufficiently small to be ignored in such experiments,
- any variation in individual susceptibility is adequately incorporated in the within-isolate dose-response function,
- there is no effect of food matrix,[83]
- the tested isolates are effectively a random sample from all *C. perfringens* affecting RTE foods,
- any given RTE food serving will be affected principally by a single clone of *C. perfringens*, so that the dose of *C. perfringens* obtained from a given food serving corresponds to the isolates tested,
- the distribution of potencies to cause human diarrhea is lognormal, and
- the uncertainties in the distributional parameters are adequately modeled by normal distributions.

It is possible that some or all of these assumptions might have influenced the results obtained. For example, while the typical subject in these studies was an adult healthcare worker, it is possible that some group in the general population may be at materially different risk to develop diarrhea following exposure to a given dose of *C. perfringens*. Similarly, most studies used either meat or a dairy product as the vehicle for exposure of human subjects, although sometimes that vehicle was introduced into a regular meal. It is unclear how modification of such vehicles, or the wide variety of RTE foods, would influence the likelihood of developing diarrhea following exposure to *C. perfringens*.

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[83] A food matrix effect is likely, but probably not discernible in the available data (see footnote 78). A weaker assumption, that any matrix effects are dominated by the between-strain variation, is sufficient here.

6. Risk Characterization

6.1. Variation of the risk of diarrhea with growth during stabilization

6.1.1. Primary results

The model was run with multiple fixed values of growth during stabilization to evaluate the effect of variation in growth during stabilization in terms of estimates of annual *C. perfringens* illnesses. Estimates of illnesses were obtained using two approaches: 1) the uncertainty incorporated in the model was omitted, with all uncertainty parameters set at their maximum likelihood estimates (MLE), and 2) the uncertainty was included and the full uncertainty distribution evaluated, the median of this distribution being used as a summary estimator of central tendency. The reason for this approach is indicated below. Figure 6-1 and Table 6.1 show how these two estimators of risk per serving vary as the growth during stabilization increases from 0.5 $\log_{10}$ to 3.5 $\log_{10}$. The range in median estimate for rate of illness is from approximately 1.9 illnesses per million servings up to 3.9 illnesses per million servings. The total number of servings of RTE and partially cooked foods in the U.S. per year is estimated to be approximately 55.7 billion (Section 3.15.1), so these estimates correspond to a range of approximately 106,000 diarrheas per year up to 217,000 per year for 0.5 $\log_{10}$ to 3.5 $\log_{10}$ growth, respectively.

Table 6.1   Estimates for annual numbers and rate of illnesses.

Growth ($\log_{10}$)	Annual number of illnesses (55.7 billion servings)			Rate per million servings		
	MLE estimate[a]	Median estimate[b]	Curve fit[c]	MLE estimate[a]	Median estimate[b]	Curve fit[c]
0.5	111,000	103,000	105,000	1.99	1.86	1.88
1	120,000	115,000	113,000	2.15	2.07	2.02
1.5	128,000	122,000	123,000	2.29	2.18	2.21
2	139,000	135,000	137,000	2.50	2.42	2.46
2.5	156,000	157,000	155,000	2.81	2.82	2.78
3	181,000	177,000	178,000	3.25	3.17	3.19
3.5	207,000	208,000	208,000	3.72	3.73	3.73

[a] One billion servings simulated at each growth, with all parameters set at the maximum likelihood for uncertainty

[b] Geometric mean of 600 values for each growth, with each value corresponding to an uncertainty simulation of 30 million servings.

[c] The best-fit curve to the median estimate, taking account of uncertainties (see equation (6.1) and Figure 6-1).

Mead *et al*. (1999) estimated approximately 250,000 cases of *C. perfringens* food poisoning annually from all food sources, suggesting that illness attributable to RTE and partially cooked foods would be some fraction of this total. Mead *et al*.'s (1999) methodology, however, required considerable extrapolation (a factor of 38) from the number of reported illness to the total

number of illnesses. This was done using the only available observations, based on unvalidated analogies with other diseases. Assuming that federally inspected plants are meeting the current 1 $\log_{10}$ stabilization performance standard, the median estimate of 120,000 illnesses at 1 $\log_{10}$ growth obtained here by modeling[84] falls within Mead *et al.*'s estimate. However, there is no available epidemiology that would allow validation of the model estimate for the number of *C. perfringens* illnesses due to consumption of RTE and partially cooked foods; furthermore, as explained below (Section 6.4.1), the number of illnesses due to hot-held foods has been underestimated by the model.

Figure 6-1   Variation in risk of diarrhea with growth during stabilization (MLE and median).

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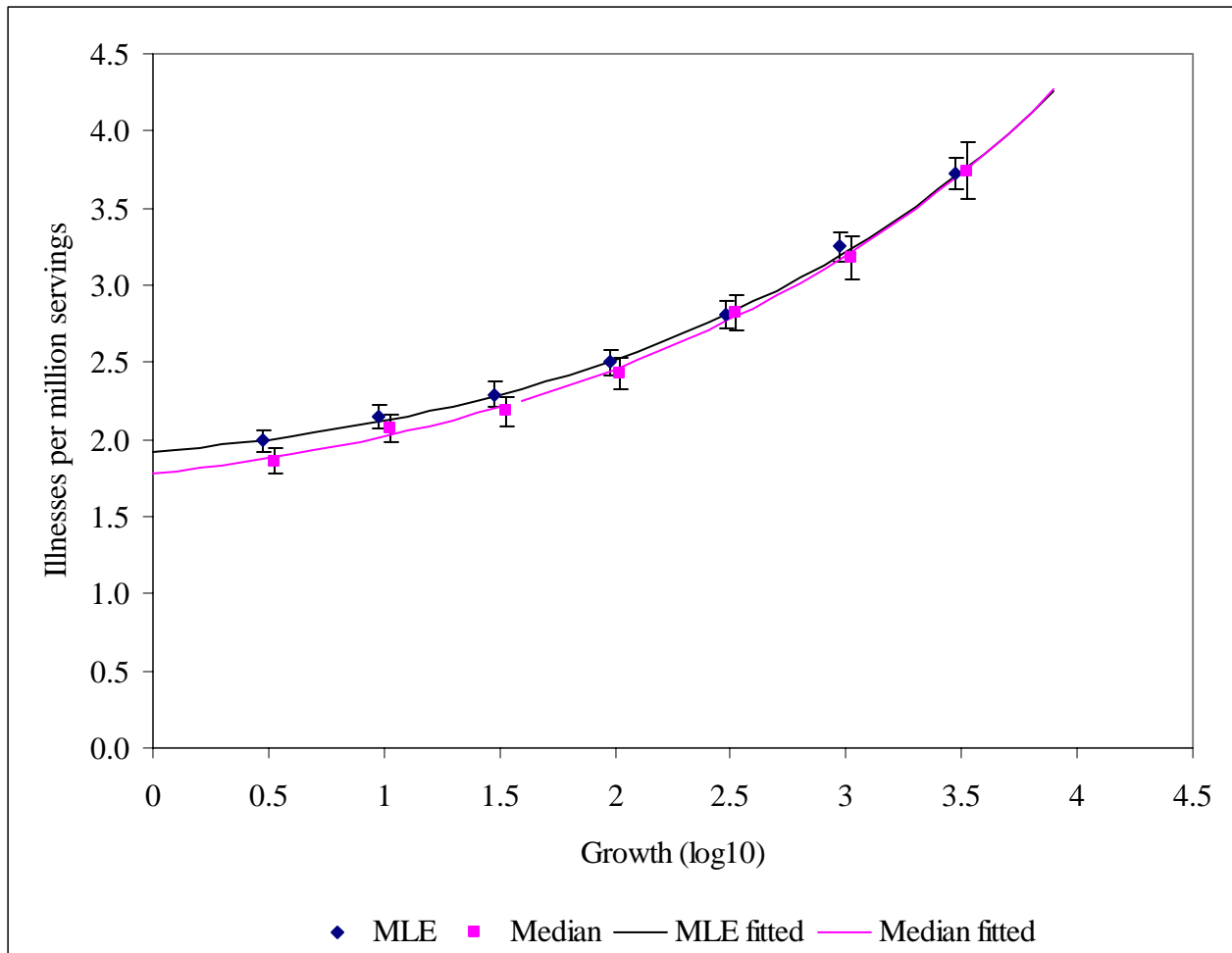
[84] The modeling is for a fixed growth during stabilization, see Section 3.12, whereas we can expect variation in growth among plants meeting a 1 $\log_{10}$ standard. The median in the latter case would be smaller than the median estimated for a fixed 1 $\log_{10}$ growth during stabilization, assuming that every plant strictly met the standard.

