A Risk Assessment for *Clostridium perfringens* in Ready-to-Eat and Partially Cooked Meat and Poultry Products

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by

Edmund Crouch, Ph.D.
Cambridge Environmental, Inc.
58 Charles Street, Cambridge, MA 02141

and

Neal J. Golden, Ph.D.
The Risk Assessment Division
Office of Public Health Science
Food Safety Inspection Service
USDA

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Contributing scientifically to this document from the FSIS *C. perfringens* Risk Assessment Team were:

Margaret Coleman, M.S.[1]
Anne Courtney-Radcliff, Ph.D.[1]
Uday Dessai, Ph.D., M.P.H
Eric Ebel, D.V.M.[1]
Abdel-Razak Kadry, D.V.M., Ph.D., D.A.T.B.
Michael Kasnia, R.S.
Janell Kause, M.P.H., M.P.P.
Heejeong Latimer, Ph.D.
Wayne Schlosser, D.V.M., M.P.H.
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[1] Previous employees of FSIS.

Executive Summary

The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) conducted a quantitative risk assessment of *Clostridium perfringens* (*C. perfringens*) in ready-to-eat (RTE) and partially cooked meat and poultry products. The purpose of the risk assessment was twofold: 1) evaluate the public health impact of changing the allowed maximal growth of *C. perfringens* during manufacturing stabilization (cooling after the cooking step) of RTE and partially cooked meat and poultry products; and 2) examine whether steps taken to limit the growth of *C. perfringens* occurring in RTE and partially cooked foods would also be adequate to protect against growth of *Clostridium botulinum.*

Public Health Regulatory Context

The bacterium *C. perfringens* grows well on meat and poultry products in the absence of oxygen, and grows best at relatively high temperatures. Since *C. perfringens* is ubiquitous in the environment, sources of raw meat[2] are occasionally contaminated with this organism, either in the form of vegetative cells or as spores. Vegetative cells are destroyed during heating in the production of RTE foods, though may survive the incomplete cooking used to prepare partially cooked foods. Spores, on the other hand, are not destroyed by heat and other processes applied to RTE foods. Rather, heat can activate spores to germinate and develop into vegetative cells capable of growth during the stabilization processes of RTE food manufacture.

Consuming foods contaminated with high levels of certain strains of *C. perfringens* vegetative cells (those known as type A, that produce the *C. perfringens* enterotoxin, CPE) may lead to diarrheal illness. Illness is generally mild, and typically self-limiting, lasting one or two days. Symptoms include diarrhea, nausea, and some abdominal pain. No known foodborne illnesses have been caused by the ingestion of *C. perfringens* spores; rather, it is necessary to consume the vegetative cells for illness to occur.

As the public health regulatory agency responsible for ensuring the safety and wholesomeness of meat, poultry, and egg products in the United States, FSIS has taken steps to address *C. perfringens* in Agency regulated products. On January 6, 1999, FSIS published a final rule in the Federal Register (FSIS Docket No. 95-033F; 64 FR 732) establishing performance standards for *C. perfringens* in cooked beef, roast beef, and cooked corned beef products; fully and partially cooked meat patties; and certain fully and partially cooked poultry products, in an effort to address the public health risk posed by *C. perfringens*. The production requirements for these products included performance standards limiting multiplication of *C. perfringens* to a maximum of 1-$\log_{10}$ (a factor of 10)[3] within the product during RTE food manufacture.

On February 27, 2001, FSIS published a proposed rule: *Performance Standards for the Production of Processed Meat and Poultry Products* [66FR12590, February 27, 2001]. The intent of this rule with regard to *C. perfringens* was to extend the existing performance standards to all RTE and all partially cooked meat and poultry products.

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[2] Throughout this document, "meat" generally means meat or poultry, except for specific cases that should be clear in context, *e.g*. where referring to an experiment on a specific meat.

[3] In this standard jargon, growth is expressed on a base 10 logarithm scale. So 1-$\log_{10}$ corresponds to a factor of 10, 2-$\log_{10}$ corresponds to a factor of 100, 3-$\log_{10}$ to 1000, 1.7-log10 would be a factor of 50, and so forth.

The risk assessment was initiated in FY2003 in response to specific FSIS risk management questions formulated for the Risk Assessment Division, to garner information in response to public comments on the FSIS proposed rule released in 2001. Several comments requested greater evaluation of the current performance standard that limits multiplication of *C. perfringens* to a maximum of 1-$\log_{10}$ within the product. To better understand those concerns, FSIS requested public input as part of the proposed rule for RTE meat and poultry products (66FR12601, *op. cit.*) and initiated a risk assessment.

Risk Management Questions

The risk assessment was designed to addresses the following risk management questions:

1. What is the impact on the probability of human illness if the allowable growth of *C. perfringens* is raised from 1-$\log_{10}$ (that is, 10-fold) during stabilization to 2-$\log_{10}$ (that is, 100-fold) or 3-$\log_{10}$ (that is, 1000-fold)?
2. What would the relative growth of *C. botulinum* (relative to the growth of *C. perfringens*) be for each of these stabilization standards?

Structure and scope of the current risk assessment

The *C. perfringens* risk assessment is a plant-to-table probabilistic risk assessment. The risk assessment incorporates a data-driven model that tracks *C. perfringens* spores and vegetative cells on raw meat and poultry products from the processing plant through the point of consumption. The risk assessment uses a computer program to perform Monte Carlo simulations on meat-containing food servings selected from the Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 2000). The selection of servings was made to limit analysis to those servings that could contain RTE or partially cooked foods, and that were considered capable of supporting growth of *C. perfringens* (omitting, for example, shelf-stable foods and foods high in salt and nitrite).

For each such food serving, the original amount of contamination by spores and vegetative cells of *C. perfringens* is obtained, the resultant amount after manufacture (including stabilization step(s)) is calculated, and the amount of contamination is tracked as spores germinate and vegetative cells grow and die during storage between manufacture and retail, during storage between retail sale and preparation, and during preparation. Ultimately, the number of vegetative cells consumed in the serving, the likelihood of those cells to cause illness, and whether that particular serving actually causes illness, is calculated for each serving. The Monte Carlo simulation also provides information on the certainty of the risk assessment estimates.

Risk Assessment Outputs

The primary results of the risk assessment are summarized as follows:

1. Approximately 79,000 illnesses per year in the U.S. from RTE and partially cooked meat and poultry products (at 1-$\log_{10}$ growth).

2. A change in growth during stabilization from 1-$\log_{10}$ to 2-$\log_{10}$ and 3-$\log_{10}$ results in a median 1.23 and 1.59 fold increase in annual diarrheal illness, respectively.

3. Improper cold storage of RTE and partially cooked meat and poultry products at retail and the home accounts for approximately 90% of the predicted *C. perfringens* foodborne illness. Improper hot-holding of RTE and partially cooked meat and poultry products accounts for approximately 8% of the predicted illnesses at 1-$\log_{10}$ growth during stabilization, although the risk assessment probably underestimates this fraction.

4. Stabilization at processing plants accounts for 0.05% and 0.4% of predicted illnesses at 1-$\log_{10}$ and 2-$\log_{10}$ allowable growth, respectively. Therefore, relatively few predicted illnesses are associated with stabilization at processing plants.

5. The growth rate of *C. botulinum* is observed to be higher at low temperatures in laboratory experiments, and it probably grows at temperatures below the minimum temperature for *C. perfringens* growth. Any measures taken to reduce or prevent growth of *C. perfringens* will not necessarily have the same effects on growth of *C. botulinum*.

Uncertainty and sensitivity analysis

In addition to obtaining a single estimate of the number of illnesses per year, the Monte Carlo simulation takes account of the known uncertainties in the data and assumptions used as model inputs. That is, how sure we are of the result of the number of illnesses each year. The uncertainty estimate is an underestimate of our true ignorance, since it does not incorporate unknown uncertainties.

Sensitivity to a particular parameter or assumption in the risk assessment was examined by running scenarios in which all inputs except one were set to baseline values. The remaining input was changed by a substantial amount, making it comparable to its likely upper or lower bound. By doing so, the relative contribution of each parameter to the final estimate of annual illnesses can be assessed and the drivers of risk determined.

Research Needs

Based on sensitivity analyses, areas for further research include:

1. The categorization of foods as RTE and partially cooked foods based on the CSFII.
2. Growth characteristics of *C. botulinum* in heat treated products.
3. The fraction of RTE and partially cooked foods that are hot-held.
4. The prevalence and concentration of type A, CPE-positive *C. perfringens* spores in spices and herbs.
5. Maximum *C. perfringens* vegetative cell density in various meat and poultry products.
6. Consumer re-heating and hot-holding time behavior.
7. Additional retail and consumer storage times and temperatures of RTE and partially cooked foods.

8. The prevalence and concentration of type A, CPE-positive *C. perfringens* spores in raw meat and poultry products.

Conclusions

The risk assessment for *C. perfringens* in RTE and partially cooked meat and poultry products is based on scientific evaluation of all available evidence. The risk assessment received stakeholder input through public comment and underwent peer review consistent with current Office of Management and Budget (OMB) guidelines. The model is a tool to evaluate the effect of interventions and risk management options, rather than predict the absolute number of illnesses.

Most of the human health risks associated with *C. perfringens* in RTE and partially cooked meat and poultry products are associated with improper consumer and retail refrigeration and, to a lesser extent, consumer hot-holding of these products. While the risk assessment indicates that few predicted illnesses are associated with growth during stabilization corresponding to the current regulatory limit on cooling practices at processing plants, there is an increase in predicted illnesses as this growth is increased.

1. Scope and Mandate

1.1. Scope

This risk assessment was initiated in FY2003 in response to FSIS risk management questions provided to the Risk Assessment Division to gather information in response to public comments on the Food Safety and Inspection Service (FSIS) proposed rule: *Performance Standards for the Production of Processed Meat and Poultry Products* [66FR12590, February 27, 2001[4]]. Several comments called into question the validity of the current performance standard that limits multiplication of *Clostridium perfringens* (*C. perfringens*) to a maximum of 1-$\log_{10}$ within the product (USDA, 1999). To better understand those concerns, FSIS requested public input as part of the proposed rule for ready-to-eat (RTE) meat and poultry products (66FR12601, *op. cit.*). In addition to the public request for data, FSIS initiated the planning and development of this risk assessment to answer the following risk management questions:

1. What is the impact on the probability of human illness if the allowable growth of *C. perfringens* is raised from 1-$\log_{10}$ (that is, 10-fold) during stabilization to 2-$\log_{10}$ (that is, 100-fold)?
2. What is the impact on the probability of human illness if the allowable growth of *C. perfringens* is raised from 1-$\log_{10}$ during stabilization to 3-$\log_{10}$ (that is, 1000-fold)?
3. What would the relative growth of *C. botulinum* (relative to the growth of *C. perfringens*) be for each of these stabilization standards?

This risk assessment will answer the above risk management questions for ready to eat (RTE) and partially cooked foods modeled from post lethality (that is, just after a treatment designed to kill the organisms) to consumption. The report will also provide information on the risk assessment model developed, the data considered and ultimately used, underlying assumptions, risk assessment outputs, and a sensitivity analysis. This report is organized to include the following sections:

1. Public Health and Regulatory Context
    a. Public health background
    b. Policy context
2. Hazard Identification
    a. *C. perfringens*
    b. Sources of *C. perfringens*
    c. Epidemiology of disease caused by *C. perfringens*
    d. Factors affecting survival and growth
    e. Pathogenesis
3. Exposure Assessment
4. Limitations of the Exposure Model
5. Hazard Characterization
    a. Data evaluation
    b. Deriving the dose-response function
6. Risk Characterization

[4] Available at http://www.fsis.usda.gov/OPPDE/RDAD/ProposedRules01.htm (Accessed 3/4/04)

   a. Results
   b. Uncertainty
   c. Risk Management Questions
   d. Sensitivity analysis
7. Research Needs
8. References
9. Appendix A Food Categories Modeled
10. Appendix B Food Category list
11. Appendix C Foods commonly hot-held
12. Appendix D Meat content of servings
13. Appendix E Using the program

1.2. Public Health and Regulatory Context

This section provides background information on the health risks posed by *C. perfringens* and the regulatory context for this pathogen in FSIS-regulated RTE and partially cooked meat and poultry products.

1.2.1. Public Health Background

C. perfringens is an anaerobic, Gram-positive, spore-forming rod shaped bacterium that generates a toxin when vegetative cells sporulate in the digestive tract of people thus causing human illness (Craven, 1980). It is widely distributed in the environment and frequently occurs in the intestines of humans and many domestic and feral animals. Spores of the organism persist in soil, sediments, and areas subject to human or animal fecal pollution.

Of all *C. perfringens* strains, only around 5% are capable of producing the toxin (McClane, 2001). *C. perfringens* poisoning is estimated to be one of the most common foodborne illnesses in the U.S. Mead *et al.* suggest there are approximately 250,000 cases of *C. perfringens* annually in the U.S. (Mead *et al.* 1999). Outbreaks are typically associated with meat and poultry products and a review of the 57 outbreaks reported to the CDC between 1992 and 1997 (CDC, 2000) reveals that outbreaks may be seasonal with peaks occurring from March through May and October through December.

C. perfringens poisoning is characterized by intense abdominal cramps and diarrhea which begin 8-22 hours after consumption of foods containing large numbers of *C. perfringens* (typically greater than 10^8 per gram, but as low as 10^6 per gram). The illness is usually over within 24 hours but less severe symptoms may persist in some individuals for 1 or 2 weeks (FDA, 1992). Since 1992 a few deaths have been reported as a result of dehydration and other complications. The young and elderly are the populations most sensitive to illness from *C. perfringens* (Mead *et al.*, 1999). Those under 30 years of age are likely to get sick and recover, while elderly persons are more likely to experience prolonged or severe symptoms and, unlike children, possible complications (*e.g.*, infection exacerbated by diverticulosis).

In most instances, temperature abuse has been associated with foods believed to be responsible for causing illness whether these foods are prepared by institutions, restaurants or at home (CDC, 2000). Spores may germinate during heating and the resultant cells can multiply to high levels (10^6 per gram or more) if food containing the cells is (1) hot-held for extended periods at

insufficiently hot temperatures, (2) improperly cooled, or (3) improperly stored. Large cuts of meat, gravies, stews, and highly spiced foods are most frequently implicated (FDA, 1992). The majority of poisonings do not appear to be from RTE products produced in FSIS regulated establishments, but rather from products prepared from raw meats and poultry and from products such as chili, tacos and enchiladas prepared from raw products in advance by consumers or in restaurants or institutions and held for extended lengths of time at temperatures that will support growth. "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), 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). Inadequate cooking was the next most commonly identified contributing factor and was reported in only 23 of those 74 outbreaks from 1973 through 1987, and 65% of outbreaks where it was positively identified as contributing or non-contributing from 1973 through 1987 (Bean and Griffin, 1990; CDC, 1996, 2000).

1.2.2. Policy Context

To protect public health, on January 6, 1999, FSIS published a final rule in the Federal Register (FSIS Docket No. 95-033F; [5] 64FR732) that established performance standards for *C. perfringens* in some RTE and partially-cooked foods. The production requirements for these products included performance standards that limit multiplication of *C. perfringens* to a maximum of 1-$\log_{10}$ within the product (USDA, 1999).

On February 27, 2001, FSIS published a proposed rule in the Federal Register entitled, "Performance Standards for the Production of Processed Meat and Poultry Products." The intent of this rule with regard to *C. perfringens* was to extend the existing performance standards to all RTE and partially heat treated meat and poultry products.

In light of comments received on the proposed rule, which called into question the validity of the current performance standard, FSIS planned to conduct a risk assessment and evaluate the effectiveness of various potential performance standards to mitigate the risk of illness from *C. perfringens* in RTE and partially cooked meat and poultry products.

This report addresses the risk management questions listed above, which were presented to the Risk Assessment Division of USDA by the Office of Policy, Program & Employee Development (OPPED) of FSIS on January 13, 2003.

[5] Available at http://www.fsis.usda.gov/OPPDE/RDAD/FinalRules99.htm. (Accessed 3/3/2004).

2. Hazard Identification for *Clostridium Perfringens*

2.1. Effects and incidence

Infection with *C. perfringens* may lead to two distinct human enteric diseases: (*i*) *C. perfringens* type A food poisoning and (*ii*) necrotic enteritis, also referred to as Darmbrand or Pig-Bel (McClane, 2001). Necrotic enteritis is rare in industrialized societies and is not the focus of this risk assessment.

C. perfringens food poisoning is frequently either not recognized or not reported; consequently, the true prevalence of this disease may be considerably underestimated (McClane, 2001). Nonetheless, current estimates suggest *Clostridium perfringens* causes approximately 250,000 illnesses, 41 hospitalizations, and 7 deaths in the United States *per annum*. All cases are believed to result from ingestion of contaminated food, and as such, *C. perfringens* has been ranked fourth (behind *Campylobacter* spp., non-typhoid *Salmonella*, and *Shigella* spp.) as the most common bacterial cause of foodborne illness (Mead *et al*., 1999).

2.2. Epidemiology of outbreaks

The most common vehicles implicated in outbreaks of *C. perfringens* foodborne illness have been beef and poultry. Products such as stews, gravies, and Mexican foods have also been recognized as important disease vehicles (CDC, 2000). To date, of the total 153 reported outbreaks between 1990 and 1999 with identified etiology and vehicle (see Section 2.4), only one has been confirmed as having been caused by an RTE product, turkey loaf (CDC, 2000; personal communication, R.F. Woron, NY State Department of Health, August, 2002). The level of *C. perfringens* cells that appears to be necessary for disease is substantial (*e.g.* around 10^7 cells per gram of food); levels this high are nearly always associated with temperature abuse of foods (McClane, 1992).

Identification of *C. perfringens* foodborne illness outbreaks has traditionally relied upon symptom presentation, determination of incubation period, and implication of temperature-abused foods. However, this has not been an exact science, especially given the similarities of these criteria to those of other types of foodborne illness, *e.g.* those caused by *Bacillus* spp. (McClane, 2001).

Bacteriological criteria for demonstrating *C. perfringens* foodborne illness include either: (*i*) the presence of 10^5 *C. perfringens* spores gram^{-1} stool from two or more infected individuals and/or (*ii*) 10^5 *C. perfringens* cells gram^{-1} in implicated food (CDC, 2000). Detection of *C. perfringens* enterotoxin (CPE) in feces of multiple ill individuals is further recommended for confirmation of *C. perfringens* foodborne illness (CDC, 2000; FDA, 1992).

2.3. Clonal characteristics of *C. perfringens* from outbreaks

There has been limited investigation of the clonal relationships between isolates of *C. perfringens* taken from foods involved in outbreaks, and from patients in those outbreaks. Ridell *et al.* (1998) used pulsed field gel electrophoresis (PFGE) after DNA restriction to determine the clonality of 39 *C. perfringens* strains originating from 14 outbreaks where at least two isolates were available. For outbreaks with toxigenic *C. perfringens* isolated in feces:

- In three outbreaks where more than one isolate was taken per feces sample, the PFGE patterns were identical, suggesting monoclonality.
- In two outbreaks where more than one isolate was taken per feces sample, the PFGE patterns were similar (different by 1 or 2 bands), again suggesting monoclonality.
- However, in two outbreaks where more than one isolate was taken per feces sample, the PFGE patterns were different, providing evidence that more than one strain could be responsible for an outbreak.

For outbreaks where toxigenic *C. perfringens* was identified in foods, only one outbreak had two samples from the same food. PFGE patterns were not identical, but were very similar.

Miwa *et al.*, 1999 (Japan) studied a single outbreak and identified two *C. perfringens cpe*-positive[6] serotypes in the implicated food and in feces from patients. The two serotypes were found at different frequencies in the food and feces.

Lukinmaa *et al*. (2002) used PFGE after DNA restriction to compare genotypes of *C. perfringens* isolates from outbreaks. From six outbreaks where more than one isolate was taken from humans and found to be *cpe*-positive, five were found to have isolates with an identical intra-isolate PFGE patterns. In the one outbreak with two *cpe*-positive strains of differing PFGE patterns, one of the strains could not actually produce the toxin, suggesting that it may not have been involved in the outbreak (however *in vivo* animal tests were not done). Two outbreaks from foodstuffs where multiple *cpe*-positive isolates were taken demonstrated identical PFGE patterns.

In summary, these papers suggest that monoclonality is generally observed. When more than one *cpe*-positive strain was identified, the maximum number identified was two. However:

- The sample size of isolates is small and therefore other strains could be missed.
- Techniques used to isolate strains could create bias.
- Most of the information reviewed is from feces and not from foods. Selection within the host could therefore be a problem.