The error bars in Figure 6-1 show the numerical precision due to running only a finite number of Monte Carlo iterations, and are presented solely to demonstrate that a sufficient[85] number of serving simulations have been run to be sure of the smooth variation with growth during stabilization. The interpolating lines are a smooth fit to the results for 7 growth values, suggesting that as allowable growth varies from 0.5 $\log_{10}$ to 3.5 $\log_{10}$ there is no evidence of a threshold event (and none would be expected from the structure of the model used). Both MLE and median estimators are plotted to illustrate the almost identical trends, and support the use of the MLE estimators to evaluate the sensitivity of results to inputs included in the sensitivity analysis.

6.1.2. The principal cause of illnesses

Examination of the results obtained during the running of the experiments[86] shows that the key to understanding the variation with growth during storage for the major fraction of illnesses predicted by the model is the storage temperature (between manufacturer and retail, or during consumer storage). If the storage temperature is below T_{min} (the minimum temperature for growh, see Section 3.11.1) then essentially nothing happens, and illness is very unlikely. If it is above T_{min}, however, then the length of storage is usually sufficiently long that any initial number of *C. perfringens* vegetative cells are predicted to grow to stationary phase, and illness becomes much more likely as a result if the product is eaten cold or not heated to a sufficiently high temperature. Thus most illnesses are predicted to occur as a result of what can only be described as broken refrigerators.

It follows that growth during stabilization has only a small overall effect. Only a small fraction of the servings are stored at a temperature just above T_{min} and in which a few initial cells would not quite have grown all the way to stationary phase by the end of storage. Only in such servings is the number of cells in the serving as it is eaten affected by the growth during storage. In addition (see Section 6.3.3 below), as the growth during stabilization increases substantially, a few illnesses can be caused by concentrations of cells that arise entirely due to that growth (with no further growth during storage).

This description of the major predicted cause of illnesses indicates that the principle determinants of illness are the initial concentrations (prevalence and count) of *C. perfringens* in servings, the distribution of storage temperatures, the distribution of times during storage, and the maximum concentration of *C. perfringens* that can be achieved in the serving. Other factors, such as death rates during cold storage, can have very little effect. Even the growth rate achieved at temperatures close to T_{min} is unimportant so long as it is sufficiently high (as it appears to be from the analysis of Section 3.11) that a large amount of growth can occur during typical storage times; although for some foods this emphasizes the potential importance of the assumption made in Section 3.11.5.2 that the effect of nitrite is to uniformly lower growth rates rather than change the range of temperatures over which growth can occur.

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[85] The results shown are based on 1 billion servings at each plotted growth for the MLE (a total of 7 billion servings for the 7 growth points plotted), and 600 uncertainty iterations each of 30 million servings for the median estimates (a total of 126 billion servings for the 7 growth points plotted).

[86] The outputs from multiple runs of the program are available in the worksheet CP_results.xls

6.2. Uncertainty estimates

6.2.1. Uncertainty not incorporated in the model

Before discussing the uncertainties estimated in this risk assessment, it is necessary to emphasize that many sources of uncertainty have not been incorporated, and that the total size of the unincorporated uncertainties is unknown. Section 4 discusses various limitatations of the exposure modeling, and Section 5.4 the further uncertainties of dose-reponse modeling. To emaphasize this point, examination of the "what-if" scenarios of Section 6.5 and some of the sensitivity results in Section 6.6 shows that the absolute size of the risk estimates depends crucially on some of the assumptions made in the modeling. All of the results depend on the model being an accurate representation of what happens in reality, and there are many places in the modeling where what happens has not been adequately investigated (or, in some cases, investigated at all).

6.2.2. Uncertainty incorporated in the model

The uncertainty (to the extent included in the modeling) of the results is illustrated by Figure 6-2, which shows the median estimate and the empirical 90% confidence interval for the rates of diarrhea for fixed growth during stabilization for seven such growths between 0.5 $\log_{10}$ and 3.5 $\log_{10}$.

Figure 6-2   Uncertainty estimates for rate of diarrhea for fixed growth during stabilization.
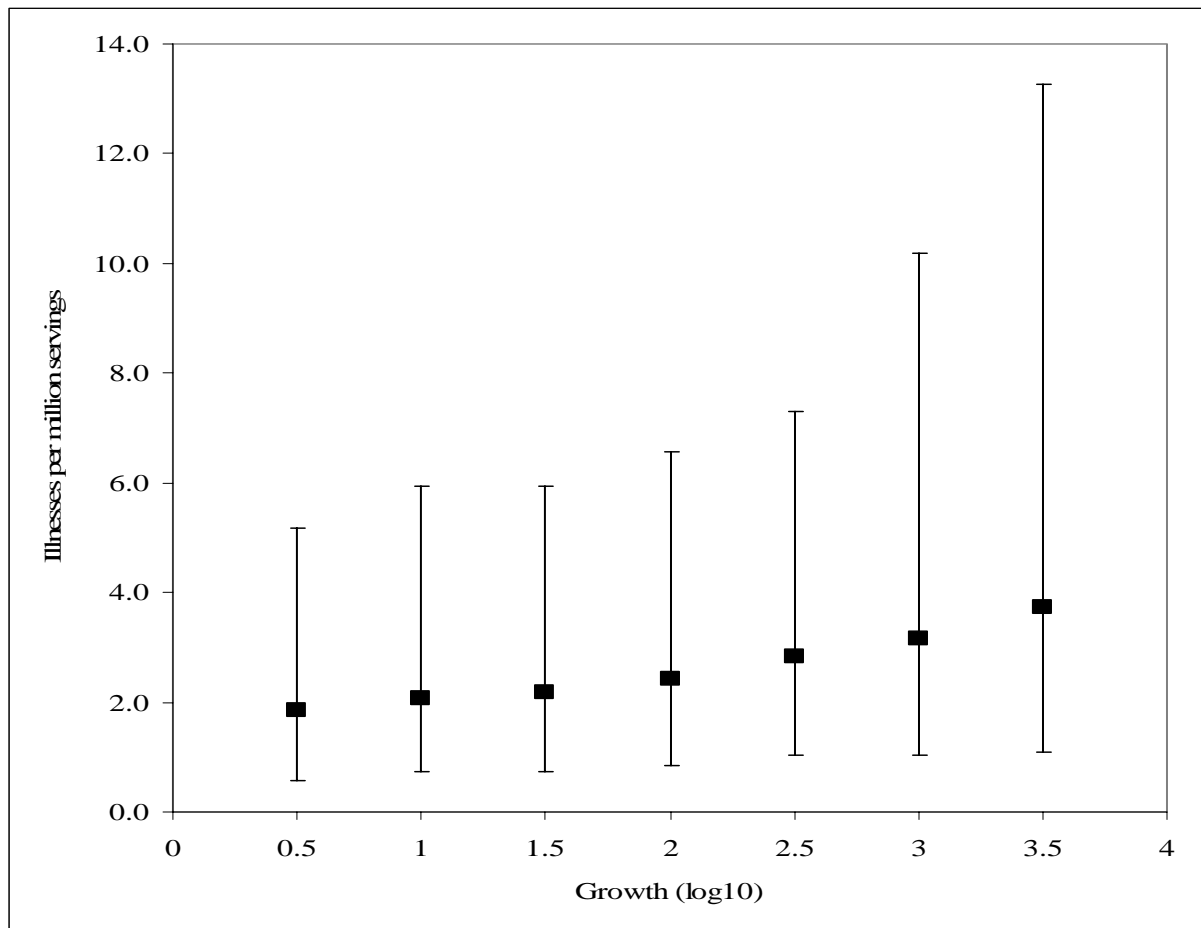
The uncertainty ranges for risk shown in Figure 6-2 are derived from the uncertainty distributions obtained in the Monte Carlo simulations, which are approximately lognormal. Figure 6-3 shows the uncertainty distributions for the 7 growths during stabilization on a plot that would be a straight line for perfectly lognormal distributions.[87] The deviations from straight lines shown are close to what would be expected for perfectly lognormal distributions, so the median estimates of the distributions can be adequately estimated by taking the geometric average of the 600 samples (and this is the estimate given in the previous sections as "median," Figure 6-1, Figure 6-2, square symbols).