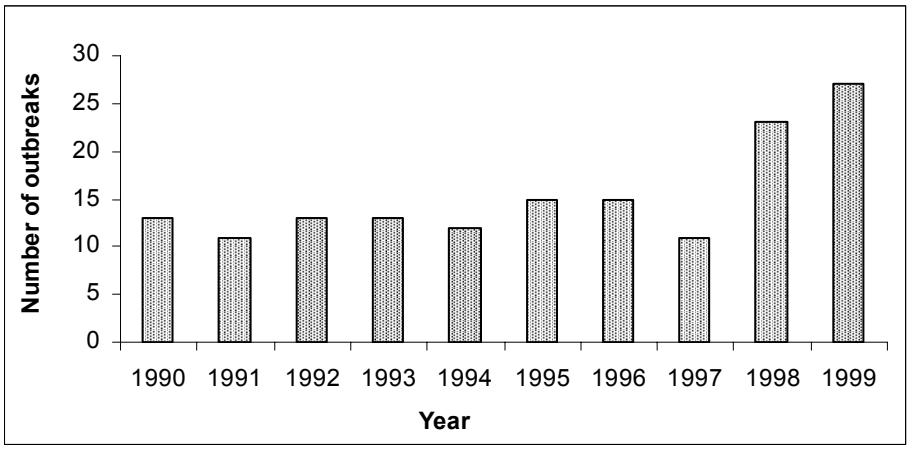
2.4. Outbreaks of *C. perfringens* foodborne illness

Data were obtained from: (*i*) the CDC, based on reports from 30 states (CDC, 2002), (*ii*) the outbreak report from the Center for Science in the Public Interest (DeWaal *et al*., 2001), and (*iii*) personal communications with state health departments. One hundred fifty-three *C. perfringens* outbreaks resulted in 9209 cases of illness in the U.S. between 1990 and 1999. The following is a summary of the data thus obtained.

The number of reported *C. perfringens* outbreaks from 1990 to 1999 is indicated in Figure 2.1.

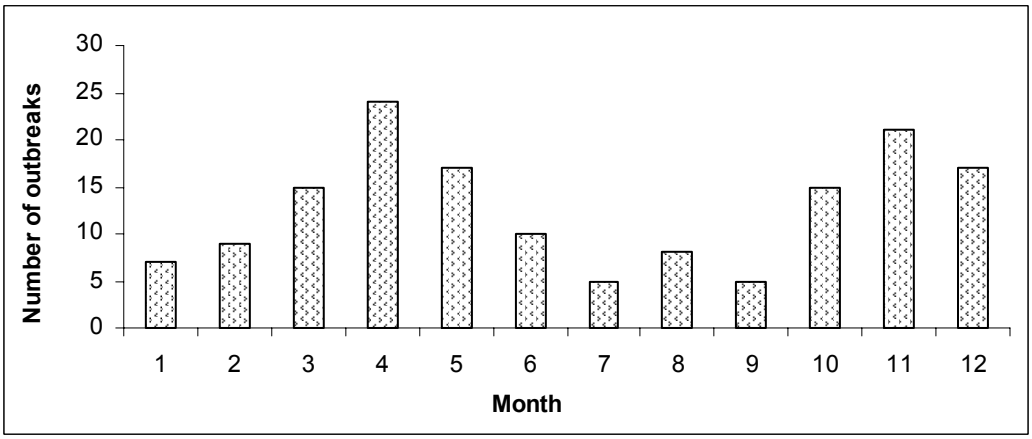
[6] CPE refers to the fully formed *C. perfringens* enterotoxin protein, *cpe* refers to the gene encoding CPE.

Figure 2.1 Temporal distribution (year) of *C. perfringens* outbreaks (1990-1999).



April and November have been peak months of reported *C. perfringens* outbreaks (Figure 2.2).

Figure 2.2 Temporal distribution (month) of *C. perfringens* outbreaks (1990-1999).



The highest number of reported outbreaks occurred in New York State, followed by Wisconsin, and Illinois (Figure 2.3) while the highest number of individual cases of *C. perfringens* foodborne illness occurred in Wisconsin, followed by Illinois and New York State (Figure 2.4). Note that these differences could be due to the differences in epidemiological investigation programs from state to state.

Figure 2.3 Geographical distribution (state) of *C. perfringens* outbreaks (1990-1999).

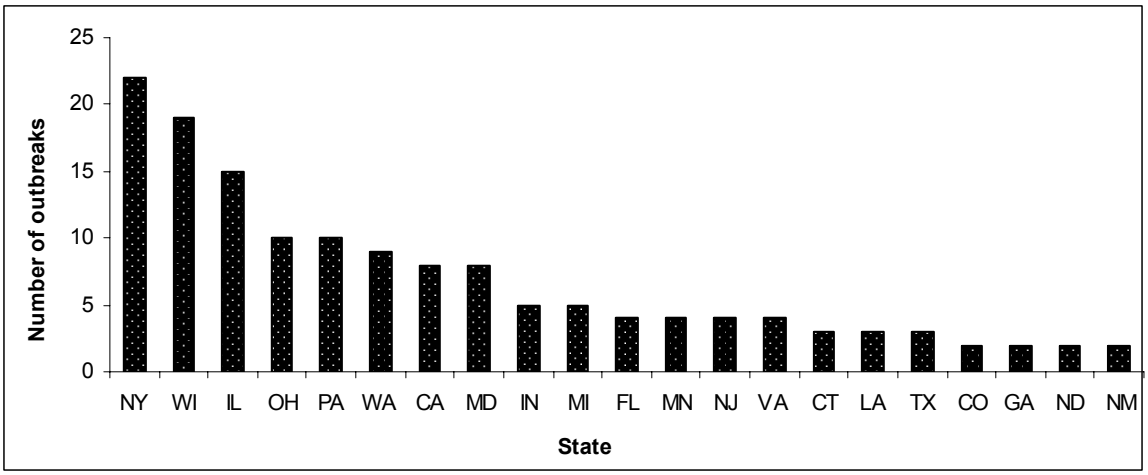
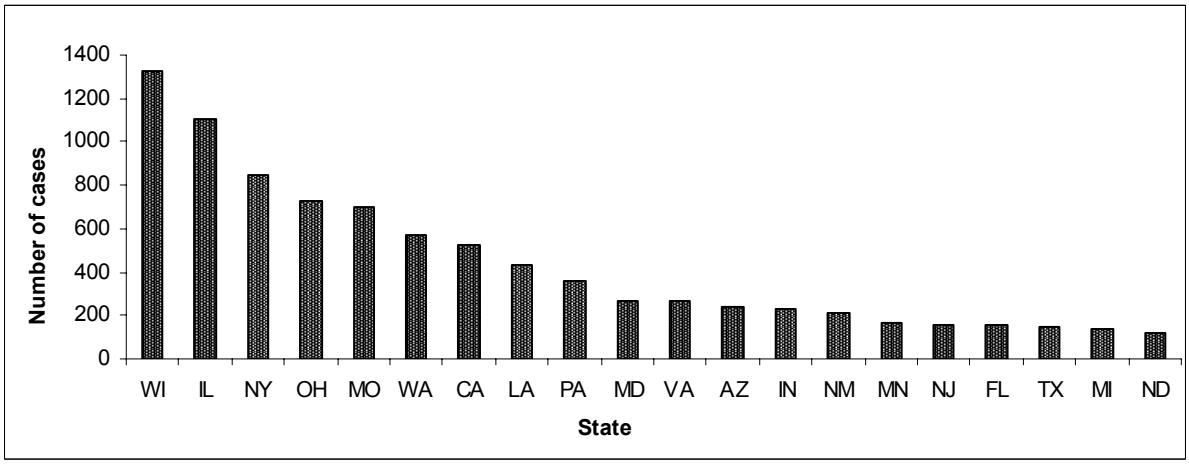
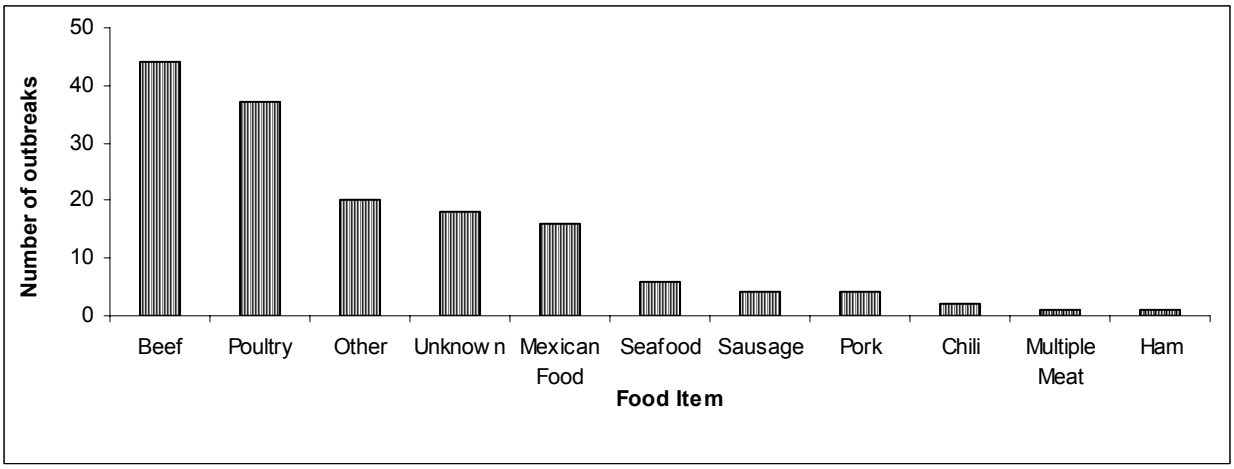


Figure 2.4 Geographical distribution (state) of *C. perfringens* cases (1990-1999).



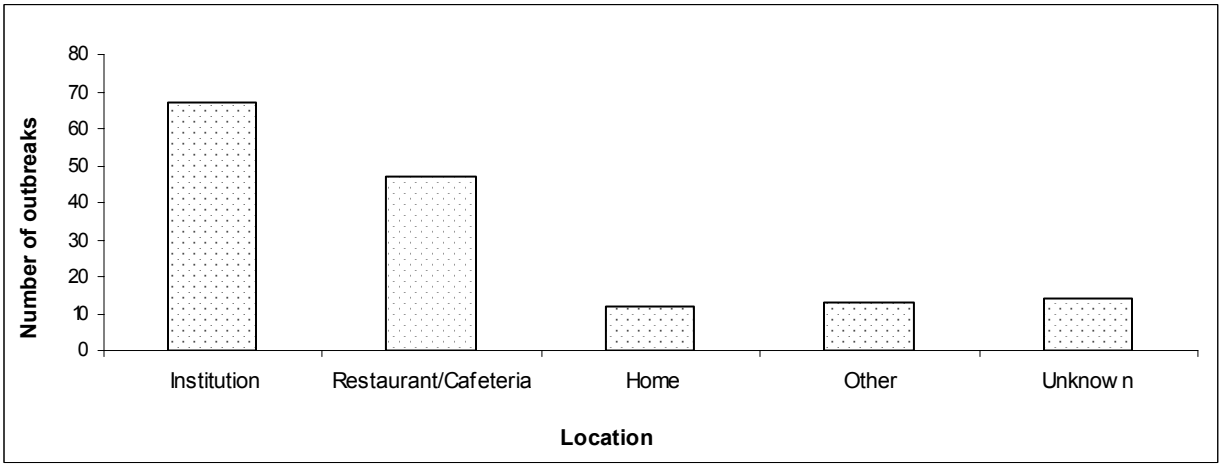
Forty four *C. perfringens* outbreaks (28.8%) were associated with consumption of foods containing beef, and 37 outbreaks (24.2%) were associated with poultry (Figure 2.5).

Figure 2.5 Distribution of food item for *C. perfringens* outbreaks (1990-1999).



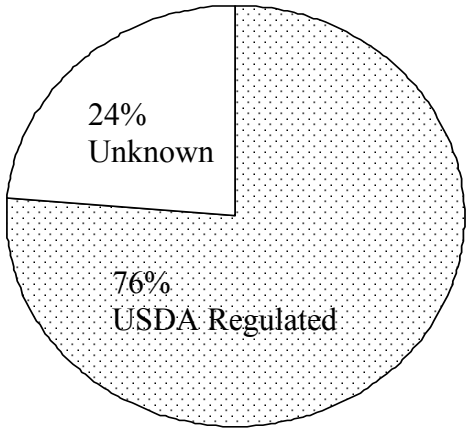
As shown in Figure 2.6, institutions (schools, hospitals, nursing homes, banquet halls, churches, and work sites) were the source of most (46.5%) *C. perfringens* outbreaks followed by restaurants/cafeterias (33.1%).

Figure 2.6 Location of *C. perfringens* outbreaks (1990-1999).



USDA-regulated food products were responsible for 76% of total *C. perfringens* outbreaks while 24 % of the food sources are unknown (Figure 2.7).

Figure 2.7 The proportion of USDA regulated foods associated with *C. perfringens* outbreaks (1990-1999).



Because of the relatively mild disease symptoms, public health authorities may not be made aware of outbreaks involving few people thus skewing the number of cases in any given outbreak observed toward higher numbers. Also, institutions frequently prepare large meals in advance, after which they are held and re-heated. Consequently, temperature abuse is more likely to occur in these settings, and thus it is not surprising that large *C. perfringens* outbreaks are often linked to institutional settings (McClane, 2001).

2.5. Clinical presentation

Persons suffering from *C. perfringens* type A food poisoning generally experience severe abdominal cramps and diarrhea; headache, vomiting, and fever may occur, but these symptoms are considered rare. Symptoms typically develop anywhere from 8 to 16 hours after ingestion of contaminated food, are self limiting and resolve sometime during the next 24 hours (McClane, 2001). In more severe cases intensive supportive therapy, including re-hydration, may be indicated. The relatively short duration of symptoms is thought attributable to two main factors: (*i*) diarrhea associated with the disease likely flushes most *C. perfringens* cells from the affected person's small intestine, and (*ii*) *C. perfringens* enterotoxin (CPE) preferentially binds to receptors in villus tip cells which, because they are the oldest intestinal cells, undergo rapid turnover in otherwise healthy individuals (Sherman *et al*., 1994).

Steps in the pathogenesis of *C. perfringens* type A food poisoning are as follows:

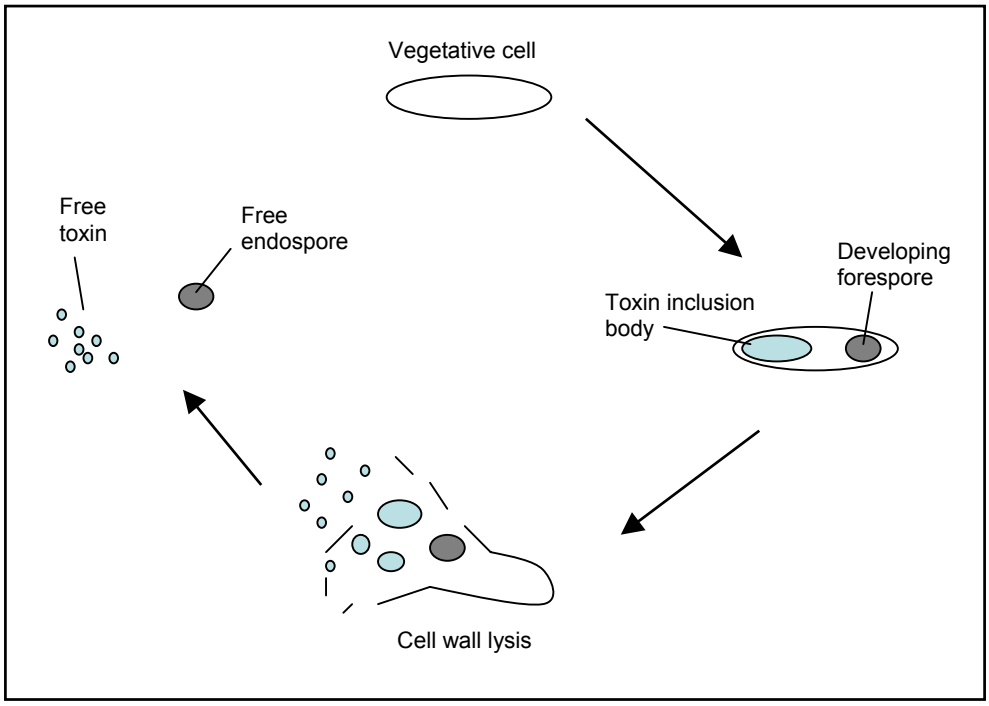
i. Vegetative cells actively multiply to a high level in food (*e.g.* $>10^7$ colony forming units (CFU) $gram^{-1}$ food).

ii. Vegetative cells are ingested during food consumption and sporulate in the small intestine.

iii. Sporulating cells synthesize CPE, which upon lysis of the mother cell is released into the intestine. (The events of bacterial sporulation are shown in Figure 2.8)

iv. CPE binds to toxin-specific receptors in the small intestinal lumen thereupon facilitating morphological damage and ultimately, abdominal cramps and diarrhea (McClane, 1992).

Figure 2.8 Simplified schematic of the bacterial sporulation process. Adapted from Boyd and Hoerl (1991).



3. Exposure assessment

3.1. Outline of the approach

The object of this exposure assessment is to evaluate the number of type A, *C. perfringens* enterotoxin (CPE) positive vegetative cells of *C. perfringens* that are eaten by consumers in RTE and partially cooked foods, the frequency with which such cells are eaten, and the changes in these quantities that would be made by changes in the regulations on allowable growth of *C. perfringens* during production of RTE and partially cooked foods. The exposure assessment is used with the hazard characterization to estimate the number of diarrheal illnesses that might result from the ingestion of such vegetative cells.

The exposure assessment starts with the servings of RTE and partially cooked foods that are eaten by individuals. RTE and partially cooked foods eaten in the U.S. have been identified in CSFII (1994-1996 and 1998) (USDA, 2000) as described in Section 3.4 and Appendix A. From CSFII, we also use the individual servings of those foods to represent the servings of RTE and partially cooked foods eaten in the U.S.

To estimate *C. perfringens* in RTE and partially cooked food servings that are eaten, the occurrence and concentrations[7] of *C. perfringens* spores and vegetative cells are tracked from the manufacturing plant to the consumer. Spores and vegetative cells of *C. perfringens* are present on raw meat[8] products entering food manufacturing plants, and on spices used in some foods; these are believed to be the principal sources of *C. perfringens* in RTE and partially cooked foods. Within the food manufacturing plants, cooking of RTE foods will kill the vegetative cells, but will activate the spores to germinate; whereas partial cooking may permit survival of a fraction of the original vegetative cells. Germinated spores and surviving vegetative cells will grow while the food is cooled after cooking until the food is cool enough to prevent such growth. It is primarily this cooling step after cooking that is the target of current regulations and possible changes in regulations.

Subsequent processing, storage, and transport steps will change the concentrations of any vegetative cells present in the foods to some extent, primarily due to cell growth at warmer temperatures and cell death at lower temperatures, and there may be slow germination of some remaining spores. Then consumer preparation of the food before it is eaten may also affect the concentrations of *C. perfringens* cells, again primarily through the temperature variations experienced by *C. perfringens* cells in the food.

To estimate how often and how many *C. perfringens* vegetative cells reach the consumer, we have to take account of the types of RTE and partially cooked foods eaten, the serving size, the frequency with which they are eaten, and the number of *C. perfringens* cells in each serving. Every serving of RTE or partially cooked food is likely to be different from the next one, and every such serving may be treated differently before finally being eaten, so we have to account

[7] The term "concentration" is used throughout this chapter to represent colony forming units (CFU) per milliliter (ml) or per gram (g).

[8] Throughout this document, "meat" generally means meat or poultry, except for specific cases that should be clear in context, *e.g.* where referring to an experiment on a specific meat.

for this variation between servings. Moreover, we are uncertain about many of the factors that are involved in the calculations, and need to keep track of how uncertain the results are.

To track both the variation between servings and the uncertainty, this assessment uses the probabilistic technique called Monte Carlo analysis. To evaluate the variation from serving to serving, a large number of individual servings are tracked from manufacturing plant to consumer, and the estimated number of *C. perfringens* cells eaten in each serving is recorded. At each point where a calculation is performed using some quantity that varies from serving to serving, the value used for that quantity is randomly selected from a variability distribution for that quantity. For example, the concentration of *C. perfringens* spores in raw meat varies from time to time and place to place, so the concentration of such spores in the raw meat that goes into any given serving will also vary. For each serving that is tracked through the calculations, a random selection is made of the concentration of *C. perfringens* spores in the raw meat from a pre-calculated representation of the distribution (the variability distribution) of such concentrations. As another example, each serving of RTE or partially cooked food differs in size and composition, so each such serving tracked through the calculations is selected at random from the servings of RTE and partially cooked foods recorded in CSFII and considered representative of what is eaten in the U.S. (an empirical variability distribution).

Recording how many *C. perfringens* cells are ingested in each serving tracked in the way described allows construction of a probability distribution that describes the variability of the number of such cells eaten per serving, and also, using the hazard characterization (dose-response relationship), the calculation of the probability for each tracked serving to cause diarrheal illnesses through the ingestion of *C. perfringens* cells. Adding these probabilities across all the tracked servings leads to an estimate of the total number per year of diarrheal illnesses caused in the U.S. by *C. perfringens* in RTE and partially cooked foods,[9] and the variation of this number with the allowed growth of *C. perfringens* during manufacturing processes, the primary desired end point of the assessment.