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[87] The "normal coordinate," the inverse normal of the rank of the sample (Cunnane, 1978), is plotted for each of the 600 samples against the natural logarithm of the number of diarrheas estimated in that sample in the Monte Carlo simulation of 30 million servings at each growth

Figure 6-3   Uncertainty distributions at fixed growth during stabilization.
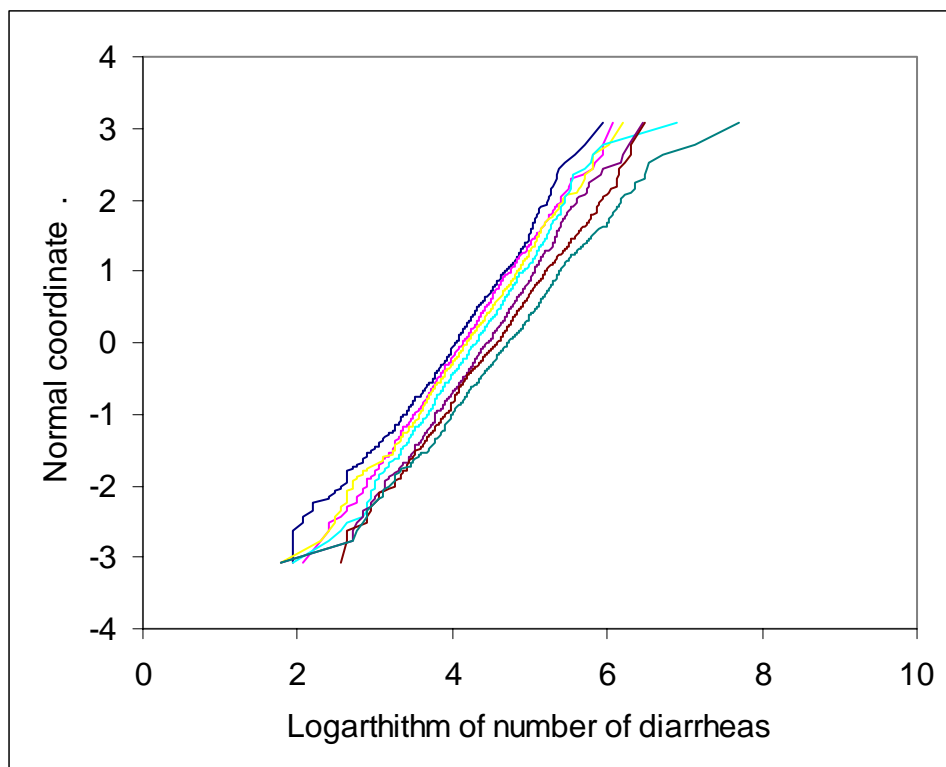
Examination of these uncertainty distributions at different growths during stabilization shows that they have essentially identical standard deviations of about 0.659 on a natural logarithmic scale (the lines of Figure 6-3 are parallel within the uncertainties involved), and the variation of the median estimate of rate of illness with growth shown in Figure 6-1 and Figure 6-2 can be well-fitted by a quadratic curve for the logarithm of the rate as a function of growth. Combining these observations, the uncertainty results can be summarized by an empirical equation for the rate of illness *R* that incorporates both the median estimate and the uncertainty. That empirical equation is:

$$R = R_0 \exp\left(\beta g + \gamma g^2 + \varepsilon\right) \tag{6.1}$$

where

R_0 = 1.77×10^{-6} per serving,
β =   0.0977,
γ =   0.0327,
g =   growth, expressed as $\log_{10}$, so that $g = \log_{10}(G_c)$ (see Section 3.12),
and ε is normally distributed with mean 0 and standard deviation 0.659 (so $e^\varepsilon = 1.93$).

One implication is that the uncertainty increases in proportion to the median rate of illness, so that for all values of growth during stabilization the uncertainty can be accurately expressed as

the same multiple of the median, specifically a factor 1.93.[88] In this sense, the uncertainty is independent of the growth during stabilization.

The median estimate is obtained from Equation (6.1) when $\varepsilon = 0$, and any desired confidence limits may be obtained by setting ε to the corresponding value (*e.g.* for 10% and 90% confidence limits, set $\varepsilon = 0.695 \times (-1.2816) = -0.845$ and $0.695 \times 1.2816 = 0.845$ respectively). The corresponding equation then shows the variation with growth at this percentile of the uncertainty distribution.

6.3.   Sources of illness-causing *C. perfringens*

6.3.1.   Meat or spice as source of the *C. perfringens*

In tracking the growth of *C. perfringens* in the model, it is possible to identify the origin of the vegetative cells that ultimately cause illness. Table 6.2 shows model predictions of the fraction of illness-causing servings in which the *C. perfringens* originated from meat, from spices, or from spores germinating during storage (and the model does not determine whether from meat or spices), for illnesses that occurred with no hot-holding or after hot-holding (in the latter case the *C. perfringens* growth occurs during the hot-holding period). Where vegetative cells from both meat and spices contribute to the serving, it is not possible to distinguish the source of the particular cells that multiply (this is the "unknown" entry in Table 6.2).

Table 6.2   Source fractions by meat, spice or germinating spores.

	Fraction of all	Normalized fraction
	Not hot held	
Meat	0.60	0.63
Spices	0.35	0.37
Unknown	0.002	0.002
Germinating spores	0.006	0.006
Total	0.96	1
	Hot held	
Meat	0.001	0.027
Spices	0.039	0.97
Unknown	0.0002	0.005
Total	0.04	1

The fractions shown in Table 6.2 are averaged across simulations for growths during stabilization of 0.5 to 3.5 $\log_{10}$. However, these fractions do not change substantially with changes in the growth during stabilization in this range.

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[88] This is a property of the uncertainties incorporated in the modeling. It does not necessarily hold true for any uncertainties not so incorporated — see Section 4.

6.3.2. The source of *C. perfringens* by food category

The type of food in which *C. perfringens* multiplication occurs is also tracked in the model, and the particular food type of the food servings that cause illness in the simulation may be tabulated. Table 6.3 shows the simulated fractions of illnesses caused by growth within each food type examined. In this case, there is some variation in the relative fractions within each food type as the growth during stabilization changes.

Table 6.3   Fraction of illnesses by each food category, for growth of 0.5 through 3.5 $\log_{10}$ during stabilization.

Growth	Fraction by food category[a] observed in the simulation								
	1	2	3a	3b	3c	3d	4a	4c	4d
0.5	0.46	0.48	0	0	0	0	0.026	0.003	0.036
1	0.49	0.46	0	0	0	0	0.012	0.0009	0.034
1.5	0.49	0.46	0	0.0004	0	0	0.018	0.003	0.027
2	0.52	0.43	0.0008	0.0008	0	0.0008	0.015	0.0008	0.031
2.5	0.53	0.43	0.0007	0.0007	0.0014	0.0014	0.014	0.0014	0.026
3	0.54	0.41	0.0006	0.0055	0.0022	0.0058	0.009	0.0012	0.028
3.5	0.53	0.38	0.0046	0.0107	0.0043	0.0207	0.015	0.0011	0.034

[a] Food categories are defined in Table 3.1.
Note: 0.0004 corresponds to 1 simulated illness, so the small fractions in this table are subject to considerable uncertainty. The zeros are present because insufficient simulations (1 billion per growth value) were performed, not because they cannot possibly lead to illness.

6.3.3. Illness due entirely to *C. perfringens* growth during stabilization

Most of the illnesses are simulated to occur as the result of extreme *C. perfringens* growth during home or retail storage at temperatures that allow growth. A small fraction, however, are simulated to arise not because of growth during storage, but purely as a result of the initial number of cells present in the food serving immediately after stabilization — that is, present due to growth during stabilization of the intial number of cells present immediately after the heat step. In the simulations, the food servings producing these illnesses are not subject to any temperatures that cause growth of vegetative cells after stabilization — indeed, there are some losses of vegetative cells during cold storage, but nevertheless there are sufficient vegetative cells present at the time of consumption to occasionally cause illness. The rate of occurrence of such illnesses is around 1 in a billion servings at a growth of 1 $\log_{10}$, increasing to about 10 in a billion at 2 $\log_{10}$, and 70 in a billion at 3 $\log_{10}$ (Figure 6-4). A good approximation[89] to the rate is given by

$$r = r_0 \exp\left(ag + bg^2\right) \qquad\qquad\qquad (6.2)$$

---

[89] There is considerable uncertainty in the rate where this formula predicts rates below about 3 in a billion, that is at growths below about 1.5 $\log_{10}$, because the rate estimates are based on only 1 billion serving simulations at each growth. All food categories are included in the simulation, but the simulated number of illnessess is too small to obtain a reliable breakdown by category.

where

   r is the rate of illnesses, per serving
   $r_0 = 0.39 \times 10^{-9}$,
 $a = 1.09$,
   $b = 0.21$,
and g is the $\log_{10}$ growth during stabilization.

To obtain the estimated number of illnesses per year, replace r_0 in Equation (6.2) with 21.6, giving, for example, an estimated numbers of illnesses due entirely to growth during stabilization shown in Table 6.4.

Table 6.4 Numbers of illnesses per year (*i.e.* in 55.7 billion servings) due entirely due to growth during stabilization.

Growth (log10)	Number of illnesses
0.5	39
1	79
1.5	179
2	450
2.5	1258
3	3915
3.5	13566

Figure 6-4   Rate of illnesses due entirely to growth of *C. perfringens* during stabilization.
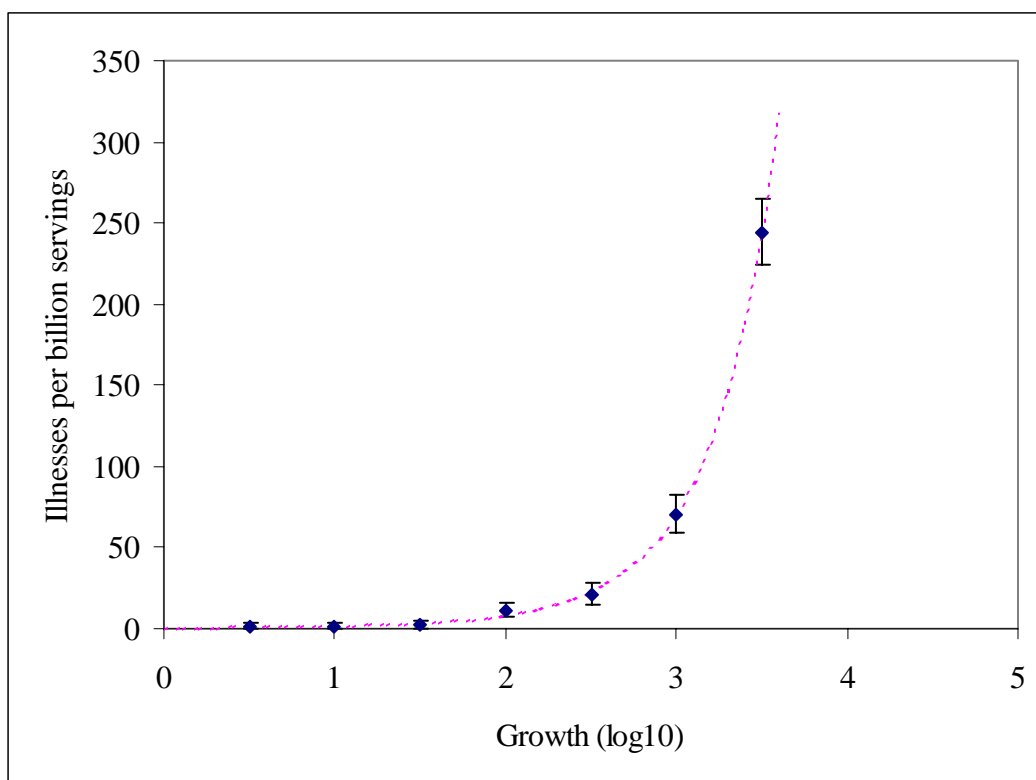
6.3.4. Source by storage temperature

Approximately 93% of the illnesses predicted by the model occur as a result of growth of *C. perfringens* vegetative cells during storage, primarily between manufacture and retail, with some also during home storage. This growth occurs because of storage for prolonged periods at temperatures above the minimum temperature for growth. Figure 6-5 shows the fractions of illnesses due to storage at various temperatures, estimated by selecting those modeled illnesses where growth of vegetative cells by a factor of 1,000 or more occurred during storage.[90] The large peak at 60 °F is due to the predominance of this temperature being recorded in the temperature surveys (the temperatures shown are those recorded during the relevant surveys, see Section 3.13.3), and is probably an artifact of the survey (due to a tendency to record the nearest mark on the thermometer, or to rounding of the temperature reading before recording it).

Figure 6-5 shows that the model predicts that the majority of illnesses are caused by improper storage, since all the temperatures shown correspond to inadequate refrigeration.

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[90] These are averages across estimates for seven growths during stabilization (0.5 through 3.5 at steps of 0.5 $\log_{10}$) at the MLE for uncertainty; there is not much variation with growth during distribution. Altering the selection criterion from a factor of 1,000 to a factor of 100 or 10,000 makes very little difference.

Figure 6-5   Fraction of illnesses caused by storage at abnormally high temperatures.
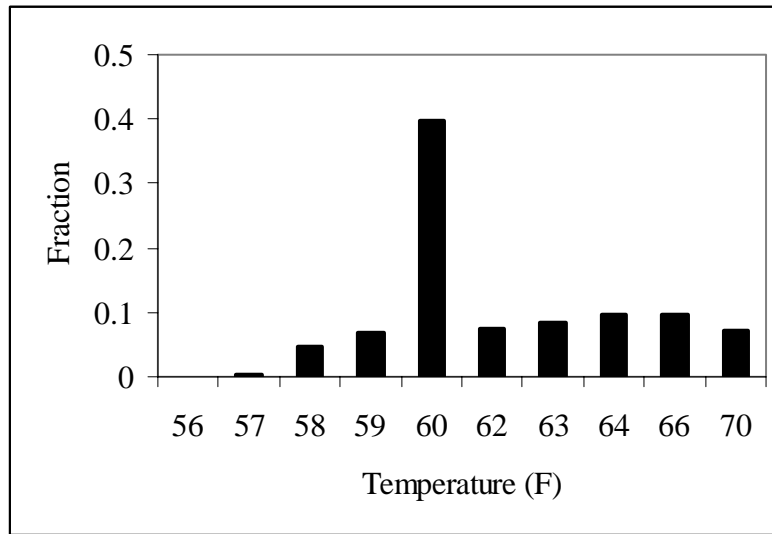
6.4.   Response to Risk Management Questions

6.4.1. What would the effect be on human illness due to *C. perfringens* of allowing up to 3 $\log_{10}$ growth during stabilization?

The number of illnesses (diarrhea) will increase with increasing relative *C. perfringens* growth. The model-estimated change is from approximately 2.0 illnesses per million servings, corresponding to approximately 113,000 illnesses per year in the U.S., at 1 $\log_{10}$ growth during stabilization, through 2.5 illnesses per million servings at 2 $\log_{10}$ growth during stabilization, corresponding to approximately 137,000 illnesses per year, to approximately 3.3 illnesses per million servings, corresponding to approximately 178,000 illnesses per year at 3 $\log_{10}$ growth during stabilization. These values are at the median of the uncertainty distribution (*i.e.* there is about 50:50 chance to be above or below these values, if all the assumptions going into the model are correct). At the upper 90th percentile of the uncertainty distribution (for the uncertainties included in the model), the number of illnesses would be about a factor 2.33 higher for all growth rates during stabilization, ranging from approximately 262,000 per year at 1 $\log_{10}$ growth, through 319,000 at 2 $\log_{10}$ growth, to 414,000 at 3 $\log_{10}$ growth. As growth during stabilization changes from 0.5 $\log_{10}$ to 3.5 $\log_{10}$, the relative change in expected illnesses is the same at any percentile of the uncertainty distribution, and the relative uncertainty is the same (a factor of 1.93 at 1 standard deviation) for any growth during stabilization.

The estimated illnesses described include those occurring because of growth of *C. perfringens* during hot-holding. The estimated rate of such events is about 1 in 10 million servings, corresponding to about 5,500 illnesses of the numbers given above, or about 4% of the illnesses at a 1 $\log_{10}$ growth during stabilization, but the number of hot-holding-related illnesses is independent of the growth during stabilization. However, it is very likely that the model under-estimates the number of illnesses due to *C. perfringens* growth during hot-holding, because it treats each serving as independent. Effectively, each illness attributed by the model to abusive

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hot-holding may represent multiple illnesses from one hot-holding event (since hot-held food servings will usually be heated together and cross-contaminate other servings). The factor by which the model underestimates illnesses may approach the average number of servings heated and mixed together during hot-holding.[91] Therefore, the extent to which abusive hot-holding contributes to *C. perfringens* food poisoning cannot be accurately estimated by this risk assessment. However, it is clear that improper hot-holding does contribute to the annual burden of *C. perfringens* illnesses and is likely a risk factor.