In addition, however, many of the calculations involve quantities about which there is considerable uncertainty. Continuing the example given, we only know the variability distribution of concentrations of *C. perfringens* in raw meat within a substantial uncertainty. The pre-calculated representation of the variability distribution of concentrations is itself uncertain, because of the limited number of observations upon which it is based; and similarly to a greater or lesser extent for many other of the important quantities used. In this risk assessment, the pre-calculated representations of variability distributions for such uncertain quantities are chosen to be mathematical distribution functions that are described by a limited set of parameter values; and the uncertainties in the quantities are represented by assigning uncertainty distributions to the parameters of those variability distributions.

To evaluate the effect of uncertainties, the whole procedure described for evaluating variability is repeated many times, each time selecting different estimates from the uncertainty distributions of

[9] This assessment examines only the effect of *C. perfringens* present in the raw materials for RTE and partially cooked foods. It is possible that there might be external contamination of some food servings, but that is not examined here. Such contamination would presumably not be affected by the amount of growth allowed during cooling and stabilization after initial cooking of foods, so is not a prime focus of the risk assessment.

the parameters of the variability distributions. For each set of (variability) parameter values, we obtain the variability distribution for the number of *C. perfringens* cells eaten and for the number of diarrheal illnesses in the U.S. each year. From the many such distributions, we build up an uncertainty distribution for the variability distributions (more accurately, for descriptors of the variability distributions) and for the numbers of illnesses in the U.S. each year.

Not all variability distributions are assigned uncertainty distributions and handled in this way. For example, for food servings we assume that the large number of observations is sufficient to reduce uncertainty to trivial levels; and indeed in this case the pre-calculated variability distribution itself is chosen to be the empirical observed distribution, and the same empirical distribution is used for all the uncertainty calculations.

Finally, for some parameters that are or may be important in the risk assessment, we do not have sufficient information to determine variability and/or uncertainty distributions with any reliability — if there are no experimental measurements of the quantity of interest, for example, or if the available measurements are not representative. In such cases we attempt to specify how variable or uncertain the quantity may be (by choosing probability distributions) based on the few available measurements or guesswork. The extent to which the risk assessment is compromised by these guesses is then evaluated by performing sensitivity analyses on the results — essentially by choosing alternative guesses and seeing how much the results are changed.

3.2. Principle steps in the assessment

The assessment proceeds by tracking RTE and partially cooked meat and poultry products through the following steps (see also Figure 3.1):

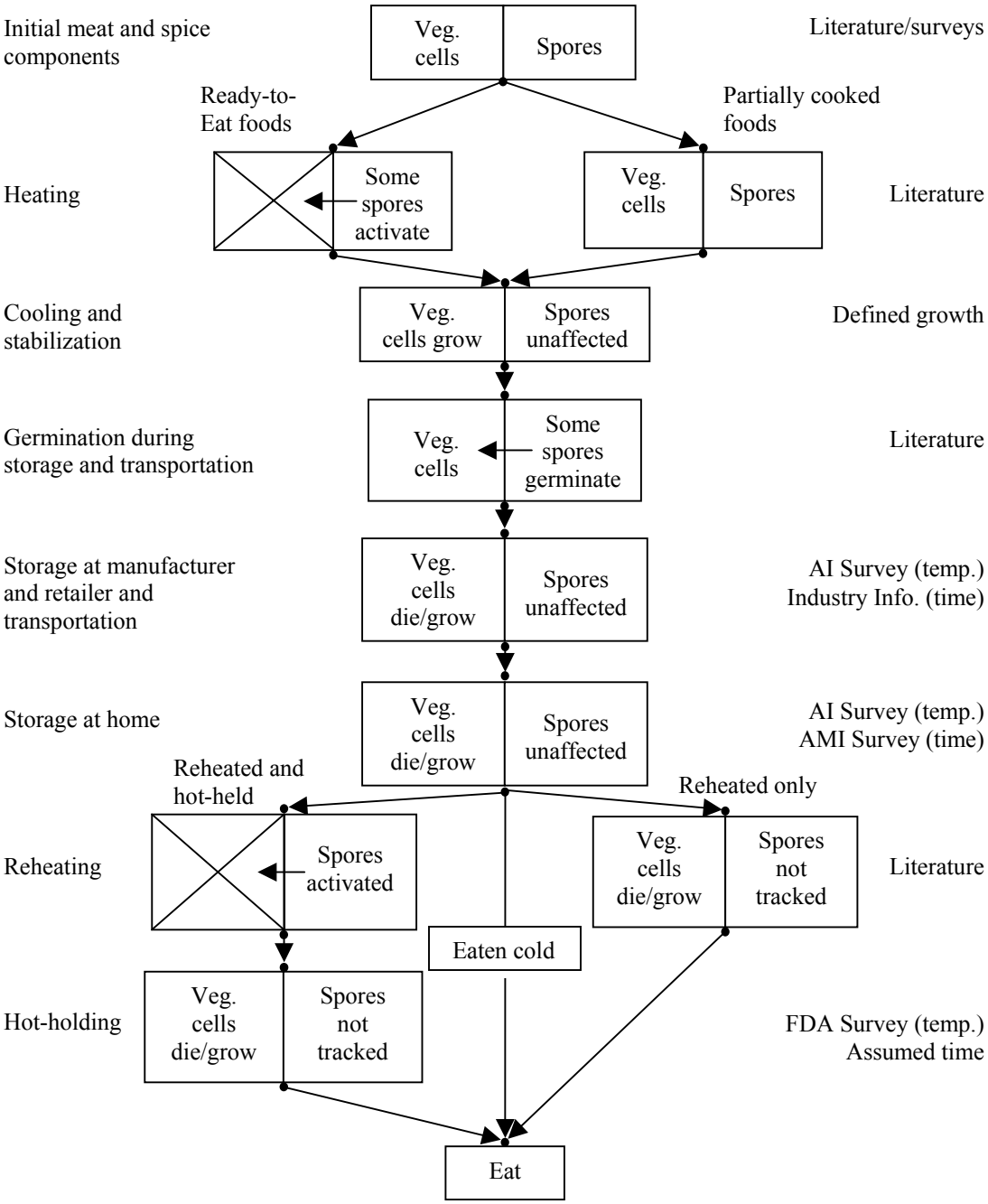
- *Processing (chilling and secondary cook steps and associated chilling)*. Fully or partially cooked foods are prepared from raw materials, cooked, then cooled and stabilized (possibly with more than one cooking and stabilization step). These processes are labeled "Heating" and "Cooling and stabilization" in Figure 3.1).
- *Transportation and storage*. The effect of storage times and temperatures for RTE and partially cooked commodities are taken into account through two stages of storage — between manufacturer and retail sale ("Storage at manufacturer and retailer and transportation" in Figure 3.1), and after retail sale and before consumption ("Storage at home" in Figure 3.1). Germination during transport and storage is assigned its own step ("Germination during storage and transportation" in Figure 3.1).
- *Preparation (reheating).* The effect of preparation of RTE and partially cooked commodities prior to consumption is examined ("Reheating" in Figure 3.1). Some foods are eaten re-heated for hot-holding ("Reheated and hot-held" in Figure 3.1), some are eaten cold ("Eaten cold" in Figure 3.1), and some are reheated for immediate eating ("Reheated only" in Figure 3.1).
- *Hot-holding.* The effect of holding some foods at elevated temperatures for extended periods is included ("Hot-holding" in Figure 3.1.

Figure 3.1 illustrates the above steps, showing where vegetative cells and spores are tracked in the model, and where spores may germinate to contribute to vegetative cell counts. In Figure 3.1 titles to the left refer to steps in the model; titles to the right refer to the source of data for

parameters in that step. For each pair of boxes the left side describes what happens to vegetative cells, and the right side describes what happens to spores. Horizontal arrows indicate the activation and germination of spores into vegetative cells. An X-ed out box indicates complete killing of vegetative cells present before that step, but not the killing of those vegetative cells produced from spores within that step (complete killing of all pre-existing cells is assumed in the initial processing lethality step, in the heating that precedes hot-holding, but not necessarily in consumer cooking procedures).

Figure 3.1 identifies manufacturing, retail, and home explicitly. However, these labels are for convenience only, and are intended as generic indicators of the movement of all food servings; they are not meant to exclude, for example, the food service sector. Initial consideration was given to treating food service operations separately, since additional food preparation steps (including further cooling, cold storage, and heating steps) might be involved in such operations, but there were insufficient data to allow distinguishing this sector from retail and home in the risk assessment.

Figure 3.1 Flow chart for modeling survival/growth of *C. perfringens* in RTE and partially cooked meat and poultry products (see text for explanation).



AI survey: Audits International/FDA (1999).
AMI survey: American Meat Institute (2001).
FDA survey: FDA (2000).

The calculations performed in the assessment for each serving can be summarized as:

- Obtain the numbers n_v, and n_s present immediately after initial processing (and before chilling, stabilization, and any secondary cooking steps) in the serving of, respectively, type A, CPE-positive vegetative cells, and type A, CPE-positive spores that could germinate during storage or preparation.

$$n_v = P\left(wC_m f_m f_{vmA}\right) + \sum_j P\left(wC_{sj} f_{sj} f_{vsA}\right)$$
$$n_s = P\left(wc_m f_m f_{smA}\right) + \sum_j P\left(wc_{sj} f_{sj} f_{ssA}\right) \quad \textbf{(3.1)}$$

where $P(z)$ denotes a Poisson sample with expected value z, and the inputs to the calculation are:

w mass of the food serving (Section 3.4),

C_m the concentration of *C. perfringens* vegetative cells in the meat product constituent of the serving immediately after initial processing (Section 3.5 for RTE products, Section 3.7 for partially cooked products),

f_m fraction of the serving weight that is meat product (Section 3.4),

f_{vmA} fraction of *C. perfringens* vegetative cells present immediately after the initial lethality step and originating in the meat product constituent that are type A, CPE-positive (Section 3.10),

j an index indicating a specific spice constituent (in the implementation, the index j is an integer in the range 0 to 3 inclusive),

C_{sj} concentration of vegetative cells or germinating spores in the spice constituent indexed by j of the serving immediately after initial processing (Section 3.8),

f_{sj} fraction of the serving weight that is the spice indexed by j (Section 3.4),

f_{vsA} fraction of *C. perfringens* vegetative cells or germinating spores present immediately after the initial lethality step and originating in spices that are type A, CPE-positive (Section 3.10),

c_m concentration of spores in the meat constituent of the serving immediately after the initial processing step (Section 3.6),

f_{smA} fraction of *C. perfringens* spores contributed by meat constituents and germinating during storage and transport or preparation that are type A, CPE-positive (Section 3.10),

c_{sj} concentration of spores in the spice constituent indexed by j of the serving after the initial processing step (Section 3.8), and

f_{ssA} fraction of *C. perfringens* vegetative cells germinating during storage and transport or preparation from spores contributed by spices that are type A, CPE-positive (Section 3.10).

If it were possible to distinguish the fractions of type A, CPE-positive spores that might germinate during storage from the fraction that might germinate during preparation, a more complex approach would have to be adopted that took account of that distinction. However, no such distinction is currently possible (Section 3.10).

- Estimate the number of type A, CPE-positive, spores n_g in this serving that germinate during storage; and, if this serving is hot-held, the number n_p that subsequently germinate during preparation:

$$n_g = B\left(n_s, g_s\right)$$
$$n_p = B\left(\left\lfloor\left(n_s - n_g\right)l_s\right\rceil, g_p\right) \quad \textbf{(3.2)}$$

where $B(m,z)$ represents a binomial sample with probability z from a sample of size m, the [] symbol indicates the nearest integer function, and the further inputs to the calculation are:

g_s fraction of spores that germinate during storage and transport (Section 3.13.1),
l_s lethality factor for spores during storage and transport (Section 3.13.2.3), and
g_p fraction of spores that germinate during preparation (Section 3.14.3).

- Estimate the number of vegetative cells at the time of eating of the serving as:

$$N = \left\lfloor\left(\left\lfloor\left(\left\lfloor n_v G_c\right\rfloor + n_g\right)G_s\right\rceil L_p + n_p\right)G_h\right\rceil \quad \textbf{(3.3)}$$

where $\lfloor\ \rfloor$ indicates the floor function (next integer less than), [ ] indicates the nearest integer function, the output is:

N the calculated number of *C. perfringens* type A, CPE-positive vegetative cells present in the serving at the time it is eaten,

and the further inputs to the calculation are:

G_c growth factor for vegetative cell growth induced by the initial stabilization (cooling) regime (and by any other heating and cooling steps in initial processing) (Section 3.12),
G_s growth or survival factor for vegetative cells occurring during storage and transport (Section 3.13.2),
L_p lethality factor for vegetative cells occurring during preparation[10] (Section 3.14.1),
G_h growth factor for vegetative cells during hot-holding (Section 3.14.5).

Not all these calculations are necessary for all servings, depending on the type of serving (see Section 3.4) and on the results of earlier calculations (for example, if at any time the serving has no vegetative cells or spores, no further calculations are performed).

There are several approximations made in this calculation. In particular, there can only be an integer number of cells in a serving at any time, but growth and death processes are treated here as though the number of cells is not limited to be integral. After any modeled growth or death process, the number of cells is forced to be an integer by finding the next integer below or the nearest integer to the calculated value (the $\lfloor\ \rfloor$ and [ ] symbols in the above equations). These approximations are made in such a way as to have minimal effect on the calculated number of illnesses.[11]

The Monte Carlo procedure can then be described as:

Repeat some number of times {
(*This loop evaluates the effect of uncertainties*)

[10] The lethality factor L_p is always zero for hot-held foods — it is assumed that re-heating before hot-holding is sufficient to kill all vegetative cells and activate spores.

[11] In an exact calculation, the effect of the limitation to integers is negligible if there are a large number (more than a few thousand) of cells present in the serving, and it is only such cases that give rise to illness.

- Choose a sample from the uncertainty distribution describing each of the inputs[12] used in Equations (3.1) through (3.3) for *N*, taking account of any correlations.

Repeat a large number of times {

(*This loop evaluates the effect of variation between servings*)

- Select an RTE or partially cooked food serving from the CSFII database (USDA, 2000).
- Choose a sample from the variability distribution(s) describing each of the inputs on the right hand side of Equations (3.1) through (3.3) for *N*, conditional on the type of food in the serving and (if necessary) on the values already obtained from the uncertainty distributions, and taking account of any correlations.
- Calculate the corresponding sample value for each of the inputs in Equations (3.1) through (3.3) for *N* using the uncertainty and variability sample values.
- Calculate *N* from Equations (3.1) through (3.3) using those sample values and (optionally) store the calculated value.
- Sample from the variability distribution for the dose-response curve, calculate the probability for this number of *C. perfringens* to cause diarrhea using the dose-response curve, and randomly with that probability decide whether the serving would have caused diarrhea. Store the result.

} (end of the variability loop)

- (Optionally) From the stored values, construct the variability distributions for the number of cells.
- Calculate the number of diarrheas caused, and (optionally) any desired population averages from the stored variability distributions.
- Store any desired details about the variability distribution (for example, store a set of percentiles of the distribution, and the averages).

} (end of uncertainty loop)

- From the stored numbers of diarrheas and the variability distributions for numbers of cells, construct uncertainty distribution (for example, construct the uncertainty percentiles for the number of diarrheas and for each stored variability percentile)
- (Optionally) Calculate averages over the uncertainty distributions.
- Print out the results in a convenient way and interpret them.

Some of the calculations can be omitted — in particular, if the initial number of *C. perfringens* cells and spores in a serving is zero, there is no need to perform any further calculations, because in this model we assume no external contamination with *C. perfringens*.

The number of times a loop is repeated depends on what information is required, and the numerical precision[13] required of the calculations. The uncertainty loop may be performed only

[12] Some of the inputs to Equations (3.1) through (3.3), such as the growth and lethality factors, are themselves calculated quantities. In such cases, the procedure is to sample from the relevant distributions for all the inputs going into such subsidiary calculations in order to obtain a new value to uses in Equations (3.1) through (3.3).

[13] Numerical precision is that due to the limited number of times the calculations in a Monte Carlo analysis are performed. For example, in calculating the number of diarrheas we simulate a large number of servings (tens to hundreds of millions) in the variability loop, but only a few servings in a million may be calculated to cause diarrhea, so the total number of diarrheas estimated to occur may be only tens to hundreds. Repeating the same number of calculations with different random numbers would give different estimates of the number of diarrheas

once if it is desired to obtain only information on the variability — for example, the effect on the number of diarrheas expected from variations in the growth allowed during stabilization. The variability loop needs to be repeated often enough to obtain results to the precision desired. For example, to obtain the distribution of the number of *C. perfringens* cells in servings, simulation of a few million servings is sufficient to obtain numerically stable estimates. To obtain the expected number of diarrheas with high numerical precision, a larger number of servings have to be simulated (about 100 million to 1 billion gives adequate numerical stability).

3.3. General approach to deriving variability and uncertainty distributions

The following sections describe in detail how values for each input quantity in Equations (3.1) through (3.3) for *N* have been estimated. Highly technical details are placed in appendices. However, there is a common theme to all the sections. In each case, we evaluate the available observations that shed light on the quantities that are to be estimated, and select those observations that we consider representative for this risk assessment, or (in some cases) detail what information is entirely lacking.

When the data are sufficient to warrant a detailed approach, we present a mathematical model that can represent the variability distribution for the quantity, and, where possible, the evidence available to substantiate that mathematical model, and perform a formal synthesis ("meta-analysis") of experimental data presented in the published literature. As examples, the concentrations of *C. perfringens* cells and spores in meat products used in RTE and partially cooked foods are assumed to be gamma distributed, whereas the probability for spores or vegetative cells of *C. perfringens* to be type A, CPE-positive is a constant for the purposes of this risk assessment.

Using the selected observations, we fit the mathematical model for variability to obtain estimates for the parameters of that model. The fitting method of choice is to write the likelihood function for the observations conditional on the model, and the best estimates for the parameters of the variability models are then the maximum likelihood estimators.

The uncertainty for the estimated parameters is represented by the likelihood function, treated as a function of those parameters, and our intent is to use the likelihood directly for this purpose. In most cases we do this by selecting transforms of the parameters (often powers of the parameters, occasionally logarithms, or some combination or compounding of such transforms) in such a way that the profile likelihood of the transformed parameters are approximately normal.[14] The

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(technically, in a way described by a Poisson process). This variation from run to run with different random numbers represents the numerical precision. The numerical precision is thus related to the number of Monte Carlo iterations, and has no fundamental importance — it gives no information about the real uncertainties associated with the number being estimated. Numerical precision can be increased by increasing the number of Monte Carlo iterations, at the cost of increased computer time. Doubling the numerical precision requires increasing the number of iterations approximately four-fold; reducing it ten-fold requires a hundred-fold increase in the number of iterations; and generally reducing it by a factor k requires approximately k^2 as many iterations.

[14] We proceeded by plotting the profile likelihood as a function of the transformed parameter value, with the transform parameterized in some way (*e.g.* by the value of a power law). We computed the correlation coefficient between the square root of the logarithm of profile likelihood deviation from the maximum likelihood and the transformed parameter value, and maximized (or minimized, for negative correlations) this correlation coefficient with respect to the chosen transform parameters. Since this procedure is approximate, and since such correlation coefficients were always very slow functions of the transform, we rounded the transform parameter to a convenient

likelihood function is then approximated using a multinormal distribution in the transformed variables, using a numerical approximation of the information matrix. This numerical approximation was obtained with difference estimates to partial derivatives, with step sizes approximately equal to the standard deviation of the marginal distributions, ensuring that correlations present out to such deviations were reasonably well approximated. We present the results of the analyses in the text by providing the maximum likelihood estimates for the transformed parameters, and a matrix that gives the standard deviations (along the main diagonal of the matrix) and correlation coefficients (in the lower left sub-diagonal of the matrix) between the transformed parameters.

This approach is somewhat unconventional, although it uses standard statistical tools. The approximation of the likelihood by multinormals in suitably transformed variables captures the essential details of correlations between parameter estimates, and makes maximum use of the (often very limited) observations. There is an implicit reliance on asymptotic normality of likelihood functions for accurate estimation of percentage points of distributions, and more accurate estimates might be possible using, for example, bootstrap calculations. However, we believe that the advantages outweigh the disadvantages.

Most of the values used in the Monte Carlo simulation were obtained by this methodology, including:

- the concentrations of vegetative cells and spores of *C. perfringens* to be expected in raw meat and spices, and the variation in such concentrations found from sample to sample,
- the fraction of vegetative cells and spores of *C. perfringens* that are of type A and positive for the CPE toxin,
- growth rates of *C. perfringens* from spores and as vegetative cells, and how these growth rates vary with temperature, from strain to strain, and in different circumstances (*e.g.* with salt and nitrite concentration),
- survival rates of vegetative cells during cold storage, and how these vary from strain to strain,
- death rates of vegetative cells at high temperatures, and how these vary from strain to strain, and
- how the relationship between number of vegetative cells consumed and the probability of illness (the dose-response function) varies from strain to strain of *C. perfringens*.

For other required inputs, insufficient information was available in the literature to perform a meta-analysis. In these cases estimates are made by whatever approach seemed reasonable, including guesswork, and the effect of variation of these estimates evaluated. Some of the inputs treated in this way are:

- the fraction of spores that germinate under various conditions (*e.g.* during RTE preparation, and during cold storage and transport),
- storage times between manufacturer and retailer,
- the fractions of foods eaten cold, oven heated, and microwaved,
- the fraction of foods held hot after preparation, and the time for which they are hot-held, and

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choice. It was generally straightforward to obtain correlation coefficients of absolute value higher than 0.998 over a range of profile likelihood corresponding to two or three standard deviations from the maximum likelihood.

- the maximum density of vegetative cells that can grow in any particular food.

A third type of source of inputs was surveys that are treated as representative of what happens to RTE and partially cooked foods, even though such surveys were not originally designed to obtain representative samples for this purpose. Such inputs include:

- temperatures achieved during storage of RTE and partially cooked foods,
- how long RTE and partially cooked foods may be stored at home before consumption, and
- cooking temperatures.

3.4. Selection and identification of servings, treatment in this assessment, and evaluation of w, f_m, and f_{sj}

Appendix A describes how four categories of foods were identified for modeling, and how servings were selected from the CSFII database (USDA, 2000) for inclusion in the risk assessment. In short, using the recipe and ingredient databases of the CSFII, a list of foods that contained meat or poultry was constructed. From this list all raw foods were removed (since the proposed rule affects only RTE and partially cooked foods), and also removed were those foods with characteristics or ingredients that can be expected to inhibit the growth of *C. perfringens* or that are otherwise unlikely to cause human illness from *C. perfringens*. Food characteristics that make commodities unlikely to cause human illness from *C. perfringens* include those that are: (1) processed in a way that result in shelf stable products, such as dried meats and foods sold in cans and jars; (2) very high in salt (sodium chloride) content (>8%); or (3) moderately high salt content (3-8%) in combination with nitrites. Foods were then placed in categories with characteristics that were considered to be most relevant, these categories being:

1) foods containing nitrites with between 2.2% and 3% salt,
2) foods unlikely to be reheated prior to consumption,
3) foods likely to be reheated immediately prior to consumption, and
4) foods reheated prior to consumption but not necessarily immediately before consumption ("hot-held").

For the purposes of exposure and risk assessment the four food categories were further separated according to likely characteristics relevant for estimation of numbers of *C. perfringens* vegetative cells in the food as eaten, using example foods as a guide. This further separation is indicated in Table 3.1, and a full list of foods modeled (and also those omitted from modeling, together with the reasons as described in Appendix A) is given in Appendix B. All servings meeting the inclusion criteria were categorized according to Table 3.1, and are used in the risk assessment.

Table 3.1 RTE and partially cooked foods that could support the growth of *C. perfringens*.

Food Category		Examples	Characteristics	Reasoning
1 Foods likely to be reheated before consumption	**a**	Hot dogs (franks) exclusively	- 2.2-3% salt in the presence of nitrite - Frequently eaten reheated/ may be hot-held	Hot dogs are the most highly consumed commodity in this group, with information on the fraction eaten cold.
	b	Ham, sausage		

2 Foods unlikely to be reheated before consumption		Cold sliced turkey sandwich	-Unlikely to be heated prior to consumption	Poultry luncheon meat is the only RTE food confirmed as a food vehicle in a *C. perfringens* outbreak since 1992.
3 Foods expected to be reheated for immediate consumption	**a**	Chicken or turkey with BBQ sauce	- Likely to be reheated for immediate consumption - Likely to be sold as a frozen product - Contains an acidic sauce	These products are semi-homogenous mixtures with an acidic sauce.
	b	Chicken patty	- Likely to be reheated for immediate consumption - Likely to be sold as a frozen product - Partially cooked	This is the only partially cooked product identified in the CSFII listings (USDA, 2000).
	c	Beef and cheese enchilada	- Likely to be reheated for immediate consumption - Likely to be sold as a frozen product - Contains added spices	Mexican style foods (not necessarily RTE) have been implicated as the 4th most common vehicle for foodborne outbreaks of *C. perfringens*.
	d	Frozen chicken meal	- Likely to be reheated for immediate consumption - Likely to be sold as a frozen product	These products are quick frozen at a neutral pH and high water activity without the added antimicrobials such as nitrites.
4 ‡ Foods expected to be reheated and may potentially be hot-held prior to consumption	**a**	Pork BBQ or Sloppy Joe sandwich	- Likely to be reheated prior to consumption - Likely to be sold as a frozen product - May be hot-held - Contains an acidic sauce	These products are semi-homogenous mixtures with an acidic sauce.
	c	Taco meat	- Likely to be reheated prior to consumption - Likely to be sold as a frozen product - May be hot-held - Contains added spices	Mexican style foods (not necessarily RTE) have been implicated as the 4th most common vehicle for foodborne outbreaks of *C. perfringens*.
	d	Beef with gravy	- Likely to be reheated prior to consumption - Likely to be sold as a frozen product - May be hot-held	Beef with gravy is the most commonly implicated food in *C. perfringens* outbreaks when hot-held.

‡ Originally a Category 4b was defined, but was not required for this assessment. The numbering was retained to agree with previously constructed data files.

Foods in Category 1 are likely to be reheated shortly prior to consumption. This may kill *C. perfringens* vegetative cells, should these foods be contaminated. This second heat step may also

induce germination of spores and subsequent growth if the foods are maintained at non-lethal but elevated temperatures for a long period prior to consumption, as may occur in hot-holding. Foods in Category 2 are unlikely to be reheated prior to consumption. This means that any *C. perfringens* vegetative cells that are present will be consumed but also that there will be no induced germination of spores. Category 3 foods are expected to be reheated for immediate consumption and therefore would not be hot-held. Re-heating is likely to kill any *C. perfringens* vegetative cells that are present, although the probability for survival depends on the temperature and time of re-heating. *C. perfringens* spores present may also germinate, but because these foods are consumed immediately, no growth is expected or modeled. Foods in Category 4 are expected to be reheated and may potentially be hot-held prior to consumption. Consequently, vegetative cells are likely to be killed, and it is assumed in this risk assessment that reheating prior to hot-holding kills all vegetative cells present; however, any spores that germinate during the heating may have the opportunity to multiply during hot-holding.

For the 607 foods identified in the CSFII database (USDA, 2000) as potentially RTE or partially cooked, there are 26,548 servings listed, together with weights inversely proportional to the probability for the person eating that serving to have been chosen in the CSFII.[15] These 26,548 servings are assumed to be representative of RTE and partially cooked food consumed in the U.S., and were sampled with the given weights (in inverse probability to their inclusion in the database). Each serving so selected was characterized by category as shown in Table 3.1, and subsequent calculations used parameter values appropriate for that category.

In addition to its identity, each serving from the CSFII provides further information used in this risk assessment, as indicated by Equation (3.3). In particular, we obtain from the database information:

w mass of the serving,
f_m meat constituent fraction of the serving (see Appendix D),
f_{sj} fraction of the serving that is the "spice" indexed by j.

Each numbered spice (actually a composite of spices, see Section 3.8 for details) is considered separately with respect to its concentration of *C. perfringens* spores, but the properties of those spores are then assumed to be independent of the spice. One further parameter characterizing each serving is obtained, but used only indirectly — the salt content (calculated from the estimated sodium content of the serving in the CSFII database, assuming all sodium is from sodium chloride). This parameter is used to modify growth rate estimates (see Section 3.11.5.2).

3.5. Vegetative cell concentration in heat treated meat — C_m for RTE foods

The majority of food servings selected from the CSFII (USDA, 2000) for this analysis are RTE foods, and the vegetative cell concentration in heat treated meat represents a primary source of *C. perfringens* for such foods. These vegetative cells come from *C. perfringens* spores present in the raw meat; the lethality step applied during manufacture of RTE foods kills all vegetative cells of *C. perfringens* present, and activates some fraction of the spores to germinate to vegetative cells. An extensive analysis was thus applied to the estimate for this post-lethality vegetative cell

[15] All available servings were used as independent samples, using the one-day weights.

concentration (Sections 3.5.1 through 3.5.5), and the results subsequently are also used to estimate the pre-lethality spore concentration present in raw meat (in Section 3.6).[16]

3.5.1. Selection of studies

Raw meat destined to become an RTE commodity undergoes a heat treatment at the manufacturing plant that is intended to kill all vegetative *C. perfringens* cells initially on or in the meat. However, spores in the raw commodities may be stimulated to germinate upon heating. Spores therefore, serve as a source of *C. perfringens* vegetative cells in RTE commodities after heat treatment.

The fraction of *C. perfringens* spores that germinate after heat treatment, and ultimately contaminate the RTE product, depends on such factors as the time-temperature profile of the heat treatment, the strain of *C. perfringens*, the particular physical and chemical milieu provided by the food matrix, and the history of the spores. All such factors (and any others that affect germination) can be expected to vary among commodities and manufacturing plants. Some of these factors are further evaluated below.

Six studies were located and evaluated for information on the expected prevalence and levels of *C. perfringens* vegetative cells in beef, pork, and poultry products following a heat treatment (Table 3.2). The criteria used to evaluate the relevance of each study to estimate the number of *C. perfringens* vegetative cells in heat treated meats are given in the table headings.

Data from the Greenberg *et al*. (1966), Hall and Angelotti (1965), and the USDA/FSIS (1992–1996) studies could not be reliably used for subsequent quantitative modeling. The reasons for this are as follows:

1. Greenberg *et al*. (1966) was an evaluation of total putrefactive anaerobic spore-formers, not specifically of *C. perfringens*. It was examined to evaluate whether it could provide an upper bound on the number of *C. perfringens* cells that might be present after a heat treatment. However, while the heat treatment used would probably have destroyed vegetative cells, it was probably too mild compared with typical cooking procedures to represent the activation of such *C. perfringens* spores during cooking. Nevertheless, the data obtained were used qualitatively as described below (Section 3.5.2).
2. Hall and Angelotti did not enumerate *C. perfringens* in samples found to be positive. Thus, the number of cells (*i.e.*, the vegetative cell concentration) was not known.
3. The USDA/FSIS (1992–1996) baseline survey did not confirm presumptive *C. perfringens* colony counts and did not distinguish between vegetative cell and spores by including a heat step in the analysis method. Moreover, the whole meat samples measured surface concentrations on surface samples of raw meat (not concentrations in the whole volume of meat). Therefore, these data could not be used for determining the number of *C. perfringens* cells in meat following heat treatment.

[16] The analyses reported in this section are performed in the workbook CP_count_RTE_meat.xls included with the risk assessment.

Table 3.2 *C. perfringens* in meat products.

Reference	Season samples collected	Region	Lethality step[a]	Presumptive CP[b] colony confirmation	Products evaluated	Results[b]
Kalinowski *et al.*, 2003	Jan., Feb., Mar., May, June 2000	USA Turkey: AR, MO, and CO. Ground pork: IL. Pork sausage: KS.	Heated to 73.9 °C	Yes[f]	Post lethality beef, pork, turkey	1% (2/197) samples with >0.5-2-$\log_{10}$ CP spores/g. 0/197 samples with >2-$\log_{10}$ CP CFU/g
Taormina *et al*., 2003	Aug. 2001-June 2002	Four Midwestern facilities	75 °C for 15 mins	No	Post lethality beef, pork, chicken	2.5% (11/445) samples with 1.62-$\log_{10}$ CP spores/g
Hall and Angelotti, 1965	unknown	OH, USA	No	Yes[e]	Raw beef, veal, lamb, pork, chicken	58% (93/161) samples contaminated with CP
			Yes[c]	Yes[e]	Processed meats and meat dishes not requiring cooking[d]	4.7% (2/42) samples contaminated with CP
Nationwide Microbiological Baseline Data Collection Program, USDA/FSIS, 1992–1996	Varied between surveys	Nationwide	No	No	Raw surface samples from steers, heifers, cows, bulls, market hogs; and samples of ground beef, ground chicken, and ground turkey	Cows & bulls: 8.4% positive. Steers & Heifers: 2.6% positive. Market hogs: 10.4% positive. Ground beef: 53.5% prevalence. Ground chicken: 50.6% prevalence. Ground turkey: 28.1% prevalence
Greenberg *et al*., 1966	Year round	Seven regions of N. America	60 °C for 15 min.	No; evaluated all putrefactive anaerobic spore-formers, not specific to *C. perfringens*	Post lethality beef, pork, chicken	Mean of 2.8 putrefactive anaerobic spores/g, with variation by product and season. Maximum 115 spores/g.
FSIS, 2003	Sept. 27–Nov. 17, 2003	48 states and Puerto Rico	75 °C for 20 min.	Yes[f]	Ground beef samples from 546 processing plants	2/593 samples with 1 colony at the detection limit of 3 CFU/g.

a. A lethality step would be expected to distinguish spores from vegetative cells by heat killing cells and simultaneously heat activating spores to germinate.
b. CP: *C. perfringens*.
c. Foods sampled were described as "not requiring cooking," suggesting a lethality step at manufacturing plant
d. Foods include sliced sandwich meats, sandwich fillings, cocktail sausage, and dried cured beef.
e. Isolates were confirmed *C. perfringens* following analysis by sulfadiazine-polymyxin-sulfite (SPS) agar, indole-nitrite medium, and lecithinase production.
f. See text, Section 3.5.3.

3.5.2. Preliminary analysis of distribution of concentrations

The study of Greenberg *et al.* (1966) was examined for qualitative evidence about the likely shape of the distribution of post heat treatment vegetative cells of *C. perfringens*, since this study was the largest and most sensitive of those examined (each sample corresponded to a 3 gram sample of meat), and *C. perfringens* cells presumably made up some fraction of the putrefactive anaerobic spore-formers observed. Greenberg *et al*. published a graphical distribution of observed CFU/gram estimates versus the numbers of samples. That graph could be read to obtain approximate numbers of samples with given numbers of observed colonies after incubation of the sample; and such estimates were supplemented with information from the text for the upper end of the distribution. The observed shape of the distribution at its upper end appeared consistent with that expected from a gamma distribution for the concentration of spores in the meat, an observation that was confirmed by fitting[17] such a distribution (Figure 3.2; see Appendix 3.1 for the methodology, and workbook CP_count_RTE_meat.xls for calculations).

This gamma shape of distribution was used for analysis of the selected studies (below), since there were too few data in the selected studies to allow discrimination as to the distribution shape.

[17] The concentration distribution fit in Figure 3.2 is the sum of two gamma distributions, the first of which corresponds essentially to a constant concentration of 2.17 CFU/g. The scale parameter of the gamma distribution fitting the upper tail is about 5 CFU/g.

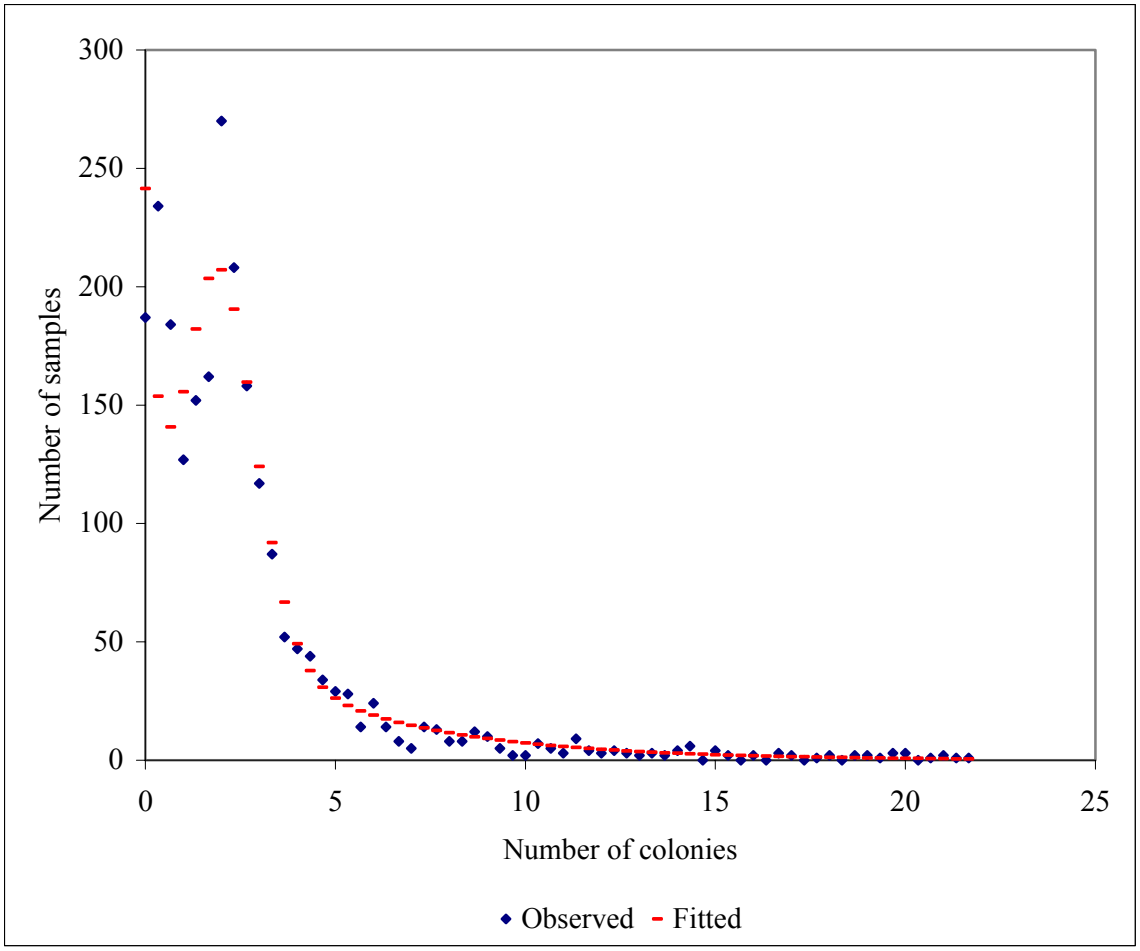


Figure 3.2 Approximate observed numbers and fitted expected numbers of samples versus numbers of colonies observed for Greenberg *et al*., 1966, illustrating the adequacy of fit of a gamma distribution.

3.5.3. Selected study data — RTE foods

The studies of Kalinowski *et al*. (2003; Table 3.3), Taormina *et al.* (2003; Table 3.4), and FSIS (2003; Table 3.2) were selected as giving the most useful information on the expected distribution of *C. perfringens* vegetative cells in post heat treated RTE commodities. As previously indicated, these vegetative cells arise from spores in the raw meat that were activated to germinate by the lethality step applied to RTE foods; all vegetative cells on the raw meats are assumed killed by this lethality step. All three studies included heat steps corresponding closely to those expected for RTE foods prior to the sampling and analysis. Kalinowski *et al*. (2003) cooked samples to a minimum internal temperature of 73.9 °C in a flowing steam chamber. Taormina *et al*. (2003) heated samples at 75 °C for 15 minutes. In FSIS (2003), samples were heated at 75 °C for 20 minutes. In all cases the same procedure was applied to all samples. Such cooking is expected to kill vegetative cells in the raw commodity and to cause near optimum germination of spores (Duncan and Strong, 1968).

In Kalinowski *et al.* (2003), presumptive *C. perfringens* colonies were confirmed as *C. perfringens* via Gram-stain, cell morphology, lactose fermentation, gelatin liquefaction, nitrate reduction, and motility reactions. Presumptive *C. perfringens* colonies on tryptone-sulfite-cycloserine media (TSC) observed in the FSIS (2003) survey were re-streaked on TSC and confirmed by Gram stain followed by API 20A® kit (bioMerieux, Inc.) according to manufacturer's instructions.[18] Taormina *et al*. (2003) did not confirm presumptive *C. perfringens* colonies. The last study is therefore used in what follows to provide an upper bound on the concentrations of vegetative *C. perfringens* cells in RTE food after the heat step. Taormina *et al.* (2003) tested more samples than Kalinowski *et al.* (2003), although less than FSIS (2003). The addition of these data contributes significantly to reducing uncertainty in the estimates.

Table 3.3 *C. perfringens* vegetative cells in raw meat blends following heat treatment (Kalinowski *et al.*, 2003)

Product type	**No. of samples examined**	**Percent of total samples**	**Number of samples with specified colony count of *C. perfringens*** [a]		
			0[b]	**1**	**20**
Ground turkey	154	78.2	154	0	0
Ground pork	11	5.6	9	1[c]	1[c]
Ground beef	6	3.0	6	0	0
Pork sausage	26	13.2	26	0	0
Total	197	100	195	1	1

a. No other colony counts were observed.

b. Corresponds to the detection limit of 3 CFU/g. For a colony count of *n* in a sample, the estimated CFU/g is 3*n*, since each plate corresponded to 1/3 g of the original meat product. Kalinowski *et al*. (2003) use 3.3*n* to estimate the CFU/g.

c. Corresponds to the two samples with estimated concentrations of 3 and 60 CFU/g. One plate had a single black colony, confirmed as *C. perfringens*. The second had 48 black colonies. Of 12 of these tested, 5 were confirmed as *C. perfringens* giving the estimate of (5/12)*48 = 20 CFU *C. perfringens* (Personal communication, R. Kalinowski, July 2003). The resulting uncertainty in actual colony count is taken into account in the analysis described in Appendix 3.1.

[18] This system screened for indole formation, urease and catalase production, gelatin and esculin hydrolysis and D-glucose, D-mannitol, D-lactose, D-saccharose, D-maltose, salicin, D-xylose, L-arabinose, glycerol, D-cellobiose, D-mannose, D-melezitose, D-raffinose, D-sorbitol, L-rhamnose, and D-trehalose acidification..