"Improper holding temperature" was cited as a contributing factor in 69 of 74 outbreaks for which at least one contributing factor was reported (of a total of 109 outbreaks identified) during 1988 through 1997 (CDC, 1996, 2000), and 97% of outbreaks in which this factor was positively identified as contributing or non-contributing from 1973 through 1987 (with 147 outbreaks with some contributing factor reported) (Bean and Griffin, 1990). However, the term "improper holding temperature" includes both storage at inappropriate temperatures as well as abusive hot-holding. Moreover, this estimate is likely biased toward institutional outbreaks that are most likely to be captured by surveillance due to the size of the outbreak. The products responsible for such institutional outbreaks are likely prepared from raw and are not RTE or partially cooked. Because of the self-limiting nature of the illness involved, many smaller outbreaks are likely not reported, and there is no reporting system for sporadic cases, so the role of hot-holding for such cases of *C. perfringens* food poisoning is unknown.

Most of the illnesses predicted by the model come from growth of *C. perfringens* during storage of food at retail or at home, and some fraction of such servings that are predicted by the model to cause illness would almost certainly be detected as spoiled and discarded without being consumed. As the allowed growth during stabilization increases, however, a fraction of the illnesses are predicted to be caused directly by the organisms present after stabilization, without further growth during storage. Such servings would not be detectable as infected or spoiled. The rate of such illnesses is predicted to be below 1 in 100 million servings for 1 $\log_{10}$ of growth during stabilization, rising to about 1 in 10 million servings for 3 $\log_{10}$ of growth (approximately 5,500 illnesses per year).

6.4.2. What would the effect of altering stabilization be on *C. botulinum*?
It is not possible to state any limits on potential *C. botulinum* growth given only stated limits on *C. perfringens* growth. Of particular concern, *C. botulinum* grows faster than *C. perfringens* below about 28 °C (82 °F), and *C. botulinum* growth is possible at temperatures below which *C. perfringens* does not grow (see Figure 3-4). To limit potential *C. botulinum* growth requires additional constraints on times spent at such temperatures, in addition to any constraints on *C. perfringens* growth.

Moreover, *C. perfringens* growth is not predictive of *C. botulinum* growth, because *C. perfringens* grows faster than *C. botulinum* at higher temperatures, and there is a range of

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[91] The average number of *C. perfringens* outbreak victims, as recorded by CDC, could be used as an estimate of the average number of servings heated and mixed together during hot-holding. However, this would probably result in an overestimate of the contribution of hot-holding due to under reporting of small *C. perfringens* outbreaks.

temperatures (50 °C and higher[92]) at which *C. perfringens* can grow but *C. botulinum* cannot. Without further specification of times and temperatures (*e.g.* limits on allowed cooling curves), it is not possible to predict growth of one organism from the other.

Even with known cooling curves, current lack of knowledge of the variation in the lag time for development of *C. botulinum* from spores in different growth media limits the predictability of the amount of *C. botulinum* growth that might occur.

6.5.   Analysis of 'what-if' scenarios:

Substantial growth of *C. perfringens* is predicted by the model at relatively low temperatures (57–60 °F, 13.9–15.6 °C, see Figure 6-5), albeit temperatures that indicate failure of refrigeration. However, the model does not include potential effects that might mitigate the effects of such failed refrigeration causing illness. Two such effects are:

* the effect of psychrotrophic spoilage organisms dominating growth at low temperatures, and
* consumer detection of *C. perfringens* spoiled ($>10^7$ cell/gram) servings prior to cooking or consumption.

6.5.1. The effect of competing psychrotrophic spoilage organisms

Aerobic and anaerobic psychrotrophic spoilage organisms have optimal growth ranges from 12–30 °C and would therefore likely establish themselves as the dominant organism at these temperatures if they are present (as opposed to *C. perfringens*, which is relatively slow-growing in this temperature range). Spore-forming psychrotrophic spoilage organisms, such as other *Clostridium* and *Bacillus* species, are present in RTE and PCF following heat-treatment at the processing plant, and vegetative cells of some therodurmic vegetative species (*Lactobacillus*, *Enterococcus*, *Micrococcus*) may also be present in some commodities (Ray, 1996). Post-heat-treatment contamination of commodities is also possible and may occur through handling, slicing and air transmission. Psychrotrophic anaerobic and aerobic bacteria have been implicated in the spoilage of RTE meats, including vacuum-packaged and gas-packaged products (Ray, 1996). The occurrence and level of such bacteria are dependent on many factors, including mode of transmission, food matrix and physiology, additives, and processing.

Ideally, experimental data and models for the growth of various possible spoilage organisms in competition with *C. perfringens* in RTE commodities would be needed to assess the impact of spoilage organisms expected to constrain growth of the pathogen under certain conditions, including low temperatures. Conducting such experiments and analysis would be complex and is beyond the scope of the current risk assessment. Although such experimental data and models do not exist for RTE meat commodities, data and models exist for growth of dominant meat spoilage organisms in more controlled culture broth matrices (Pin and Baranyi, 1998) for mixed cultures. From this study, pseudomonads appear to be good surrogates for the spoilage organisms in raw meat and poultry products. Procedures to limit pathogen growth in raw meat and poultry products on the basis of competition with pseudomonad surrogates have been published (Ross and McMeekin, 2003; Coleman *et al.*, 2003). However, additional complexities arise with cooked and partially cooked products. Therefore, in the absence of a convincing body

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[92] Data that would allow evaluation of the exact range have not been published. Published data show growth of *C. perfringens* at 50 °C whereas *C. Botulinum* showed no growth after 504 hours at this temperature.

of scientific evidence to support explicit modeling, the "what if" scenario approach presented in Figure 6-5 was developed to provide some indication of the potential antagonism of growth of *C. perfringens* by spoilage organisms. The effect of overgrowth by competing spoilage organisms would be to suppress the growth of *C. perfringens*, quite possibly completely, in some substantial fraction of cases. The fractional suppression would vary with temperature, probably being higher at lower storage temperatures.

Figure 6-5 indicates the fraction of predicted illnesses at each observed storage temperature from 57–70 °F,[93] assuming no suppression by competing organisms. These fractions are thus also the maximum fraction of predicted illnesses that would be removed by complete suppression of growth of *C. perfringens* at the corresponding temperature, and the effect of less than 100% suppression at some temperatures can be estimated by adding up reductions in these the fractions at the relevant temperatures.

Since the illnesses due to growth during storage at these temperatures constitute 93% of the illnesses predicted by the model, the effect of suppression of growth by overgrowth of spoilage organisms would have an almost directly proportional effect on the total number of illnesses. At 100% suppression between 57 and 70 °F, the total number of illnesses would be reduced to 7% of the original estimate; at 50% suppression at all temperatures between 57 and 70 °F, the total number of illnesses would be reduced to 53.5% of the original estimate.[94] The large potential impact of competition with spoilage organisms warrants inclusion of microbial ecology of RTE foods in the research needs section of this document.

6.5.2. The effect of consumer detection of high *C. perfringens* concentrations.

While *C. perfringens* in not a putrefactive anaerobe, high levels of organism in food ($>10^7$ cells/gram) will likely result in a "spoiled" food product that would probably be detectable by eye, taste, or smell, by a fraction of consumers.[95] Consumers would either not purchase such product (if the spoilage occurred prior to retail sale) or would likely be alerted to the spoilage when the food was removed from refrigeration or during preparation. In either case, the product would likely not be consumed and could therefore not contribute to illness. However, the discriminatory powers of different consumers is likely to be different for similar products contaminated with similar levels of *C. perfringens*, because of the variation between consumers in taste, smell, visual acuity, and judgment.

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[93] Temperatures were recorded to the nearest °F, but not all temperatures in this range were seen. 56 °F was observed, but no illnesses were predicted for this storage temperature in the seven billion servings simulated.
[94] The computer model allows evaluation of this "what-if" scenario by specifying a temperature below which overgrowth by other organisms occurs, and the fraction of cases in which such overgrowth occurs. However examination of Figure 6-5 is sufficient to appreciate the effect.
[95] Hauschild (1975) mentions "Foods responsible for *C. perfringens* outbreaks contain 10^6 or more vegetative *C. perfringens* cells per gram, but in spite of the contamination they appear to be quite palatable at the time of consumption." Craven *et al*. (1981) evaluated organoleptic quality of chicken after growth of *C. perfringens*. The odor of each sample was determined independently by 3 trained judges for 12 responses/treatment. Mean odor determination at 7.99 $\log_{10}$ cfu/g was significantly different compared to 7.37 $\log_{10}$ cfu/g and uninoculated control, and Craven *et al*. remark that "Apparently, as vegetative cell numbers approached 10^8/g and before sporulation and enterotoxin formation, spoilage odors were detected."

To assess this consumer behavior, the model was modified to allow incorporation of a probability to dispose of food servings just before cooking that increased from zero at concentrations below C_{min} to 90% at C_{90}, where C_{min} and C_{90} are two parameters provided to the model. At other concentrations the probability to dispose of the food is assumed to follow an exponential curve:

$$p = 1 - \exp\left(-\ln(10)\frac{\ln(C/C_{min})}{\ln(C_{90}/C_{min})}\right)$$

(6.3)

where *p* is the probability to discard the serving. This detection model was applied with parameters

C_{min} = 7 $\log_{10}$ cfu/gram, and
C_{90} = 8 $\log_{10}$ cfu/gram

which gives 99% probability of discarding food at 9 $\log_{10}$ cfu/g.

A simulation of 500 million servings at each growth rate during stabilization produced the results shown in Table 6.5. The detection of spoilage implied by equation (6.3) results in a decrease in estimated numbers of illness by a factor of about 3.5 at all growths during stabilization. Also shown in Table 6.5 is the corresponding discard rate — the rate at which servings would be discarded in this scenario.

Table 6.5   Estimated annual number of illnesses without and with detection of spoilage by consumers, and the serving discard rate.

Growth ($\log_{10}$)	Estimated annual numbers of illnesses using MLE parameter values		Discard rate per million servings
	No spoilage detection[a]	With spoilage detection	
0.5	111,000	27,000	8.4
1	120,000	35,000	9.0
1.5	128,000	37,000	9.9
2	139,000	39,000	11.0
2.5	156,000	45,000	12.1
3	181,000	52,000	13.2
3.5	207,000	62,000	14.9

[a] Estimated using one billion samples at each growth rate with default sensitivity parameters and uncertainty values set at their MLE.

6.6.   Sensitivity analysis

For several of the model parameters, experimental evidence suggests a range of values for a variability distribution, but there are too few data to adequately define that variability distribution. In other cases, the model has been simplified to use a single value, but no experiment has measured precisely the quantity of interest and extrapolations of the value from related measurements are subjective. These cases were identified in Chapter 3 for sensitivity

analyses, and are listed here together with numerical or other evidence for their effect on the results of the model.

Table 6.6 summarizes numerical estimates for the sensitivity of the total number of illnesses per year to the various parameters for which sensitivity analyses were performed. These numerical estimates are of the dimensionless sensitivity measure given by

$$\frac{\partial \ln N}{\partial \ln x} = \frac{x}{N} \frac{\partial N}{\partial x} \qquad (6.4)$$

where

N is the annual number of illnesses predicted by the model, and
x is the parameter of interest.

The value given by equation (6.4) was obtained either by direct numerical measurement (changing the size of the parameter x, running the Monte Carlo simulation, and observing the change in N), or by theoretical evaluations summarized in the paragraphs following the table and using results already obtained.

It has to be borne in mind that different parameters are uncertain to different extents, and evaluation of the relative importance of each parameter should take account of both the size of the potential variation in the parameter as well as the sensitivity shown in Table 6.6.

Table 6.6   Summary of numerical estimates of sensitivity.

Parameter of interest	Sensitivity	Method
Max. fraction germinating after two heat steps	< 0.04	t
Mean fraction of spores germinating in RTE production	0.05	t
Mean fraction of spores germinating with no heat step	0.03	t
Mean fraction of spores germinating in second heat step	~ 0.04	t
Mean fraction of spores germinating during storage	0.006	t
Mean storage time in manufacture and retail	2.0	n
Fraction of category 1 foods eaten cold	0.16	t
Fraction of heated foods heated in an oven	~ -0.12	n
Mean microwave heating time	$\pm < 0.04$	n
Mean oven heating time	$\pm < 0.06$	n
Mean fraction of category 1 & 4 foods hot-held	0.04	t
Hot-holding time	Not evaluated (less than 0.04	a
Maximum vegetative cell density in foods	0.29	n
Fraction of selected CSFII foods that are RTE and partially cooked	1.0	t
t Theoretical analysis, coupled with measured results already obtained		

n Direct numerical measurement (detection limit magnitude approximately 0.04)

a This is probably small, but would require numerical measurement using of order 10 billion samples.

6.6.1. The maximum fraction of spores that may ever germinate in two heating steps.

The default value is 0.75. This fraction primarily affects the potential maximum number of spores remaining after the first heat step that could germinate during re-heating and subsequent hot-holding. The fraction of diarrheas predicted to be due to hot-holding is approximately 4% of the total, so the sensitivity of the number of diarrheas to this fraction is less than about 0.04.

6.6.2. The fraction of spores that germinate during production of RTE

This is a variability distribution with default a triangular distribution (0.05, 0.50, 0.75). Modification of this fraction will primarily affect the number of vegetative cells initially in the serving, and to some extent the probability for a serving to contain any vegetative cells initially. The mean value of the distribution will therefore be the controlling factor. Variation of the mean value of the fracton of spores that germinate is practically equivalent to varying growth during production by the same relative amount, since both multiply the number of germinated spores. From Equation (6.2), a good estimate for the variation in number N of illnesses with growth during stabilization is

$$N = N_0 \exp\left(ag + bg^2\right) \tag{6.5}$$

where

$\quad\quad N \quad$ is the number of illnesses per year,

$\quad\quad N_0 \quad$ is the number of illnesses per year that would be expected with no growth during stabilization

$\quad\quad a = 1.09,$

$\quad\quad b = 0.21,$

and $\quad g \quad$ is the $\log_{10}$ growth during stabilization.

Thus if

$$g = \log_{10}\left(xy + z\right) \tag{6.6}$$

where a fraction $f = xy/(xy + z)$ of the growth is proportional to some parameter x, then

$$\frac{x}{N}\frac{\partial N}{\partial x} = f\,\frac{a + 2bg}{\ln 10} \tag{6.7}$$

At $g = 1$ the term on the right of equation (6.7) is $0.071f$, and at $g = 2$ it is $0.099f$, and the fraction of illnesses predicted to be caused by germinating spores in RTE foods is $f = 0.6$.[96] Thus the sensitivity of the number of illnesses to the mean estimate of the fraction of spores germinating during RTE is approximately $0.071 \times 0.6 = 0.043$ at $g = 1$ to approximately $0.099 \times 0.6 = 0.059$ at $g = 2$.

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[96] The fraction of illnesses is only approximately equal to the effective fraction of the growth rate, because of the g^2 term in equation (6.5), but the approximation is adequate here.

6.6.3. The fraction of spores that germinate without any heat step

This is a variability distribution with default a triangular distribution (0.01, 0.05, 0.10). Again, variation of the mean value of this fraction is almost equivalent to variation of the growth during stabilization. about 35% of illnesses are predicted to be proportional to this value (the percentage of illnesses arising from spores in spices). Using the same approach as in Section 6.6.2, the sensitivity of the total number of illnesses to the mean value of the fraction of spores that germinate without any heat step is about 0.03.

6.6.4. The fraction of spores that could be heat-activated that are heat activated by a second heating

This is a variability distribution with default a triangular distribution (0.0, 0.5, 1.0). It affects only the hot-hold situation, with the number of such illnesses approximately proportional to its mean value. Since the fraction of illnesses due to hot-held food is about 4%, the sensitivity of the total number of servings to the mean value of this parameter is about 0.04.

6.6.5. The fraction of spores that germinate during storage and transport

This is a variability distribution with default a triangular distribution (0.0, 0.025, 0.05). The number of illnesses caused by spores germinating in storage is approximately proportional to the mean value of the variability distribution, and the fraction of illnesses due to such germinating spores is about 0.6%. The sensitivity of the total number of illnesses to the mean value of this parameter is thus about 0.006.