Table 3.4 Putative *C. perfringens* vegetative cells in raw meat product mixtures following heat treatment (Taormina *et al*., 2003)

Product type	**No. of samples examined**	**Percent of total samples**	**Number of samples with specified colony count of *C. perfringens*** [a]						
			0[b]	**1**	**2**	**3**	**4**	**10**	**13**
Cured whole muscle	194	43.6	194	0	0	0	0	0	0
Cured ground or emulsified [c]	152	34.2	144	5 4 3	0 1 3	0 1 0	1 0 0	2 2 2	0 0 0
Uncured whole muscle	81	18.2	81	0	0	0	0	0	0
Uncured ground or emulsified [c]	18	4.0	15	1 0	0 2	1 0	0 0	0 0	1 1
Total	445	100	434	Six possible combinations[d]				2	1

a. No other colony counts were observed.
b. Corresponds to the detection limit of 10 CFU/g. For a colony count of *n* in a sample, the estimated CFU/g is 10*n* since each plate corresponded to 0.1 g of the original meat product.
c. Each row corresponds to a possible pattern of colony counts, given the published information.
d. The actual pattern of colony counts was not given for any product type, but is unambiguous for cured and uncured whole muscle, based on the published information. There are three possible combinations of values for cured ground or emulsified product, and two possible combinations for uncured ground or emulsified product, for a total of six possible combinations for all products.

While studies designed to capture any seasonal, geographical, and species variance in concentrations would be preferred for estimating the levels of *C. perfringens* vegetative cells after heat treatment, no such studies that are otherwise suitable have been conducted. The studies of Taormina *et al*. (2003), Kalinowski *et al*. (2003), and FSIS (2003) have several drawbacks related to estimating the level of confirmed *C. perfringens* in beef, pork, and poultry; the most significant are:

1. A relatively small number of samples (445, 197, and 593) were tested. To obtain useful information on the shape of the upper tail of the distribution for *C. perfringens* spore concentrations would require substantially larger samples, probably in the tens of thousands.

2. No seasonal or geographical variations can be examined in these data. The Greenberg *et al*. (1966) study demonstrated that small seasonal and geographical variations were demonstrable at that time in total putrefactive anaerobic spore-former concentrations.

3. The proportions of various meat samples (ground and whole, cured and uncured, beef, pork, and chicken) are probably not representative of the proportions used in RTE products. Greenberg *et al*. (1966) demonstrated that small variations were demonstrable at that time between different types of meat in total putrefactive anaerobic spore-former concentrations.

4. No attempt was made to enrich *C. perfringens* from putatively negative samples or to enhance the viability of any vegetative cells present in positive samples; thus the number of negative samples may have been overestimated,[19] and the number of colonies detected in positive samples may underestimate the number of viable germinated spores present.

Clearly, using these data to represent the prevalence and level of *C. perfringens* in all heat treated RTE commodities is less than ideal. Yet due to lack of any other data, and noting their shortcomings, the data of Kalinowski *et al*. (2003), Taormina *et al*. (2003), and FSIS (2003) were used to estimate the initial levels (that is, post heat treatment but prior to stabilization) of *C. perfringens* vegetative cells in beef, pork, and poultry following heat treatment.

3.5.4. Evaluation of certain types of false negatives or positives

The efficiency of the methods used by Kalinowski *et al*. (2003), Taormina *et al*. (2003), and FSIS (2003) were examined to determine if any known false negative or false positive rate should be applied to their results. Kalinowski *et al.* (2003) and FSIS (2003) confirmed presumptive *C. perfringens* colonies, suggesting a low or nonexistent false positive rate. The authors used TSC to enumerate bacteria from meat samples. To estimate the likelihood this medium might produce false negatives due to growth of non-typical colonies, Araujo *et al*. (2001) plated water samples on TSC as well as three other types of standard media (Table 3.5). These data indicate plating water samples on TSC will not result in a substantial false negative rate.

Table 3.5 Efficiency of *C. perfringens* media (Table 1; Araujo *et al*., 2001).

Medium	False negatives[a]
mCP	1/53 (1.9%)
TSC	0/28 (0.0%)
TSN	4/16 (25.0%)
SPS	2/6 (33.3)

a. False negative: number of non-typical colonies confirmed as *C. perfringens*/total number of non-typical colonies examined.

The Kalinowski *et al*. and FSIS studies utilized meat, rather than water samples, and plated on TSC; thus while Araujo's study suggests the methodology of these studies would not have produced a substantial false negative rate, it does not negate the possibility that the plating of meat samples could yield false negatives. For this risk assessment, no explicit false negative or positive rate is applied to the observed data reported by Kalinowski *et al*. (2003) and FSIS (2003).

The Taormina *et al*. (2003) study used Shahidi-Ferguson Perfringens (SFP) agar base with supplements to enumerate bacteria from their samples. This agar has been shown to have

[19] Enrichment of *C. perfringens* from samples previously considered negative for *C. perfringens* has been demonstrated by Hall and Angelotti (1965) and McKillop (1959), indicating that even viable vegetative cells may not be detected by the standard type of plate count. None of Kalinowski *et al*. (2003), Taormina *et al*. (2003), or USDA/FSIS (2003) attempted to enrich *C. perfringens* from samples putatively defined as negative for *C. perfringens*, so the actual frequency of post-lethality samples that contained *C. perfringens* cannot be stated with absolute certainty.

approximately the same sensitivity as TSC, but to be less selective (Hauschild and Hilsheimer, 1974; de Jong *et al*., 2003). Moreover, the authors did not confirm putative *C. perfringens* colonies, so their results can be expected to overestimate *C. perfringens* concentrations. Thus no false-negative rate is applied, but the observed results are treated as an upper bound on the concentrations of *C. perfringens*.

3.5.5. Analysis of selected study data for vegetative cell concentrations in RTE foods

In view of the small number of observed positive detections, for all three studies by Kalinowski *et al*. (2003), Taormina *et al*. (2003), and FSIS (2003), only the total data (Table 3.3 and Table 3.4) were used — no attempt was made to separate pork, chicken, and beef; and no attempt was made to separate whole muscle and ground meat, or cured and uncured products. This may result in underestimates of concentrations in particular products, and in an overestimate of the number of products with significant concentrations, and more generally in an underestimate of the uncertainties of concentrations.

The variability in concentrations of *C. perfringens* vegetative cells present in RTE meat products after an initial cooking step was modeled by a probability distribution for such concentrations. This probability distribution was estimated from the data of Kalinowski *et al*. (2003), Taormina *et al*. (2003), and FSIS (2003) as follows.

Data from the three studies were separately modeled with single gamma distributions for concentrations of *C. perfringens* (see Appendix 3.1 for the methodology; all analyses reported here are performed in the workbook CP_count_RTE_meat.xls accompanying this risk assessment). That is, the probability distribution for a meat sample to contain a concentration x (CFU/g) was assumed to be given by

$$p(x,a,b) = \frac{(x/b)^{a-1}\exp(-x/b)}{b\Gamma(a)} \qquad \textbf{(3.4)}$$

where a, b are the parameters of the distribution (b is a scale parameter).

This distribution shape was based on that observed for the upper end of the distribution in Greenberg *et al*. (1966) (see Section 3.5.2), although there are too few detections to allow a formal goodness-of-fit analysis for the specific datasets on *C. perfringens* from the three studies used in modeling initial density (Kalinowski *et al.*, 2003; Taormina *et al*., 2003; and FSIS, 2003). The scale parameters (b) of the three distributions so obtained are not significantly different ($p = 0.99$; likelihood ratio test between Kalinowski *et al.* and Taormina *et al*.; no such comparison is possible for the FSIS study since only one colony was ever detected from any single sample), so these scale parameters were set equal and all subsequent analyses performed simultaneously taking this equality into account. The distribution obtained from the data of Taormina *et al*. (2003) was assumed to form an upper bound on the distribution of *C. perfringens* concentration modeled by the data of Kalinowski *et al*. and the FSIS study to correspond to the lack of specificity of the Taormina *et al*. analysis method. This distributional inequality was enforced (with equal b parameters) by requiring the parameter a_T of the gamma distribution associated with the Taormina *et al*. data to be larger than the corresponding parameter a_K associated with the Kalinowski *et al*. and FSIS data. This ensures that the cumulative distribution from the Taormina *et al*. data lies entirely to the right (with higher concentrations) of the distribution from the Kalinowski *et al*. and FSIS data (Figure 3.3).

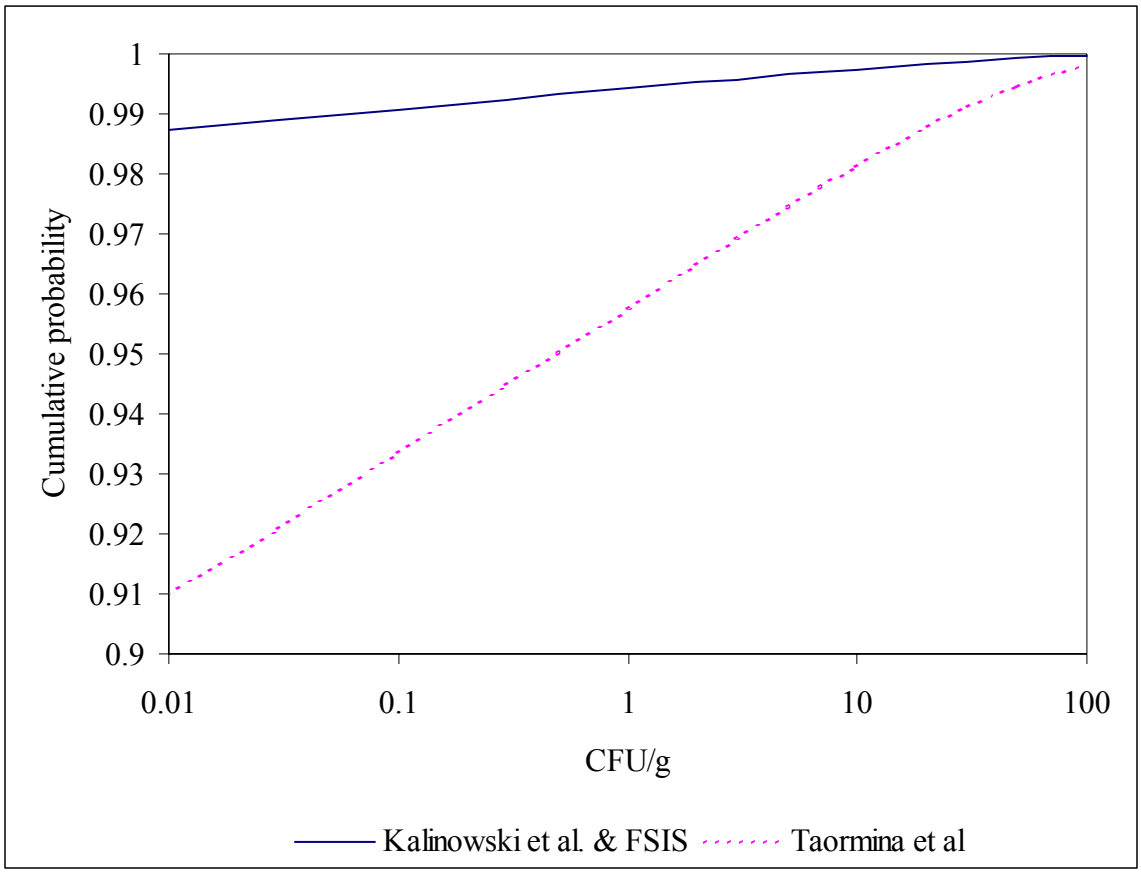


Figure 3.3 Upper end of the cumulative distributions (maximum likelihood estimates) for *C. perfringens* (Kalinowski *et al.*, 2003; FSIS, 2003) and total presumptive *C. perfringens* (Taormina *et al.*, 2003) concentrations in meat and poultry.

The maximum likelihood parameter estimates for the distribution for the concentration of *C. perfringens* in cooked meat in RTE foods are shown in Table 3.6. The parameter a_K corresponds to the distribution used for *C. perfringens* (based on Kalinowski *et al*. and the FSIS study), and a_T to an upper bound (derived from the Taormina *et al.* data). The second is given because it is needed for the uncertainty analysis.

Table 3.6 Maximum likelihood estimates for the distribution parameters for *C. perfringens* concentration in cooked RTE foods.

Power parameter a_K	0.00150	For *C. perfringens*
Scale parameter *b*	84.5	CFU/g
Power parameter a_T	0.0111	Upper bound

The uncertainties in these parameter estimates were obtained using the likelihood methodology described in Appendix 3.1. It was found that the parameters had to be transformed to obtain normal error structures, those transformations being:

Scale parameter, b	$\ln(\ln(\ln(b)))$
Power parameter a_K, Kalinowski *et al*. data	$\ln(\ln(-\ln(a_K)))$
Power parameter, a_T, Taormina *et al*. data	$\ln(-\ln(a_T))$

Table 3.7 gives the standard deviation and correlation coefficient estimates for these transformed parameters. In order to enforce the constraint on distributions, samples from the multinormal uncertainty distribution are censored if the sampled parameter values satisfy $a_K \geq a_T$ (that is, sampling is repeated until $a_K < a_T$).

Table 3.7 Standard deviation/correlation coefficient matrix for transformed parameters for *C. perfringens* concentration in cooked RTE foods.

	$\ln(\ln(-\ln(a_K)))$	$\ln(\ln(\ln(b)))$	$\ln(-\ln(a_T))$
$\ln(\ln(-\ln(a_K)))$	0.0438		
$\ln(\ln(\ln(b)))$	0.2647	0.0783	
$\ln(-\ln(a_T))$	0.1506	0.5689	0.0833

The main diagonal contains standard deviation estimates, off-diagonal entries are correlation coefficient estimates.

The maximum likelihood parameter estimates a_K and b of Table 3.6 are for a gamma distribution representing the variability of concentrations of germinated spores of *C. perfringens* in meat after any heating processes during RTE food production (and before stabilization). This distribution can also be characterized by a mean of 0.13 CFU/g and standard deviation of 3.28 CFU/g. The extremely large standard deviation, compared with the mean, results from the very long right tail of the distribution (Figure 3.3). The prevalence of vegetative cells in RTE servings obtained from this distribution depends on meat content of the RTE serving.[20] For example, the prevalence in servings containing 100 grams of meat is 1.35% at the maximum likelihood estimates of Table 3.6. It is smaller for smaller quantities of meat, and larger for larger quantities. The weighted average quantity of meat per serving evaluated in this risk assessment is 69.5 grams (2.45 oz.); the prevalence in servings with that quantity of meat is about 1.30%.

3.6. Spore concentrations in the meat fraction — c_m

The spore concentrations required are those remaining from the meat constituent of RTE and partially cooked foods after the initial processing step. For RTE foods, initial processing includes heating that will activate a large fraction of the spores to germinate (as well as killing vegetative cells). The effective spore concentration remaining in the meat constituent of RTE foods is the same fraction of the original spore concentration as the fraction of spores that do not germinate in the initial processing step (Section 3.9.4). For partially cooked foods, the initial processing step is assumed in this assessment to have no effect on vegetative cell or spore concentrations in raw meats, so the effective spore concentration in the meat constituent of the food is just that present in raw meat.

[20] The prevalence may be calculated using Equation (A3.1.3) in Appendix 3.1. It corresponds to the probability for one or more cells in a serving, hence is one minus the probability for zero cells.

3.6.1. Spore concentration c_m for RTE foods

Section 3.5 evaluated the vegetative cell concentration C_m in the meat constituents of RTE foods, based on measurements in meat that had been heated. Because the heat step kills pre-existing vegetative cells, the measured vegetative cells in heat treated meat originate from spores in the meat that are activated to germinate. The measured vegetative cell concentration C_m estimated in Section 3.5.5 is thus the concentration of spores that are activated to germinate into vegetative cells during initial processing involving a heat step (and thus a fraction of the spores originally present in raw meat). Section 3.9.4 (below) evaluates the fraction η of spores that are activated by the heat step. So in order to observe a concentration C_m of vegetative cells that were activated from spores, the original concentration of spores in the raw meat must have been C_m/η, of which a fraction $(1-\eta)$ remains un-activated after the heat step applied to RTE foods. The concentration of un-activated spores remaining in the meat constituents is thus given by

$$c_m = \frac{1-\eta}{\eta} C_m \qquad \textbf{(3.5)}$$

In the Monte Carlo procedure, for each serving an estimate of C_m is obtained from its variability distribution, and independently an estimate of η is obtained from its variability distribution, and c_m is computed as shown in Equation (3.5).

3.6.2. Spore concentration c_m for partially cooked foods.

For partially cooked foods, the vegetative cell concentration C_m is obtained independently of any estimates of spore concentrations (Section 3.7). In this case, an independent estimate of spore concentration is obtained by sampling from the distribution for C_m for RTE foods (Section 3.5), and applying the same approach as for RTE foods (Section 3.6.1) — so that the concentration of spores in this case is

$$c_m = C_{RTE}/\eta \qquad \textbf{(3.6)}$$

where C_{RTE} is here a sample from the distribution C_m for RTE foods (Section 3.5).

3.7. Vegetative cell concentrations in raw meat — C_m for partially cooked commodities

Only one category of food servings (3b, see Table 3.1) was identified as being partially cooked commodities, and there are fewer data available from which to infer concentrations of *C. perfringens* in such commodities. Consequently, the analysis of the concentration of vegetative cells for these products is somewhat less detailed than for RTE foods (Section 3.5).[21]

3.7.1. Selected study data — raw meat

Partially cooked products (see Table 3.1) are treated at temperatures lower than RTE foods, with even temperatures as low as 46 ^{o}C (used for softening and forming bacon) considered to be a partial cook. Such low temperatures are not lethal for many *C. perfringens* vegetative cells. Further, the lethal temperature employed for RTE commodities is applied in such a way that the minimum required temperature is achieved throughout the meat, while there is no such requirement for partial cook procedures. Any impact that the gradient of sublethal temperatures in partially cooked commodities may have on the level of vegetative *C. perfringens* cells and on *C. perfringens* spores is currently conjectural. While some vegetative cells may be killed and others injured, some fraction may remain unaffected. While some spores present may be

[21] The analyses reported in this section are performed in the workbook CP_count_raw_meat.xls included with the risk assessment.

activated and germinate, the fraction germinating is likely to be substantially less for partially cooked foods than for RTE foods that are cooked to higher temperatures.

No measurements of *C. perfringens* vegetative cells in partially cooked commodities are available. In lieu of such measurements, in this risk assessment it was assumed that the concentration of *C. perfringens* spores in partially cooked commodities is the same as that in raw meats. This would be true if, for example, a partial cook procedure does not kill *C. perfringens* vegetative cells nor cause germination of *C. perfringens* spores; or if the net killing of vegetative cells was offset by the germination of spores.

Seven studies were identified that determined the prevalence and levels of *C. perfringens* vegetative cells in raw meats, and these values were applied to partially cooked products (Table 3.8).

Table 3.8 Prevalence and levels of *C. perfringens* in raw meats.

Reference	**Season samples collected**	**Region**	**Lethality step**	**Presumptive *C. perfringens* colony confirmation**	**Product evaluated**	**Results**
Strong *et al*., 1963	not stated	WI, USA	No	Yes	Raw beef, veal, lamb, pork, chicken	18% (20/111) samples positive with 10–1,180 cell/g [d]
Hall and Angelotti, 1965	not stated	OH, USA	No	Yes	Raw beef, veal, lamb, pork, chicken	"Most" samples out of 36 tested with 1–100 CFU/g. One sample with 760 CFU/g. [e]
Taormina *et al.*, 2003	August 2001 — June 2002	Four midwestern plants, USA	No	No	Raw beef, pork, chicken; cured & uncured; whole and ground	(21.6%) 96/445 samples positive, mean 102 CFU/g, max 525 CFU/g.
Foster *et al*., 1977	Over 11 months (year not stated)	CA, USA	No	No[a]	Raw beef	(56%) 84/150 samples with <1 – $2.7x10^3$ CFU/g; mean=55 CFU/g
Ladiges *et al*., 1974	not stated	CO, USA	No	No[c]	Raw ground beef	(47%) 45/95 samples with 0–700 CFU/g
Bauer *et al*., 1981	not stated	GA, USA	No	Yes	Pork sausage[b]	(39%) 7/18 samples with 5–95 CFU/g

Nationwide Microbiological Baseline Data Collection Program, USDA/FSIS, 1992–1996	Varied between surveys.	Nationwide	No	No	Raw surface samples from steers, heifers, cows, bulls, market hogs; and samples of ground beef, ground chicken, and ground turkey	Cows & bulls: 8.4% positive. Steers & Heifers: 2.6% positive. Market hogs: 10.4% positive. Ground beef: 53.5% prevalence. Ground chicken: 50.6% prevalence. Ground turkey: 28.1% prevalence.

a. Presumptive *C. perfringens* on SPS agar were transferred to indole-nitrite medium. Non-motile and nitrite positive reactions were reported as *C. perfringens*. This analysis did not include gelatin liquefaction or lactose fermentation and was therefore considered incomplete (Hauschild, 1975).
b. Meat samples used were described as pork sausage samples from local area supermarkets. It is unclear if these were cooked products (suggesting a heat treatment step), or uncooked products (no heat treatment), or a mixture.
c. Presumptive *C. perfringens* colonies were additionally examined for motility and nitrate reduction. This confirmatory analysis was considered incomplete (Hauschild, 1975).
d. Omitting 11 fish samples, none of them positive.
e. It is possible, although unlikely, that some of these samples could have been cooked or otherwise processed meat products rather than raw meat.