6.6.6. The storage time between manufacturer and retailer

This is a variability distribution with default a uniform distribution (10, 30) days (mean 20 days). The results of the assessment are relatively sensitive to this default assumption. Altering the estimate to a uniform (5,20) days (mean 12.5 days) of storage results in a drop in estimated illnesses to approximately 1 in a million servings at 1 $\log_{10}$ growth, 1.2 in a million at 2 $\log_{10}$ growth, and 1.5 in a million at 3 $\log_{10}$ growth, in each case approximately 0.4 of the rates obtained using the default assumption. Figure 6-6 ("short storage") illustrates the effect of the change in assumed manufacturer to retailer storage time. The error bars shown correspond to the numerical precision of the simulated 500 million servings.

The change in mean storage time can be expressed as about –0.47 on a logarithmic scale (ln(12.5/20)), and this causes a reduction of about –0.92 (ln(0.4)) in the number of illnesses (again, on a logarithmic scale). The sensitivity is thus about –0.92/(–0.47) = 2.

Figure 6-6   Approximate variation in MLE of illness rate for sensitive parameters.
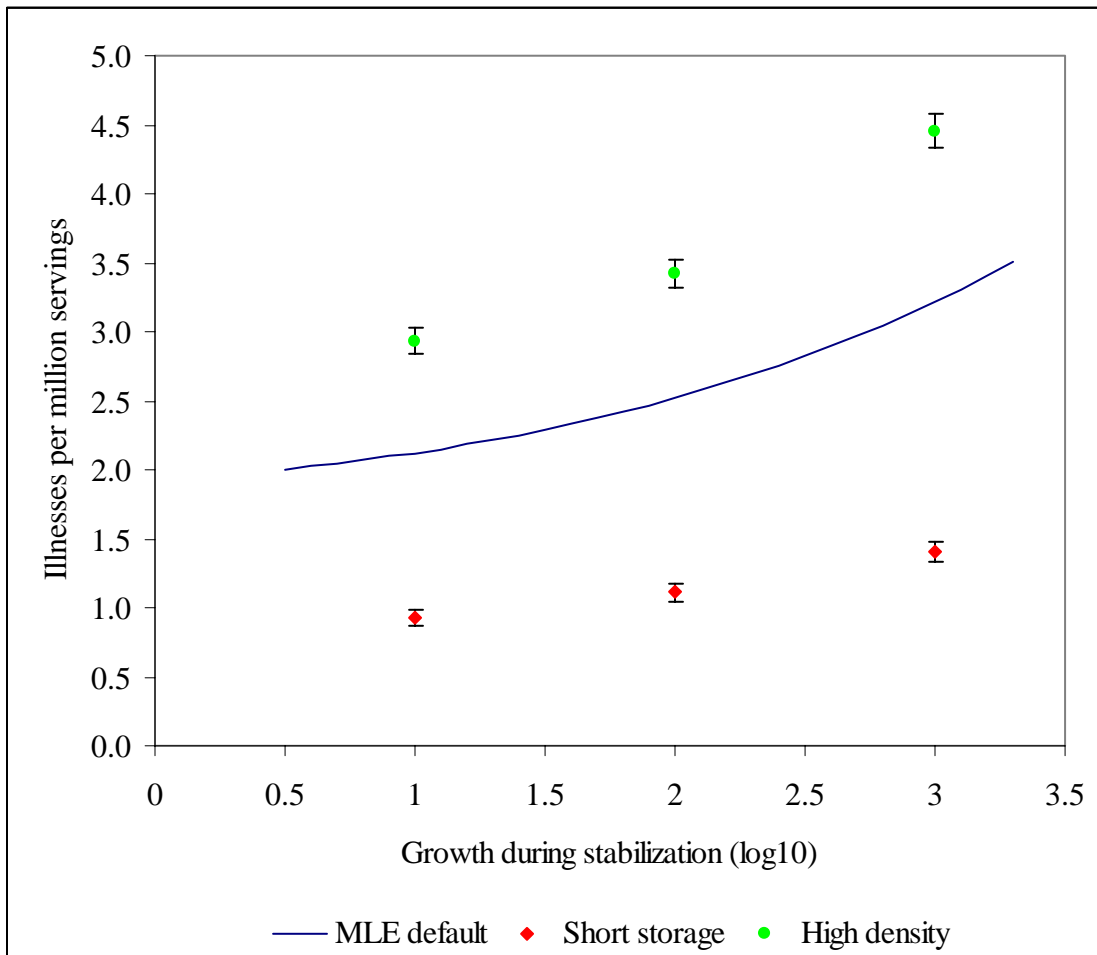
6.6.7. The fraction of category 1 foods that are eaten cold
This parameter is a fixed fraction, with default value 0.2. In the model, the number of illnesses caused by eating cold category 1 foods is proportional to this fraction, and about 16% of illnesses are predicted to occur as a result of eating cold category 1 foods. The sensitivity of the number of illnesses to this fraction is thus about 0.16.

6.6.8. The fraction of RTE and partially cooked foods that are heated in an oven
The fraction of foods that are heated in an oven (with a lower heating rate, the alternative is being heated in a microwave with a higher heating rate) is estimated by default as 0.5. Altering this fraction to 0.25 has a small effect on estimated numbers of illnesses — a increase of about 9% in a numerical simulation of 500 million servings, in which the numerical precision in the simulation is approximately 3% (expressed as a standard deviation). The logarithmic change in number of illnesses is +0.086 (ln(1.09)), for a logarithmic change in parameter value of –0.69 (ln(0.25/0.5)), giving a sensitivity of about –0.12 (although with substantial uncertainty).

6.6.9. Heating time in a microwave

This is a variability distribution with default a uniform distribution of (1, 10) minutes. Altering this distribution to a uniform (0.5, 5) minutes (a logarithmic change of about –0.69 in mean value) has an undetectable effect in a simulation of 500 million servings (in which the approximate numerical precision is 3% when expressed as a standard deviation). The sensitivity of the total number of illnesses to the mean value of the heating time in a microwave is thus zero, with an uncertainty of about $\ln(1.03)/(0.69) = 0.04$.

6.6.10. Heating time in an oven

This is a variability distribution with default a uniform distribution of (10, 30) minutes. Altering this distribution to a uniform (5, 20) minutes has an undetectable effect in a simulation of 500 million servings (in which the approximate numerical precision is 3% when expressed as a standard deviation). The sensitivity of the total number of illnesses to the mean value of the heating time in a microwave is thus zero, with an uncertainty of about $\ln(1.03)/(\ln(20/12.5) = 0.06$.

6.6.11. The fraction of Category 1 and 4 foods that are hot-held

The default value is 0.01, which is simply a guess. Hot-holding illnesses are directly proportional to this fraction. At the default value they form only a small fraction of the total (about 4%), so the sensitivity to this parameter is approximately equal to 0.04 provided the default estimate is anywhere near close. Moreover, hot-holding illnesses are not affected by growth during stabilization (under the conditions assumed by the model).

6.6.12. The hot-holding time

This is a variability distribution with default a triangular distribution (0.5, 2, 8) hours based loosely on the regulations covering hot-holding. Since predicted hot-holding illnesses are only a small fraction (about 4%) of the total, the sensitivity of total illnesses to this parameter is small (less than 4%). Moreover, hot-holding illnesses are not affected by growth during stabilization (under the conditions assumed by the model). As discussed in Section 6.4.1, the model very likely substantially underestimates hot-holding illnesses, and the underestimation has not been taken into account in this sensitivity analysis.

6.6.13. The maximum vegetative cell density

This is a variability distribution with default a lognormal distribution corresponding to a median $8 \log_{10}$ and a standard deviation 0.5 on the $\log_{10}$ scale. The results of the assessment are relatively sensitive to this default assumption. Altering the estimate to a median $8.5 \log_{10}$ with a SD of 0.5 on the $\log_{10}$ scale results in an increase in estimated illnesses to approximately 3 in a million servings at $1 \log_{10}$ growth, 3.5 in a million at $2 \log_{10}$ growth, and 4.6 in a million at $3 \log_{10}$ growth, in each case approximately 1.4 times the rates obtained using the default assumption. Figure 6-6 ("High density") illustrates the effect of the change in assumed maximum vegetative cell density. The error bars shown correspond to the numerical precision in the simulated 500 million servings. The sensitivity of the total estimated number of illnesses to the mean estimate of maximum vegetative cell density is thus approximately $\ln(1.4)/\ln(10^{0.5}) = 0.29$.