Four studies were used only in a qualitative sense. Bauer *et al*. (1981) measured *C. perfringens* in pork sausage samples, but it was impossible to determine whether the sausages were cooked or uncooked products. Ladiges *et al*. (1974) did not confirm *C. perfringens* fully, and their measurements have been superceded by later studies of ground beef. Hall and Angelotti (1965) confirmed *C. perfringens*, but reported too little information for analysis. Nevertheless, the measurements of these studies appeared consistent with the measurements that were used for this analysis. The USDA/FSIS (1992–1996) Nationwide Microbiological Baseline Data Collection Program collected representative raw meat surface samples from cows, bulls, steers, and heifers, and samples of ground raw beef and poultry, with the aim of obtaining estimates of prevalence of contamination. However, there was no confirmation of *C. perfringens*, the surficial concentrations reported for raw meat are not representative of (volumetric) concentrations in meat entering processing, and too little information was published on the concentrations in ground beef and poultry to be usable.

Three studies were used quantitatively. Strong *et al.* (1963), Foster *et al*. (1977), and Taormina *et al*. (2003) provided information on measurements performed on raw meats without any preliminary heating procedure, so the measurements are primarily of vegetative cells. Strong *et al.* (1963) confirmed *C. perfringens* fully, Foster *et al*. (1977) performed a partial confirmation, and Taormina *et al*. (2003) did not confirm presumptive *C. perfringens* colonies in their measurements. For the purposes of this risk assessment, it was assumed that the measurements of Strong *et al*. are representative of *C. perfringens* concentrations in raw meat, while those of Foster *et al*. and Taormina *et al*. provide upper bounds.

While Strong *et al*. performed their study over 30 years ago, no more recent data with fully confirmed *C. perfringens* analysis were identified. No false-negative rate was applied to the results.

3.7.2. Analysis of selected study data for partially cooked foods

The data available from the selected studies are too sparse to fully define variability distributions for *C. perfringens* concentrations in partially cooked foods. As for RTE foods, the distribution clearly has a long tail, with appreciable probabilities for relatively high concentrations of *C. perfringens* (Table 3.8). To account for this long tail, the variability distribution was modeled by gamma distributions, as for RTE foods. The same techniques as were used in the previous analysis (Section 3.5.5) were used to enforce bounds on the distribution derived from the data of Strong *et al*. (1963) using the data from Foster *et al*. (1977) and Taormina *et al*. (2003). The scale parameters for the gamma distributions are all consistent with being equal[22] (p=0.51; likelihood ratio test). With equal scale parameters, the maximum likelihood estimates for the power parameters of the gamma distributions (Table 3.9) fall in the order expected from the degree of confirmation of *C. perfringens*; lower values (corresponding to fewer organisms) for more stringent confirmation (Appendix 3.1 gives details of the methods used, and the calculations are performed in the workbook CP_count_raw_meat.xls, included with this risk assessment).

Table 3.9 Maximum likelihood estimates for parameter values for gamma distributions for concentrations in partially cooked food.

Power parameter a_s [a]	0.06835
Power parameter a_t	0.09756
Power parameter a_f	0.2078
Scale parameter b, CFU/gram	298.9

a. Subscripts s for Strong *et al.*, t for Taormina *et al.*, f for Foster *et al*. data. All are needed for the uncertainty analysis.

The parameters given in Table 3.9 correspond to a variability distribution for *C. perfringens* vegetative cell concentrations in partially cooked food with a mean of 20.4 CFU/g and a standard deviation of 78.1 CFU/g. The large standard deviation, compared with the mean, is due to the long right tail of the assumed gamma distribution — and the observations, particularly of Foster *et al*. (1977) support such a long right tail. The prevalence of vegetative cells from meat in partially cooked food servings depends on the amount of meat in the serving.[23] For example, for a serving containing 100 g (3.53 oz.) of meat, the prevalence of vegetative cells is 50.6% at the maximum likelihood values of Table 3.9.

[22] Strictly speaking, the scale parameter for the Strong *et al*. data is indeterminate — the available data provide only an upper bound on it, since Strong *et al*. provide so few statistics on their measurements.

[23] The prevalence may be calculated using Equation (A3.1.3) in Appendix 3.1. It corresponds to the probability for one or more cells in a serving, hence is one minus the probability for zero cells.

To estimate the uncertainty distributions for the parameters defining the distributions of concentrations, transformations of the parameters were found that approximately normalized the profile likelihood distributions separately. The transformations used were:

Parameter	**Transformation**
Power parameter a_s	a_s (No transformation)
Power parameter a_t	$a_t^{0.2}$
Power parameter a_f	$a_f^{0.25}$
Scale parameter b	$1/\sqrt{b}$

The estimated standard deviations and correlations for these transformed parameters (see Appendix 3.1 for the methodology used) are given in Table 3.10.

Table 3.10 Standard deviations (main diagonal) and correlation coefficients (off-diagonal) for the uncertainty distribution of transformed parameters of the distributions for *C. perfringens* concentrations in partially cooked food.

	$1/\sqrt{b}$	a_s	$a_t^{0.2}$	$a_f^{0.25}$
$1/\sqrt{b}$	0.00433	0.231	0.480	0.000
a_s	0.231	0.01714	0.111	0.140
$a_t^{0.2}$	0.480	0.111	0.01366	0.291
$a_f^{0.25}$	0.000	0.140	0.291	0.01922

These values were used to define a multinormal distribution to represent the uncertainty in *C. perfringens* concentrations in partially cooked food. Values from the multinormal for which $0 < a_s < a_t < a_f$ is not true are censored during the calculations, to enforce the lower and upper bound assumptions.

3.8. Concentrations of *C. perfringens* vegetative cells (C_{sj}) and spores (c_{sj}) in spices

Spices can contain substantial levels of *C. perfringens* spores (DeBoer *et al.*, 1985; Rodriguez-Romo *et al*., 1998; Neut *et al.*, 1985; Eisgruber and Reuter, 1987). Many spices are handled in a dry, powdered form, unprotected from the oxygen in the air, that would not be conducive to survival of *C. perfringens* vegetative cells. Spices can be irradiated or treated by chemical means to lower bacterial load. These processes destroy vegetative cells, although their effect on *C. perfringens* spores is likely variable. It is therefore expected that the great majority of *C. perfringens* associated with spices are present in spore form, rather than as vegetative cells.

The addition of spices to raw commodities typically occurs during the processing stage of RTE foods. Any *C. perfringens* spores present in the spice could therefore be stimulated to germinate during the heat treatment step and could potentially grow under favorable conditions (indeed, the studies located indicate that some spores will germinate from spices even in the absence of any heat treatment). Consequently, foods containing spices may be more contaminated than those that do not. In fact, epidemiological evidence from *C. perfringens* outbreaks suggests spiced foods, such as Mexican style foods, may be an important vehicle for *C. perfringens* food

poisoning (see *Hazard Identification*). Spices added to foods are therefore taken into account in this risk assessment.

3.8.1. Study selection for *C. perfringens* in spices.

Table 3.11 lists studies that were located that examined the prevalence and levels of *C. perfringens* spores in spices. Examination of the available studies shows that experimenters in different times and places have found substantial differences in *C. perfringens* concentrations in some spices, presumably because of differences in origin, handling, and sterilization procedures applied.

Table 3.11 Levels and prevalence of *C. perfringens* spores in spices.

Reference	Spice/herb	Levels CFU/g	Prevalence
Candlish *et al.*, 2001[a,c]	Chili powder, curry powder, white pepper, paprika, garlic powder, ginger powder, black pepper, cloves, bay leaves.	ND – 900	unknown, mean of two samples reported
Pafumi, 1986[d]	Cayenne-saromex, chinese capsicums, chives, cinnamon, cloves, coriander, cumin, fenugreek, garlic, ginger, mace, mint flakes, mixed herbs, mustard seed, nutmeg, onion powder, oregano, paprika, parsley flakes, pepper, black pepper, white pepper, pimento, turmeric	<100[f] – >10,000	0 – 67% of from 3 to 50 samples of each spice.
Rodriguez-Romo *et al.*, 1998[b]	Garlic powder, black pepper, cumin seed, oregano, bay leaves	<100[f] – 500	3 – 20% of 76 samples of each spice
Powers *et al.*, 1975[g]	Bay leaves, cayenne pepper, chili powder, cinnamon, garlic powder, mustard powder, oregano	<100[f] – 2,850	0 – 53% of 15 to 18 samples of each spice
Salmeron *et al.* 1987[d]	83 samples of black and white, whole or ground, pepper	<10 – >50	0/18, 1/17, 7/24, and 8/24 samples
Smith, 1963[h]	Whole peppercorns, cayenne pepper, white pepper, black pepper, chili pepper, paprika, red pepper	0 – 12	unknown
Strong *et al.*, 1963[b]	20 types of spices	10 – 30	3/60 (5%)
DeBoer *et al.*, 1985[b]	150 samples of spices and herbs	<100–10,000	100/150 (67%)
Neut *et al.*, 1985[b]	Spices, unspecified	>100 – <10,000	2/2 (100%)
Eisgruber and Reuter, 1987[e]	Paprika, black pepper, coriander, cinnamon and others	Not specified	21/70 (30%)
Kneifel and Berger, 1994[d]	160 samples of 55 spices	<100	1 caraway sample only [i]
Masson, 1978[h]	Paprika, curry, black pepper, white pepper, cayenne pepper,	<10 – 650	0 – 89% of from 1 to 9 samples

	and others		
Baxter and Holzapfel, 1982[b]	Various spices and herbs	Detection limit not specified	None detected
Leitao *et al*., 1974[b]	Dehydrated pepper and cinnamon	<10 – <1000	15/45 pepper, 22/42 cinnamon
Krishnaswamy *et al.* 1971[d]	black pepper, turmeric, coriander, mustard, fenugreek, red chilis, cumin, and fennel	0 – 700	Unknown

a. Presumptive *C. perfringens* colonies were stated as confirmed, however details were omitted, no reference given.
b. Presumptive *C. perfringens* colonies were confirmed.
c. n=2, however unclear if both samples were positive.
d. Presumptive *C. perfringens* colonies were not confirmed.
e. Unclear if presumptive *C. perfringens* colonies were confirmed (original not translated from German).
f. Limit of detection.
g. Partial confirmation: sulfite reduction, lactose fermentation and motility tests.
h. Unknown if *C. perfringens* were confirmed. Details not given.
i. This study is the only one in which an initial heating step was used.

Of the studies listed in Table 3.11, four stand out as providing the most useful data, and these studies are assumed to be representative in this assessment. The most representative for U.S. conditions is probably that by Powers *et al*. (1975), since it involved samples (of seven spices) from 16 different military bases in different geographical areas of the U.S., each sample was procured locally, and *C. perfringens* colonies were confirmed to some degree; although this study is now nearly 30 years old. More recently, Rodriguez-Romo *et al*. (1998) examined a total of 380 samples of five spices in Mexico, with confirmation of presumptive *C. perfringens* colonies. Further afield but still relatively recent, Candlish *et al.* (2001) examined ethnic samples in Scotland, with some degree of confirmation but few details provided. Lastly, Pafumi (1986) has the merit of providing some information on many spices, although *C. perfringens* was not confirmed in this study, and it was performed on spices imported to Australia.

3.8.2. Analysis of studies for "as measured" *C. perfringens* concentrations in spices

The data from the selected studies were used in the following manner.[24] Table 3.12 lists all the spices named in the CSFII (USDA, 2000) and occurring in the servings of 607 foods selected as RTE and partially cooked, together with the number of distinct servings containing each spice (in the total of 26,548 such servings), and the maximum percentage contribution of the spice to the total serving size. The spices for which Powers *et al*. (1975), Rodriguez-Romo *et al*. (1998), or Candlish *et al*. (2001) provide data are also listed. For those spices (oregano, mustard, garlic, cumin, cinnamon, chili, cayenne pepper, black pepper) with data provided by Powers *et al*. and/or Rodriguez-Romo *et al.*, measurements were combined (and combined with any corresponding data from Candlish *et al.*) to estimate the variability and uncertainty distributions for *C. perfringens* concentrations. Different forms of the same spice (*e.g.* powder and seed; Dijon mustard and mustard seed) were combined. Only for oregano and garlic were sufficient data available to distinguish differences in the distributions —data on mustard, cumin, cinnamon, chili, cayenne pepper and black pepper were combined. All measurements on spices not so selected were combined and treated as a single "spice" having the same variability and

[24] The analyses reported in this section are performed in the workbook CP_in_spices.xls included with the risk assessment.

uncertainty distributions, estimated from the combined data of Pafumi (1986) for all spices not previously selected.

Table 3.12 Occurrence of spices in foods in the selected CSFII servings (RTE and partially cooked).

Spice/herb	# occurrences	Max % in food	Some occurrence data provided by		
	in CSFII		Powers	Rodriguez	Candlish
Chili Powder	1223	1.02	•		•
Pepper, Black	1017	0.57		•	•
Garlic Powder	537	1.57	•	•	•
Oregano, Ground	457	0.11	•	•	
Mustard Seed, Yellow	266	0.29	•		
Dijon Mustard	139	4.62			
Ginger, Ground	135	0.13			•
Paprika	79	0.60			•
Basil, Ground	63	0.57			
Pepper, Red/Cayenne	53	4.31	•		
Sage, Ground	49	0.51			
Parsley, Dried	46	0.20			
Curry Powder	28	0.41			•
Cinnamon, Ground	25	0.13	•		
Anise Seed	24	0.05			
Cloves, Ground	24	0.05			•
Cumin Seed	24	0.05		•	
Nutmeg, Ground	18	0.17			
Allspice, Ground	15	0.08			
Onion Powder	11	0.51			
Thyme, Ground	8	0.57			
Poultry Seasoning	6	2.35			

There are too few data available to adequately determine the shape of the variability distribution for *C. perfringens* concentration in spices. For this assessment, it was assumed that the variability could be adequately modeled by a gamma distribution (Equation (3.4)), a shape consistent with that observed for the highest concentrations of spores of putrefactive anaerobes in meat (Section 3.5.2). All reported concentration measurements were assumed to be accurate

— too little information was generally provided to estimate the uncertainty in concentration estimates due to counting of a only a small number of colonies. Maximum likelihood estimates for the parameters *a*, *b* of the gamma distribution (Equation (3.4)), with *b* in CFU/g) were obtained by maximizing the sum of loglikelihoods of all reported distinct measurements. The contribution to the loglikelihood of an observed sample within a range of reported concentrations from C_1 to C_2 was taken to be

$$\ln\left(P\left(a,b,C_2\right)-P\left(a,b,C_1\right)\right)$$
$$\text{where}\quad P\left(a,b,C\right)=\frac{1}{\Gamma\left(a\right)}\int_0^{C/b}t^{a-1}e^{-t}\,dt \qquad \textbf{(3.7)}$$

while each sample with a single reported concentration *C* contributed

$$\left(a-1\right)\ln\left(C/b\right)-C/b-\ln\left(b\Gamma\left(a\right)\right) \qquad \textbf{(3.8)}$$

Uncertainty estimates were obtained by first finding a suitable transform to make the profile likelihoods for transformed variables approximately normal (see Appendix 3.1 for discussion of this approach). Power law transformations of *a* and *b* were found to be suitable:

$$u=a^{\omega_a}\quad\text{and}\quad v=b^{\omega_b} \qquad \textbf{(3.9)}$$

Re-writing the likelihood in terms of the transformed variables *u* and *v* allowed quadratic approximation of the loglikelihood using an information matrix (estimated by separately and together making increments in *u* and *v* approximately equal to their standard deviations as indicated by their individual profile likelihoods, and solving the resultant simultaneous quadratic equations for the change in loglikelihood). An estimate of the variance-covariance matrix for *u* and *v* was then obtained by inverting the information matrix. The uncertainty distribution for *u* and *v* was then estimated as a multinormal distribution with this variance-covariance matrix.

The results obtained are shown in Table 3.13 through Table 3.16. Each table displays maximum likelihood estimates (MLE) for parameters *a* (dimensionless) and *b* (CFU/gram), and the corresponding MLEs for mean and standard deviation (SD) of the distribution (the former is the product of *a* and *b*, the latter the product of *b* and the square root of *a*), the transformation power laws used (ω_a and ω_b) and the corresponding MLE for *u* and *v*. The multinormal uncertainty distribution obtained for *u* and *v* is represented by the standard deviations and correlation coefficients for *u* and *v*.

Table 3.13 Parameter estimates for *C. perfringens* in mustard, cumin, cinnamon, chili, cayenne pepper and black pepper combined.

a	0.173	Mean (CFU/g)	19.2
b (CFU/g)	111	SD (CFU/g)	46.1
ω_a	0.1	u	0.839
ω_b	-0.36	v	0.184
SD (diagonal) and correlation coefficient (off-diagonal)			
	u	v	
u	0.0356	0.884	
v	0.884	0.0261	

Table 3.14 Parameter estimates for *C. perfringens* in garlic (as a spice)

a	0.252	Mean (CFU/g)	49.5
b (CFU/g)	196	SD (CFU/g)	98.5
ω_a	0.125	u	0.842
ω_b	-0.37	v	0.142
SD (diagonal) and correlation coefficient (off-diagonal)			
	u	v	
u	0.0391	0.846	
v	0.846	0.0211	

Table 3.15 Parameter estimates for *C. perfringens* in oregano

a	0.0839	Mean (CFU/g)	72.4
b (CFU/g)	862	SD (CFU/g)	249.8
ω_a	0.11	u	0.761
ω_b	-0.33	v	0.107
SD (diagonal) and correlation coefficient (off-diagonal)			
	u	v	
u	0.0311	0.724	
v	0.724	0.0197	

Table 3.16 Parameter estimates for *C. perfringens* in all other spices

a	0.0562	Mean (CFU/g)	148.3
b (CFU/g)	2641	SD (CFU/g)	625.9
ω_a	0.08	u	0.794
ω_b	-0.25	v	0.139
SD (diagonal) and correlation coefficient (off-diagonal)			
	u	v	
u	0.0106	0.696	
v	0.696	0.0116	

In the risk assessment, to correspond to the data analysis performed, the quantities of mustard, cumin, cinnamon, chili, cayenne pepper and black pepper are combined and treated as a single spice with concentrations estimated by a gamma distribution with parameters given by Table 3.13. The quantities of garlic and oregano are treated separately (using parameter values from Table 3.14 and Table 3.15 respectively), then all other spices are combined and evaluated using the parameters of Table 3.16.

3.8.3. Vegetative cell and spore concentrations in spices

As previous stated, it is here assumed that *C. perfringens* in spices are present entirely as spores. The measurements discussed here of *C perfringens* concentrations in spices were performed without an initial heat treatment in all studies but one, so the measured concentrations may represent only a small fraction of the spores present in the spices. A heat processing step might be expected to lead to considerably higher concentrations of vegetative cells, as a larger fraction of the spores is induced to germinate.

On the other hand, Kneifel and Berger (1994) examined 160 samples of 55 spices (between 1 and 6 samples of each spice) obtained in Austria and expected to be essentially untreated by any sterilization methods. Using an initial heat treatment (80°C for 5 min) that would be expected to be highly effective at inducing spore germination, the authors detected only one positive result (in caraway, for which there were 6 samples). The detection limit was unstated, but probably was between 3 and 30 CFU/g. The failure of Kneifel and Berger (1994) to detect more *C. perfringens* is puzzling when compared with the measurements of other authors (Table 3.11). It presumably indicates either a large variability in *C. perfringens* concentrations between places and times, or it reflects the mixture of strains of *C. perfringens* on spices obtainable at that time in Austria (Section 3.9.3).

The experiments included in the quantitative analysis of Section 3.8.2 all were performed without a heat step, so presumably underestimated the total concentration of spores in the spices. In the Monte Carlo procedure, the following approach is adopted to estimate the initial number of spores and vegetative cells present in servings of food due to added spices.

For each spice j, an estimate C_j of "as measured" spore concentrations is obtained from the distributions of Section 3.8.2. An estimate ϕ of the fraction of spores that may germinate under favorable conditions without heat treatment is obtained (see Section 3.9.5), and the ratio C_j/ϕ then estimates the initial concentration of spores in that spice (the same value of ϕ is used for all spices within each serving).

For partially cooked foods, the initial concentration of vegetative cells due to spores that germinate during initial processing, C_{sj}, is assumed equal to the "as measured" concentration (so $C_{sj} = C_j$), and the remaining concentration of spores after initial processing is then given by $c_{sj} = (1/\phi - 1)C_j$.

For RTE foods, the fraction η of spores that are activated by the initial processing is estimated (Section 3.9.4), and applied to the estimate for the initial concentration of spores, so that

$$C_{sj} = \eta C_j/\phi \quad \text{and} \quad c_{sj} = (1-\eta)C_j/\phi \qquad \textbf{(3.10)}$$

The estimates obtained in this way do not track any differences in activation and/or germination rates between heat resistant strains of *C. perfringens* (among which are the type A, CPE-positive food poisoning strains) and classical strains. However, there are insufficient data to currently distinguish these differences in spices.