6.6.14. The fraction of CSFII (USDA, 2000) servings that are RTE and partially cooked
This fraction is assumed to be 0.8 (Section 3.15.2), but with no scientific basis. The estimated rates of illness are independent of this value, but the total number of illnesses is directly proportional to its value.

7. Research Needs

Examination of the risk assessment analyses and results has identified the following research or data needs. They are listed in an approximate priority order that takes some account of the relative difficulty of satisfying them.

1. Relation between CSFII foods and RTE and partially cooked foods

The CSFII does not distinguish between foods prepared from raw and RTE and partially cooked foods, so that broad inferences were necessary in selecting foods described in the CSFII for inclusion in the analysis. It is therefore unknown what fraction of foods that could be RTE and partially cooked selected from CSFII are in fact RTE and partially cooked foods (see Section 3.15.1). This mostly affects the estimate of total number of servings per year of RTE and partially cooked foods, rather than the distribution of sizes and types of servings. It was assumed that 80% of foods selected from CSFII were actually RTE and partially cooked foods, and the estimate of number of illnesses is directly proportional to this fraction. To obtain an independent estimate of the total number of servings produced by the RTE/partially cooked foods industry, a market or industry survey would be needed.

2. Growth characteristics of C. botulinum in heat-treated products

Proteolytic *C. botulinum* A and B are present in RTE and partially cooked foods and can cause illness due to the production of botulinum toxin during stabilization. The amount of bacterial growth needed to produce toxin in foods is unknown, so the aim is generally to prevent any growth. Evaluation of the available studies on *C. perfringens* and *C. botulinum* indicated that growth rates were dependent on the growth medium used in the studies, but that lag time was even more sensitive. However, no studies on *C. botulinum* in cooked meat and poultry products were identified that allowed adequate determination of lag times in particular (See Section 6.4.2). Studies are needed to better quantify the variability of lag time, growth rates and time to toxin production in cooked beef and poultry products. This study should include variables such as: strain variation, food matrix and physiology (including *pH*, salt concentration, and water activity), temperature, additives (*e.g.* nitrites, phosphates) and the effect of competing microflora.

3. Percentage of RTE and partially cooked foods that are hot-held

Outbreak observations suggest that improper hot-holding is a contributing factor to *C. perfringens* outbreaks. This notion is supported, although not well modeled, by the current risk assessment. The risk assessment assumes that 1% of meat-containing *C. perfringens* growth-supporting RTE and partially cooked food servings of categories 1 and 4 are hot-held (see Section 3.15.2). However, the actual percentage of foods that are hot-held is unknown. A nationally representative fraction of RTE and partially cooked foods that are hot-held is therefore needed. To reduce the uncertainty of this estimate, it may be possible to design a survey directed toward consumers and institutions (restaurants, hospitals, nursing homes, schools, prisons, and grocery stores) expected to be the principle users of hot-held RTE and partially cooked foods.

4. Prevalence of type A CPE positive C. perfringens spores in spices and herbs

Outbreak observations suggest that heavily spiced foods, such as some Mexican style foods, may be a contributing factor to *C. perfringens* outbreaks. The current risk assessment considers the

role that *C. perfringens* contaminated spices may play; however, the literature data used may not be representative of current *C. perfringens* spore levels and prevalence (see Section 3.8). A nationally representative survey to elucidate the prevalence and level of *C. perfringens* type A enterotoxin positive spores in spices and herbs used in RTE and partially cooked foods is needed to better identify the role of spices in *C. perfringens* food poisoning.

5. *Maximum C. perfringens vegetative cell density in different foods*
The maximum *C. perfringens* vegetative cell density is assumed to by 8 $\log_{10}$ with a variability of 0.5 on a $\log_{10}$ scale, based on an informal evaluation of just three experiments (see Section 3.11.5.6).

6. *Consumer re-heating and hot-holding time behavior*
The level of *C. perfringens* vegetative cells consumed in a serving is the primary determinant of the probability of illness. The duration at certain temperature at which a contaminated product is held will affect the final level of *C. perfringens* in a serving by allowing growth, survival or death of these bacteria. The risk assessment assumes re-heating times will vary due to heating methods: 1) 50% of RTE and partially cooked foods are assumed cooked by microwave in a time that varies uniformly from 1 to 10 mins., and 2) 50% of RTE and partially cooked foods are assumed cooked by oven in a time that varies uniformly from 10 to 30 mins. For hot-holding times, a minimum of 0.5, median of 2.0 and maximum of 8.0 hrs. (triangular distribution) was assumed (see Sections 3.14.2 and 3.14.4). To more accurately determine the final level of *C. perfringens* in servings, a survey of consumer re-heating and hot-holding times, methods, and temperatures is needed for RTE and partially cooked foods.

7. *Storage of RTE and partially cooked foods*
Following stabilization, RTE and partially cooked foods are moved through stages of storage and transportation before the sale of the product. During these processes, variation in times and temperatures may alter the level of *C. perfringens* in a contaminated serving. The risk assessment currently does not distinguish between manufacturer, distributor and retail storage and transportation between these locations, and assumes the duration to be uniformly distributed between 10 and 30 days for all foods considered. Additionally, Audits International (1999) data on selected products in retail refrigerator cabinets is assumed representative of the entire storage time between manufacturer and retail (see Sections 3.13.3). To better determine the effect of storage and transportation on *C. perfringens* food poisoning illnesses, a survey investigating time and temperature data for each specific section of storage and transportation is needed.

8. *C. perfringens spores in raw products*
Some studies have evaluated the levels of *C. perfringens* spores in some raw products used for production of RTE and partially cooked foods. However, these studies examined too few samples to determine the upper end of the distribution of levels that may occur, or to distinguish between different raw products or detect geographical or temporal variations; and none of the studies has evaluated the fraction of *C. perfringens* spores or vegetative cells that are type A, enterotoxin positive in these raw products (see Section 3.5). A more extensive survey is needed to identify the upper bound of *C. perfringens* spores in all raw products destined to become RTE and partially cooked foods. A very large nationally representative survey conducted over all

seasons to estimate the prevalence and levels of *C. perfringens* type A, enterotoxin positive spores in raw whole and comminuted/ground meat and poultry products is needed.

9. Additional data needs

The following are of somewhat lower priority than those listed above, and are not listed in any priority order.

- ***Storage times in consumer refrigerator/freezers***

The estimated storage time in consumer refrigerators and freezers is based on a small survey asking a non-representative sample of consumers for the mean time of storage of deli meats and hot dogs, and another non-representative sample of consumers for the most recent time of storage of hot dogs (see Section 3.13.3). A survey of a representative sample of consumers is needed to obtain the distribution of storage times for all RTE and partially cooked food products.

- ***Fraction of type A, CPE+ spores that germinate under various conditions***

The fraction of *C. perfringens* spores that germinate after heating varies very strongly with heating temperature and time, and with the strain of the spore. Too little is known of the temperature, time, and strain variation, or of processing conditions, to allow prediction of the fraction of type A, CPE+ spores that will germinate during processing of either RTE or partially cooked foods based on knowledge of processing conditions. It is similarly currently impossible to predict accurately the fraction that will germinate under mild conditions, or during storage at various low temperatures (see Section 3.9). Experiments on (multiple) type A, CPE+ strains are needed, preferably under field conditions, to obtain reliable data on this fraction. In addition, such studies need to proceed to a second heat treatment to evaluate the fraction of spores surviving the first heat treatment that germinate during the second. The origin of any differences between type A, CPE+ *C. perfringens* found in raw products and spices needs also to be elucidated.

- ***Quantitative estimate of the variation of growth rate with nitrite and salt content of foods***

The variation of growth rates of *C. perfringens* with nitrite and salt concentrations is currently not well mapped, particularly in food matrices, only crude cut-off values being available (see Section 3.11.5.2). In particular, the effect of salt and nitrite concentration on the temperature range for growth is not known. Factorial experiments in food matrices using varied nitrite and salt concentrations would supply considerably more information.

- ***Growth rate experiments in more strains of C. perfringens, and in more food matrices***

Current growth rate estimates for *C. perfringens* depend on measurements in very few strains, typically those selected to be fast growing (see Section 3.11 in general, and Section 3.11.4 in particular). Experiments on growth rates and their temperature-dependence for many strains of *C. perfringens* type A CPE+ are needed. Similarly, growth rate estimates are available only for few food substrates. The effect of variation of meat content on growth rate and its temperature dependence needs to be evaluated.

8. References

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