3.9. The fraction of spores that germinate

The fraction of *C. perfringens* spores that undergo germination in foods under particular conditions may depend on multiple factors, including (1) the presence of food additives, (2) physiologic properties of the food matrix, (3) strain variation, and (4) the temperature and duration of heat treatment. These factors are described below; however, there were insufficient data published on them to evaluate germination rates as a function of any of them but temperature and time. For the combined factors of temperature and time, there may be sufficient data available to make an estimate of the germination fraction as a function of them, but lack of information on temperature/time relationships for initial processing or final preparation of RTE and partially cooked foods vitiates the usefulness of any such approach (Section 3.9.4).

3.9.1. The effect of common food additives on germination

The effect of two commonly used food additives, nitrites and salt (NaCl), on germination of *C. perfringens* spores was evaluated. There is evidence to suggest that the level of nitrite in foods does not affect germination of *C. perfringens* spores. Labbe and Duncan (1970) found that addition of 20,000 ppm sodium nitrite to laboratory growth media did not inhibit germination of heat-resistant *C. perfringens*. By way of comparison, the allowable sodium nitrite in foods is 200 ppm. No effect of nitrite on spore germination was modeled in this risk assessment.

Similarly, the addition of salt to foods is not likely to affect germination of *C. perfringens* spores. Hobbs (1962) reported that *C. perfringens* spores could germinate in 5% sodium chloride (probably on raw meat covered with brine), but gave no details of the experiments. Germination of *Clostridium sporogenes* spores were not inhibited by 1–3% salt; >3 to <6% salt was required to alter germination kinetics and 6–10% salt was required to inactivate a portion of germinating spores. In addition, Mundt *et al.* (1954) found that *C. sporogenes* spores were capable of germination in 8% salt. These data, although not from *C. perfringens*, suggest that moderate

levels of salt (2–3%) in food do not greatly influence the frequency of *C. perfringens* spore germination. No effect of salt on spore germination was modeled in this risk assessment.

Whether nitrites and salt may act synergistically to inhibit the germination of *C. perfringens* spores is an open question. As described in Section 3.11.5, nitrites and salt have been shown to act synergistically to inhibit the growth of *C. perfringens* vegetative cells in foods. No evidence has been identified explicitly evaluating the effect of such a synergy on spore germination. For this risk assessment, no effect of salt and nitrite at concentrations encountered in the foods examined on germination of *C. perfringens* spores was modeled.

3.9.2. The effect of physiologic properties of the food matrix on germination.

Several factors, including the presence of oxygen, water activity, and pH of the food, were considered.

C. perfringens is an anaerobic bacterium that is unable to grow in the presence of oxygen. Studies using heat-sensitive strains of *C. perfringens* suggest the fraction germinating will be affected by the presence of oxygen (Ahmed and Walker, 1971). However, while heating tends to reduce the oxygen available in a food matrix, data on any effect on *C. perfringens* germination are lacking. For this risk assessment, no effect of oxygen was modeled.

Water activity refers to the water available for biological processes. Kang *et al.* (1969) plated heat-activated *C. perfringens* spores on media with varying water activity. The water activity levels were controlled by the addition of three solutes in separate experiments. Spores germinated and grew even in low water activity environments; however, based on these data, it was not possible to distinguish between the effect that reduced water activity has on germination and on growth (see Section 3.11.5.5 for further details). Moreover, *Clostridium botulinum* spores were able to germinate at water activity levels below those that permitted growth of vegetative *C. botulinum* cells (Baird-Parker and Freame, 1967; Williams and Purnell, 1953). It is therefore reasonable to suppose *C. perfringens* spores are capable of germinating at water activities below those that allow vegetative cell growth. Observed water activities in foods similar to those evaluated in this risk assessment show values above any threshold that might affect germination (Section 3.11.5.5). Therefore, no effect of water activity on germination of *C. perfringens* spores was modeled.

There is some evidence to suggest that pH affects germination rates of *C. perfringens* spores. Experiments using heat-resistant spores of *C. perfringens* showed that as the pH of the solution increased, the optimal temperature for germination decreased (Craven, 1988). For instance, optimal germination was observed for spores at pH 5.6 and 75 °C for 20 minutes. However, at pH 5.6, germination fell by 2.3 fold at 65 °C. At pH 6.6, a similar fraction of germinated spores was observed after both 65 and 75 °C for 20 minutes. However, in these studies Craven (1988) quantified change in germination by measuring reduction of optical density values rather than by enumeration; and the relation of this measurement to the delay time modeled here is not known. Consequently, any separate effect of pH on germination of *C. perfringens* spores could not be reliably modeled in this risk assessment (in addition, pH values for the food servings used in the risk assessment were not measured; and any effect of pH on germination would affect only the modeling of hot-holding).

3.9.3. The effect of heat treatment temperature and duration, and strain, on germination

There is some evidence to suggest that *C. perfringens* that cause food poisoning are more resistant to heat than those strains not associated with human disease (Roberts, 1968), and there may be some correlation between heat-sensitivity and the effect of heat on the fraction of spores that germinate. For example, spores from one strain characterized as heat sensitive germinate to the greatest extent when exposed to 65-70 °C for 10-20 minutes. For two strains characterized as heat-resistant, spores germinated best for heating in the range of 70 to 80°C for 10 minutes (Duncan and Strong, 1968). For any single strain, there is a clear and very large variation in germination rate for different heat treatment temperatures and times of exposure to that temperature (temperatures above about 50°C are required to produce any activation), and this variation varies substantially between strains (Roberts, 1968; Craven and Blankenship, 1985; Tsai and Riemann, 1974; Duncan and Strong, 1968).

While these data suggest there is a difference between heat sensitive and heat resistant strains of *C. perfringens,* the literature contains results on only a few strains, so it is not currently possible to parameterize this difference. Therefore, data from heat sensitive and resistant strains were used to evaluate heat-activated *C. perfringens* spore germination.

In interpretation of these data, experimental techniques and definitions are important. Some experimenters measured the absolute initial number or concentration of spores (by total spore counting, or by an optical method calibrated by total spore counting), and then measured spores that germinated by colony counts after incubation on suitable media. Such measurements will be referred to as "absolute" in what follows. Other experimenters measured both the effective initial number of spores and the number that germinated by techniques that depended entirely on incubation on suitable media, so may have entirely omitted any spores that never germinated under the conditions of the experiments. Such measurements will be referred to as "relative" in what follows.

- Wynne and Harrell (1951) used an uncharacterized strain of *C. perfringens* in a relative method that indicated 98.5% germination rate after a single heat treatment, with subsequent 1.5% further germination after a second heat treatment, the combined effects of two heat treatments being defined as 100%. The exact methodology is not clear, and raw results are not given. This is the only experiment identified that attempted to recover spores that had not germinated after incubation after the initial heat treatment with a subsequent heat treatment. It is thus the closest available match to the expected sequence of events for some RTE foods — an initial cooking step during manufacture, followed by a re-heat during preparation.

- Wynne *et al*. (1954) again used an uncharacterized strain (possibly the same one) of *C. perfringens* in a relative approach to estimate 94% and 100% relative germination rate after a single heat treatment in two experiments. Measurement in this case was of spores, rather than vegetative cells produced by germinated spores, and recovery of spores was generally by incubation for 2 to 3 days with no second heat treatment — vegetative cells instead were destroyed by contact with oxygen. However, one test performed with a second heat treatment could be interpreted (along with the 100% relative germination rate test, in which

there were no recovered spores) as showing approximately 0.2% additional germination after a second heat treatment.

- Ahmed and Walker (1971) used changes in optical density to estimate an alteration in spores that correlated with subsequent germination (as measured by colony counting), as a function of time after heat treatment using *C. perfringens* strain S45. It appears that the method used was an absolute one (the calibration methodology used was not described in sufficient detail to make a complete determination). They measured a maximum optical density change corresponding to approximately 47% germination within 25 minutes after heat treatment at 75 °C for 20 minutes, with a smaller maximum change for 80 °C heat treatment. The optical density change increased approximately linearly with time after heat treatment until it saturated, and for lower heat treatment temperatures the optical density change was apparently still progressing at the end of the experiments.

- Tsai and Riemann (1974) measured the activation of five strains (NCTC 8798, S79, 80535, ATCC 3624, and BP6K; the first three strains listed are associated with food poisoning, the last two are classical or well studied, but not associated specifically with foodborne illness) for various time and temperature combinations of heat treatment. The maximum germination rates, measured with an absolute method (absolute optical count of initial number of spores; colony counts for germinating spores) ranged from 30% to 70%.

- Craven and Blankenship (1985), using strain NCTC 8679 and a relative measurement method, observed maximum activation with heat treatment at 75 °C for any period longer than about 5 minutes, and defined such conditions as giving 100% activation. Addition of lysozyme increased activation to 105%, (significantly higher than without lysozyme) so that relative activation without lysozyme was at most actually 100/105 = 95%. Using an absolute method, the same authors measured an absolute activation (corresponding to 100% relative activation on their scale) of 61 ± 19%.

The experiments described were conducted in laboratory media and water, using heat-resistant and heat-sensitive (or unknown) *C. perfringens*. A study investigating *C. perfringens* germination in meat indicated a very large relative fraction of spores germinating after heat treatment (but not lysozyme treatment), although no quantitative estimates could be derived (Barnes *et al.*, 1963), and only two studies were found that used heat-resistant strains.

3.9.4. Spore germination fractions after heat treatment — η and g_p

The spore germination fractions required in the model could be either relative fractions or absolute fractions, so long as both are well-defined and used consistently (use of relative fractions would be justified if there are spores that do not germinate in meat products under any conditions met in food processing, storage, transport, and preparation). Two fractions are required; the first (symbolized by η above) for initial processing, and the second (g_p in Equation (3.2)) for reheating during food preparation. It is likely that these fractions vary with the strain of *C. perfringens*, and with conditions of heat treatment, neither of which can currently be modeled.

To encompass the range of measurements described above, the varied heat treatments expected, and the variation in *C. perfringens* strains, η is modeled as varying from 5% to 75% (of the initial total number of spores, corresponding to absolute measurements in Section 3.9.3) with a triangular distribution with a mode of 50%. The effect of these assumptions about distribution shape and values is evaluated using a sensitivity analysis.

Only one experiment (Wynne and Harrell, 1951) effectively measured g_p, and that with near-optimum initial heat treatment for the strain tested. In that circumstance it appeared that few spores remained after the initial heat treatment that could be activated by subsequent heating. If the conditions of the original heat treatment are not optimal, however, and any re-heating approaches optimal conditions, it appears likely that a larger fraction than measured by Wynne and Harrell (1951) could be activated by the second heating. The estimate for g_p is thus conditioned on η — it is treated as variable from 0 to $(0.75-\eta)/(1-\eta)$ (the upper limit corresponding to the assumption that there is an upper bound of 75% in the total fraction of spores that might be activated by up to two heat treatments), with a triangular distribution with mode half way between zero and the upper limit. The effect of these assumptions about distribution shape and values is evaluated using a sensitivity analysis.

3.9.5. Spore germination in favorable conditions without heat treatment

The fraction of spores (symbolized by ϕ above) that germinate in favorable conditions (but without heat treatment) is required to interpret the experiments on spices (Section 3.8.3). The following studies were used to estimate the fraction of spores that germinate in favorable conditions.

- Barnes *et al.* (1963) measured 3% apparent germination and growth (relative to recovery after heat activation) of spores prepared by lysozyme treatment of a spore and vegetative cell suspension of *C. perfringens* F2985/50. However, subsequent incubation at 37 °C led to less than 3.5 logs of growth in the following 24 hours in either raw or cooked meat, suggesting a much extended delay period for any viable remaining vegetative cells or germinating spores. In other tests examining the effect of storage temperature, raw beef blocks were inoculated with a suspension of spores and vegetative cells and stored at constant temperature. Barnes *et al.* (1963) indicate a failure of spores to germinate at all temperatures tested. However, these tests could not distinguish between germination and death of spores, and for temperatures below 15 °C have been assumed to correspond to (see Section 3.13.2) to spore death.

- Roberts (1968) observed that culture counts of unheated spore suspensions were 0.13–3.6% of the microscopically determined total spore count for four or five heat-resistant strains (NCTC 8238, 8239, 8798, 8797, and perhaps 9851; the paper is not clear), but 31–46% for two classical strains (NCTC 3181, 8084). However, it was also observed that the spore preparation method, involving inactivation of vegetative cells by oxygen, was not completely effective, so some of the culture count may have been due to surviving vegetative cells.

- Ahmed and Walker (1971) indicated the presence of some microscopically visible germination after storage of spores frozen for 1 or 2 months (temperature not specified).

- Tsai and Riemann (1974) measured recoveries from spore preparations of 4%, 6% and 8% for three food-poisoning associated *C. perfringens* strains that were not heat treated, and 10% and 13% for two classical strains, although it is not clear to what extent the spore preparations were free of vegetative cells. These recoveries are colony counts for germinating spores, but the initial number of spores was apparently measured optically, so these are absolute recoveries.

- Craven and Blankenship (1985), using type A strain NCTC 8679, observed that 4% to 6% (relative to recovery after heat treatment of 75 °C for 20 minutes) of a spore suspension without heat treatment (<1% vegetative cells, stored desiccated) formed colonies on TSC. Addition of lysozyme increased colony counts to about 10% of the spores in this experiment. The absolute recovery corresponding to 100% relative recovery was 61 ± 19%, so the absolute germination rate is approximately 2% to 4%.

As for spore germination with heat treatment, spore germination fractions without heat treatment are expected to vary with strain of *C. perfringens* and with conditions. To encompass the measurements described above, ϕ for type A, CPE-positive strains is modeled as variable with a triangular distribution ranging from 1% to 10%, with a mode of 5%. A sensitivity analysis is performed on these parameters and distribution shape to determine the effect of this set of assumptions.

3.10. The fraction (f_{vma}, f_{smA}, f_{vsA}, and f_{ssA}) of *C. perfringens* cells that are type A, CPE-positive

C. perfringens food poisoning is caused by *C. perfringens* type A, CPE-positive (see *Hazard Identification*), and is not typically associated with other types of *C. perfringens*. Measurements and estimates of concentrations in foodstuffs (above) have been made without regard to the type of strain, or to toxin production potential. Consequently, it was necessary to estimate the fraction of *C. perfringens* cells and spores that are type A, CPE-positive. As seen below, no data were available to distinguish how such fractions might vary throughout the preparation of foods, nor to distinguish between vegetative cells and spores in raw meat (presumably the measurements in spices were of spores). Thus no data are available to distinguish the fractions identified as f_{vmA} and f_{smA} in Equation (3.1), nor to distinguish the fractions identified there as f_{vsA} and f_{ssA}. In the analysis that follows, each pair of fractions is assigned a single value. These fractions represent the probabilities for any *C. perfringens* isolate found in food to be type A, CPE-positive. It is possible that such probabilities vary in systematic ways, perhaps geographically or temporally. However, in this analysis they are treated as independent of the particular serving of RTE or partially cooked food — they are not variable, only uncertain.[25]

3.10.1. Selection of studies measuring prevalence of type A strains, prevalence of CPE-positive strains, or both

Experimental measurements that may allow some inference about the proportion of type A and/or CPE-positive strains are summarized in Table 3.17. The studies by Kokai-Kun *et al*. (1994), Skjelkvale *et al.* (1979) and Rodriguez-Romo *et al*. (1998) measured only the fraction of

[25] The analyses described in this section are performed in the workbook CP_typeA.xls included with the risk assessment.

samples that were positive for the *C. perfringens* enterotoxin gene (*cpe*) rather than those that were type A. Songer and Meer (1996) and Daube *et al.* (1996) measured both genotype and *cpe* status (presence of DNA for the CPE toxin[26]), the former also demonstrating excellent agreement between *cpe* status and CPE toxin production in classically characterized cell lines.

The first four studies listed in Table 3.17 were measurements of isolates from mammalian or food samples, whereas the last (Rodriguez-Romo, 1998) was of isolates from spices. The first four studies were therefore considered most appropriate to use for estimating the prevalence of type A, CPE-positive strains in raw meats, while the last was used only for estimation of prevalence in spices.

Table 3.17 Proportion of *C. perfringens* environmental isolates that were type A.

Reference	**Source**	**No. Samples**	**% *cpe*-positive**	**% both *C. perfringens* type A and *cpe*-positive**	**% of *cpe*-positive not *C. perfringens* type A**	**Experimental Method**
Songer and Meer, 1996	USA; primarily human and mammal isolates	616	8.1 % (50/616)	7.1 %	12 % (6/50)	PCR analysis
Daube *et al.*, 1996	Belgium; primarily human and mammal isolates	2,659	1.8 %	1.6 %	12.2 % (6/49)	Colony hybridization with DNA probes
Kokai-Kun *et a*l., 1994	Canada and USA; primarily human and mammal isolates	454	3.5 %	3.1%[a]		PCR analysis
Skjelkvale *et al*., 1979	UK and Norway; mammal feces, meats and foods	168 (not associated with outbreaks or infections)	1.2%	1%[a]		Functional enterotoxin assay
Rodriguez-Romo *et al.*, 1998	Spices in Mexico	188	4.3%	3.7%[a]		Dot-blot with DNA probes.

[a] Percent *cpe*-positive *C. perfringens* type A adjusted by the percent of *cpe*-positive strains not *C. perfringens* type A (~12%).

The summary proportions in Table 3.17 may overestimate or underestimate the proportion of *C. perfringens* type A spores capable of causing *C. perfringens* food poisoning for several reasons, including:

[26] CPE refers to the fully formed *C. perfringens* enterotoxin protein. *cpe* refers to the DNA gene encoding the CPE toxin.

1) The studies did not evaluate whether the isolates containing *cpe* actually produce the enterotoxin (CPE). It is therefore possible that some of the isolates were not capable of causing disease (Kokai-Kun *et al*., 1994). This would result in an overestimate of the proportion of *C. perfringens* type A spores capable of causing *C. perfringens* food poisoning.

2) The studies did not distinguish between *C. perfringens* type A cells that harbored *cpe* on a plasmid and those that harbored *cpe* on the chromosome. Cells of the former are thought to cause sporadic gastrointestinal illness that is not related to food poisoning. Therefore, these cells harboring *cpe* on the plasmid most likely do not represent *C. perfringens* spores capable of causing foodborne disease (Sarker *et al*., 2000). This would result in an overestimate of the proportion of *C. perfringens* type A spores capable of causing *C. perfringens* food poisoning.

3) Isolates were obtained in a non-random fashion, with often unidentified fractions of them from humans, mammals, or food samples associated with intestinal, if not diarrheal, illness. In particular, the Songer and Meer (1996) isolates appear to have been heavily biased to CPE-positive strains (at least 44% of the isolates from Pennsylvania were identified as CPE-positive; the sources were listed as human, human food or unknown, with no statement as to association with human disease). Daube *et al.* (1996) indicated that of 769 samples (providing their 2659 isolates), 76 were associated with diarrhea (37/46 in humans, although clostridial disease was not suspected), 458 with enterotoxemia, and 10 with necrotic enteritis. This could result in either an over or underestimate of the proportion of *C. perfringens* type A spores capable of causing *C. perfringens* food poisoning depending on how representative these studies are of the prevalence of *C. perfringens* type A spores in meats.

4) The proportion of environmental *C. perfringens* isolates that are of type A may not accurately mirror that found in meat products either before or after initial processing. This could result in either an over or underestimate of the proportion of *C. perfringens* type A spores capable of causing *C. perfringens* food poisoning depending on the true prevalence.

Very few isolates were stated to be derived solely from human foods not associated with disease outbreaks. A subset of 45 of the isolates in Daube *et al.* (1996) was identified as coming from 32 samples of human food not associated with human disease episodes; all isolates in this subset were type A and *cpe*-negative. Of 17 isolates from human food identifiable in Songer and Meer (1996), all were type A, and at least one was *cpe*-positive. However, association with or independence of disease was not reported for these isolates. Of 168 isolates from meat carcasses, minced beef, food and feces not associated with disease, and pig feces, 2 were *cpe*-positive, both in pig feces (Skjelkvale *et al.*, 1979).

In view of the non-randomness identified above in the Songer and Meer (1996) isolates, these data were not used. Selected data from Daube *et al*. (1996), Kokai-Kun *et al*. (1994), and

Skjelkvale *et al*. (1979) were used: to increase the representativeness of the data from these papers, only isolates associated with cattle, sheep, pigs, fowl, and human food (not associated with food-poisoning outbreaks) were analyzed to estimate the proportion of type A, CPE-positive cells associated with meat and meat products. For spices, the only data available are from Rodriguez-Romo *et al*. (1998), and these were used.

There are limited other data that may be correlated with the proportion of *C. perfringens* type A present in foods (Table 3.18). These studies estimated the frequency of *C. perfringens* heat resistant strains in raw and processed meats. These data were not used for the reasons stated below:

1) *C. perfringens* strains were not typed.

2) *C. perfringens* strains were not analyzed for the *cpe* gene or the CPE toxin.

3) Though heat resistance is correlated with those *C. perfringens* strains that cause *C. perfringens* food poisoning, heat resistance alone does not predict the potential to cause human disease.

4) Changes have occurred in the slaughter and processing conditions during the past 35 years that may have affected the fraction of *C. perfringens* type A present.

Table 3.18 Proportion of heat resistant *C. perfringens* among food samples.

Reference	Source	Samples	Heat-resistance	% heat resistant *C. perfringens* spores
Hall and Angelotti, 1965[a]	OH, USA	Raw and processed meats	Spores "resisted heating at 100 °C for 30 min or more."	1.9% (2/108)
McKillop, 1959[b]	Scotland, UK	Raw beef, sausage and chicken	Samples "immersed in a bath of boiling water for 15 mins."	3.6% (2/55)
Bauer *et al*., 1981[a]	GA, USA	Pork	Spores surviving heating at 95 °C	6% (2/34) at 30 min 0% (0/34) at 60 min
Hobbs and Wilson, 1959[b]	Imports to UK from 4 unknown countries	Veal, beef, lamb, mutton, pork	Meat sample jars were “steamed for one hour.”	11% (76/722) Boneless 1.5% (3/195) Carcass
Weadon, 1961[b]	UK	Raw meats	Meat sample jars placed in “shallow water bath kept constantly boiling for 1 hr.”	18% (130/714)

a. Authors isolated *C. perfringens* vegetative cells, induced sporulation, then tested for heat resistance.
b. Authors heat exposed samples, and then tested samples for presence of *C. perfringens*.

3.10.2. Analysis of selected studies for the fraction of *C. perfringens* in raw meat and spices that are type A, CPE-positive

To estimate the fraction of *C. perfringens* cells and spores in raw meat and spices that are type A, CPE-positive, selected data from Daube *et al*. (1996), Kokai-Kun *et al*. (1994), and Skjelkvale *et*

al. (1979) were used for raw meat; and from Rodriguez-Romo *et al*. (1998) for spices. No data specifically distinguishing spores from vegetative cells were located; the fractions were assumed to be identical.

Daube *et al.* (1996) typed their isolates using gene probes, and similarly identified those isolates that were *cpe*-positive. In view of the good agreement observed between genotype and phenotype (Songer and Meer, 1996) for all toxins (including CPE), the genotype was assumed in this analysis to correspond to the phenotype (for both the type A/non-type A and CPE/non-CPE dichotomies), although in principle (at least for CPE) the two may be different because *cpe* could be located on a plasmid rather than in the chromosome (Sarker *et al*., 2000). Kokai-Kun *et al*. (1994), Skjelkvale *et al*. (1979) and Rodriguez-Romo *et al*. (1998) provided data only on *cpe* status. The selected information (the set of all data on isolates associated with cattle, sheep, pigs, fowl, and human food and not associated with food-poisoning outbreaks) is summarized in Table 3.19.

Table 3.19 Summary of selected data analyzed for fraction of *C. perfringens* expected to be type A, CPE-positive.

Source of data	Type	Number of isolates	
		cpe-positive	*cpe*-negative
Daube *et al.* (1996)	Type A	8	1780
	Non-A	4	20
Kokai-Kun *et al.* (1994)	Unknown	5	201
Skjelkvale *et al*. (1979)	Unknown	2	166
Rodriguez-Romo *et al.* (1998)	Unknown	8	180

Preliminary analysis showed that the *cpe*-positive fractions in the first three studies are homogeneous, and this was subsequently confirmed by the analysis described below. It was assumed that among the individual cells of *C. perfringens* infecting meat products (either as spores or vegetative cells) there is a fraction A^+ that are type A, *cpe*-positive, a fraction nA^+ that are non-A, *cpe*-positive, a fraction A^- that are type A, *cpe*-negative, and a fraction $nA^-=1-(A^+ + nA^+ + A^-)$ that are non-A, *cpe*-negative. Similarly, for spices there are corresponding fractions S^+, nS^+, S^-, and $nS^-=1-(S^+ + nS^+ + S^-)$. Then the observations in Table 3.19 are binomial samples, allowing the corresponding loglikelihood for their observation to be written using suitable combinations of these probabilities. The contribution to the loglikelihood from each entry in Table 3.19 can be written as

$$r \ln\left(pN/r\right) \qquad \textbf{(3.11)}$$

where r is the observed count, N is the total number observed in the study, and p is a suitable combination of the probabilities A^+, nA^+, A^-, nA^-, S^+, nS^+, S^-, and nS^- (see Table 3.20).

For spices, there are insufficient data to estimate what fraction of the isolates are type A or non-A. It was assumed that within each *cpe* category (+ and –), the relative fraction of type A and

non-A were the same as for meat and other foods (the first three studies listed). That is, the additional constraints

$$nS^{+} = S^{+}\, nA^{+} / A^{+}$$
$$nS^{-} = S^{-}\, nA^{-} / A^{-} \qquad \textbf{(3.12)}$$

were imposed. It then follows that

$$S^{-} = \frac{1 - S^{+}\left(1 + nA^{+}/A^{+}\right)}{1 + nA^{-}/A^{-}} \qquad \textbf{(3.13)}$$

Table 3.20 Probabilities for each entry in Table 3.19.

Source of data	Type	Probabilities	
		cpe-positive	*cpe*-negative
Daube *et al*. (1996)	Type A	A^{+}	A^{-}
	Non-A	nA^{+}	$1-(A^{+}+nA^{+}+A^{-})$
Kokai-Kun *et al.* (1994)	Unknown	$A^{+}+nA^{+}$	$A^{-}+nA^{-}=1-(A^{+}+nA^{+})$
Skjelkvale *et al*. (1979)	Unknown	$A^{+}+nA^{+}$	$A^{-}+nA^{-}=1-(A^{+}+nA^{+})$
Rodriguez-Romo *et al.* (1998)	Unknown	$S^{+}+nS^{+}$	$S^{-}+nS^{-}=1-S^{+}+nS^{+}$

With these assumptions, maximum likelihood estimates for the independent parameters A^{+}, nA^{+}, nA^{-}, and S^{+} were obtained (any four parameters can be treated as the independent ones, and the maximum likelihood estimates are just the obvious values obtained as ratios of the values in Table 3.19, but only A^{+} and S^{+} are of direct interest here). Using the loglikelihood contributions normalized as in Equation (3.11) ensures that the loglikelihood behaves approximately as a χ^2_6 variate, allowing a test for homogeneity between the studies. They are homogeneous by this test (p=0.54).

Uncertainty estimates were obtained by first finding a suitable transform to make the profile likelihoods for A^{+} and S^{+} approximately normal (see Appendix 3.1 for discussion of such transformations). The transformations selected are

$$u = \left(\frac{A^{+}}{1-A^{+}}\right)^{0.4} \quad \text{and} \quad v = \left(\frac{S^{+}}{1-S^{+}}\right)^{0.25} \qquad \textbf{(3.14)}$$

which give excellent normal approximations to the profile likelihoods at least out to 4.5 standard deviations. The maximum likelihood estimates for A^{+}, S^{+}, u, and v are given in Table 3.21.

Table 3.21 Maximum likelihood estimates for the fractions of cells that are type A, CPE-positive.

A^+	0.00579
S^+	0.0284
u	0.128
v	0.413

Re-writing the likelihood in terms of u and v allowed quadratic approximation of their local joint profile likelihood using an information matrix (estimated by separately and together making increments in u and v equal to about 1.5 times the standard deviations indicated by their individual profile likelihoods, re-optimizing with respect to the nuisance parameters nA^+ and nA^-, and solving the resultant simultaneous quadratic equations for the change in loglikelihood). An estimate of the variance-covariance matrix for u and v was then obtained by inverting the information matrix. The resultant estimates for standard deviations and the correlation coefficient are given in Table 3.22.

Table 3.22 Standard deviations (main diagonal) and correlation coefficient (off-diagonal) for the uncertainty distribution of u and v.

	u	v
u	0.0156	0.257
v	0.257	0.0417

3.11. The growth of *C. perfringens* and *C. botulinum*

3.11.1. Modeling growth of *C. perfringens* and *C. botulinum* as a function of temperature and time

Modeling of growth for *C. perfringens* is discussed in technical detail in Appendix 3.2. The methods of that appendix are used here. A model for growth of *C. botulinum* is needed to respond to one of the questions to be answered (Section 1.1), and this is formulated in exactly the same way as for *C. perfringens*.

Growth from spores of *C. perfringens* at fixed temperatures after a heat treatment and in suitable surroundings may be characterized by a delay period t_m during which the activated spore converts to a vegetative state and prepares for cell division. The resultant vegetative cell then enters the growth phase in which cell division occurs regularly, causing an exponential increase with time in cell density, until the density of vegetative cells becomes so high that some aspect(s) of the environment becomes unfavorable for further growth (for example, the cells might run out of food, or produce mutually self-inhibitory chemicals). The growth phase is characterized by a doubling time (the time for cell density to double) or a growth rate (the ratio of the rate of increase in cell density to the cell density itself). The growth rate, symbolized by μ and measured in units of inverse time, is used here. Subsequent behavior, after the vegetative cells have reached the stationary phase at high cell density, is of less concern to this risk assessment.

Cell densities would generally decline somewhat, and in suitable conditions the vegetative cells might start sporulating. In favorable environments, such as meats, cell densities in the stationary phase may reach 10^8 to 10^{10} cells per gram. Where necessary in this risk assessment, it is assumed that cells remain at the same high density in stationary phase — although in foods, *C. perfringens* at such cell densities generally imparts a definite "off" odor and taste.

As discussed in Appendix 3.2, the delay period t_m and the growth rate μ depend on the history of the spore or vegetative cell's environment. The temperature of the environment has a major effect on both, although it is generally believed that μ at any time depends principally on the temperature at the same time, whereas t_m depends strongly on temperature history. For constant temperatures, this risk assessment uses a primary growth[27] model of the form

$$C_s(t) = C_0\left(1 - I\left(a+1, at/t_m\right)\right)$$

$$C_v(t) = f\left(t, T, C_0, \mu, t_m, C_m, a\right) \equiv C_m \frac{z(t)}{1+z(t)} \qquad \textbf{(3.15)}$$

$$z(t) = \frac{C_0}{C_m} e^{\mu t}\left(\frac{a}{a+\mu t_m}\right)^{a+1} I\left(a+1, t\left(\mu + a/t_m\right)\right) \qquad \textbf{(3.16)}$$

where I is the incomplete gamma integral

$$I(\alpha, x) = \frac{1}{\Gamma(\alpha)}\int_0^x w^{\alpha-1} e^{-w}\, dw \qquad \textbf{(3.17)}$$

and the various terms are

- $C_s(t)$ the spore cell density at time t,
- $C_v(t)$ the vegetative cell density at time t,
- f the mathematical function representing the primary model,
- C_0 the initial spore density (cells/gram),
- T the temperature, with $\mu = \mu(T)$ and $t_m = t_m(T)$,
- C_m the maximum density of cells that can be supported, and
- a an additional variance parameter of the model that indicates how variable t_m is between individual spores under similar conditions (the standard deviation of t_m is approximately $t_m/\sqrt{a}$).

The secondary models describe how μ and t_m vary with temperature; both are of Ratkowsky form,[28] the first for μ and the second for $1/t_m$. These curves may be characterized by maximum and minimum temperatures, the location of the maximum of the curve, and the magnitude of the curve at the maximum (see Appendix A3.2.4). The models take the form

$$\mu = \mu(T) = A_m \frac{(1-x)^2\left(1-\exp\left(-\theta_m x\right)\right)}{N_m} \qquad \textbf{(3.18)}$$

[27] The "primary" model is the fixed temperature model that relates cell density to time. The "secondary" models describe how the parameters of the primary model vary with temperature. The primary model here is "model 3" of Appendix 3.2

[28] The Ratkowsky form is used because that is the form used in the majority of the literature. L. Huang (personal communication 2004) has pointed out that the Ratkowsky shape may be inadequate for modeling the variation of growth rate, particularly at temperatures near T_{max}.

and

$$1/t_m = 1/t_m(T) = A_t \frac{(1-x)^2 \left(1-\exp(-\theta_t x)\right)}{N_t} \quad \textbf{(3.19)}$$

where

$$x = \frac{T_{\max} - T}{T_{\max} - T_{\min}} \quad \textbf{(3.20)}$$

is a location on the curve and the terms are:

T	the temperature,
$T_{\max}$	the maximum temperature for growth or progression through the delay period,
$T_{\min}$	the minimum temperature for growth or progression through the delay period,
θ, N	functions of the location of the maximum of the curve (see Equations (A3.2.33) and (A3.2.34)).

3.11.2. Method of evaluation of growth rates of *C. perfringens* and *C. botulinum*

The primary model (Equations (3.15) and (3.16)) was used to fit measured growth of *C. perfringens* at fixed temperatures. Data on estimated cell densities as a function of time were obtained (personal communications, 2003, with L. Huang, H. Marks, and V.K. Juneja) for the experiments described by Juneja *et al.* (1999) in broth; Juneja *et al.* (2001) in cooked cured beef; Juneja and Marks (2002) in cooked cured chicken; Huang (2003) in cooked ground beef; and Juneja and Marks (1999) for *C. botulinum* in reinforced Clostridial medium (RCM) supplemented with oxyrase enzyme. These experiments were performed with the sterile growth medium initially inoculated with spores that were then activated to germinate with a heat treatment. Growth media were maintained at constant temperatures thereafter, and samples taken (either by sub-sampling liquid media, or the use of multiple small samples of meat media) at appropriate intervals to measure cell counts by plating.

It was assumed in these experiments that what was measured (as CFU/g) was the sum of vegetative cell and remaining spore densities, $C_s(t) + C_v(t)$ in the notation of Equation (3.15), and that the logarithms of the experimentally estimated CFU/g have normal measurement errors[29] with equal standard deviations at all cell densities. For each temperature replicate in each experiment (with multiple temperatures), the values of C_0, μ, and t_m were estimated. For each experiment, the parameters C_m, a, and the common standard deviation for the measurement errors were estimated. The method of estimation used was maximum likelihood — all parameters associated with a given experiment were obtained simultaneously by maximizing the likelihood with respect to all those parameters. The original investigators' censoring of the measurement data was used — where original authors censored whole replicates for microbiological or experimental reasons (*e.g.* suspected overgrowth, bad thermostat) the same censoring was performed. Where replicates were dropped from analysis by the original authors because there were too few data points to support their analysis approach, the same was generally done, unless those data could sustain the current analysis approach. For Juneja *et al*. (2001) the data above the early exponential part of the growth curve were not censored as in that

[29] In this analysis, the measurement error is assumed to measure the deviations (assumed random) from an ideal mathematical form that occur for the time points within each replicate growth curve.

original paper (which used an approximation to the growth curve only valid in the early portion of the curve), since the growth curve used here tracks the growth curve above that region.[30]

This approach allowed evaluation of maximum likelihood estimates for all the parameters for each experiment, except for the variance parameter a. The likelihood function is a very slow function of a, because the experiments are not sensitive to its value — its value affects only the shape of the growth curve between the initial constant spore density (during the delay period) and the period of exponential growth. A value of $a = 100$ was selected (corresponding to an assumption of about 10% standard deviation in the delay t_m among individual spores).[31]

For subsequent evaluation of the secondary models, the maximum likelihood estimates for all the other parameters (except a) were obtained, and the information matrix for $\ln(\mu)$ and $\ln(t_m)$ estimated numerically for each temperature replicate at fixed values for C_0 for that temperature replicate, and for the experiment-wide C_m and the standard deviation of measurement errors.[32] This information matrix measured the variation in $\ln(\mu)$ and $\ln(t_m)$ to be expected based on the measurement errors only.

Mathematically, for a replicate (a single growth versus time curve at fixed temperature and identical initial conditions) with index i within an experiment (multiple growth curves, possibly including multiple replicates at each temperature), it is expected that

$$\ln\left(C_{ij}\right) = \ln\left(f\left(t_j, T_i, C_{0i}, \mu_i, t_{mi}, C_m, a\right)\right) + \varepsilon \qquad \textbf{(3.21)}$$

where C_{ij} is the CFU/g after time t_j in a replicate experiment at temperature T_i, f is the primary model, and ε is normally distributed error term with mean zero and standard deviation σ.

C_m, a, and σ are experiment-wide parameters, while C_{0i}, μ_i, and t_{mi} apply to this replicate (numbered i). The term σ represents the experimental error. The conditional loglikelihood for the expectation represented by Equation (3.21) (given C_{0i}, μ_i, and t_{mi}) is:[33]

$$J = \sum_i J_i = -\sum_{i,j}\left(\ln\sigma + \frac{\left(\ln\left(C_{ij}/f_{ij}\right)\right)}{2\sigma^2}\right)$$

where **(3.22)**

$$f_{ij} = f\left(t_j, T_i, C_{0i}, \mu_i, t_{mi}, C_m, a\right)$$

Now find maximum likelihood estimates for all the parameters, and compute the information matrix for each $\ln(\mu_i)$ and $\ln(t_{mi})$ at fixed values for the other parameters. Then for each replicate

[30] Except for points in two replicates, both at 21.1°C. The last point in the first replicate and the last 3 in the second replicate were censored (as was done by the original authors). The first one dropped 2 logs between 48 and 54 hours, the second 1.94 logs between 39 and 44 hours and stayed down at 48 and 53 hours.

[31] Further analysis testing the effect of varying values of a might be appropriate.

[32] This underestimates the uncertainties slightly through failure to take account of the co-variance of these other parameters. However, the effect appears to be small.

[33] It would be preferable to start with the experimental colony count data and explicitly convolve the Poisson uncertainty associated with counts with an additional experimental uncertainty. The analysis given here corresponds to starting with estimates of CFU/g obtained from those colony count data.

approximate the interesting part (*i.e.* just the part involving μ_i, and t_{mi}) of the conditional likelihood by a normal that looks like:

$$\exp(J_i) \sim |B_i|^{1/2} \exp(-x_i' B_i x_i)$$
$$\text{where} \qquad \textbf{(3.23)}$$
$$x_i = (\mu_i - \mu_i^*, t_{mi} - t_{mi}^*)'$$

and * denotes maximum likelihood estimate, ‘ denotes transpose, and B_i is the information matrix for $\ln(\mu_i)$ and $\ln(t_{mi})$.

The Ratkowsky equations for μ and $1/t_m$ (secondary models) were estimated by assuming that $\ln(\mu)$ and $\ln(t_m)$ have normally distributed variabilities about the Ratkowsky equations (in addition to their uncertainties of measurement). These variabilities are taken to represent the experiment-to-experiment variation in μ and $1/t_m$, and are subsequently used as surrogates for variations that are expected in different food media, between different strains, and under different conditions (except temperature). The variabilities are represented in the analysis by a variance-covariance matrix that allows evaluation of any correlation between the variation in μ and the variation in $1/t_m$. To estimate the parameters of the Ratkowsky equations, and the magnitude of the experiment-to-experiment variability variance-covariance matrix components, the total variation in $\ln(\mu)$ and $\ln(t_m)$ is estimated by a variance-covariance matrix equal to the sum of the experiment-to-experiment variability variance-covariance matrix, and the inverse of the information matrix representing experimental errors. All parameters of the Ratkowsky equations and the experiment-to-experiment variability variance-covariance matrix were then estimated by maximum likelihood. There are nine parameters involved for each experiment — $T_{\min}$, $T_{\max}$,[34] two parameters each for the Ratkowsky curves for each of μ and $1/t_m$, two variances and one covariance for the experiment-to-experiment variability.

Mathematically, it was assumed that

$$\ln(\mu_i) = \ln(R(T_i, X_m, A_m, T_{\min}, T_{\max})) + \eta$$
$$\ln(1/t_{mi}) = \ln(R(T_i, X_t, A_t, T_{\min}, T_{\max})) + \phi \qquad \textbf{(3.24)}$$

where R is the secondary model (of Ratkowsky form) with parameters $T_{\min}$, $T_{\max}$,[35] X (location of maximum) and A (height at the maximum), with subscripts m and t distinguishing values for μ and t_m. The terms (η,ϕ) represent variability from replicate to replicate, and are assumed to be jointly normal with zero mean and variance-covariance

$$Q = \begin{pmatrix} s_m^2 & c_{mt} \\ c_{mt} & s_t^2 \end{pmatrix} \qquad \textbf{(3.25)}$$

Then the loglikelihood (not conditioned on μ_i, and t_{mi}) for replicate i can be approximated by the loglikelihood for a normal form with variance-covariance matrix $Q + B_i^{-1}$ (this comes from the relevant convolution integral over μ_i, and t_{mi}). Summing these over all replicates gives a loglikelihood for the whole experiment. The nine parameters $T_{\min}$, $T_{\max}$, X_m, A_m, X_t, A_t, s_m, c_{mt},

[34] Based on previously published analyses, $T_{\min}$ and $T_{\max}$ were assumed to be equal for the Ratkowsky equations for μ and t_m.

[35] It was assumed that the same maximum and minimum temperatures apply to the growth rate μ and the delay time t_m.

and s_t are then estimated maximizing that loglikelihood, and the uncertainties in the parameters (and the correlations between those uncertainties) by computing the inverse of their information matrix.

3.11.3. Results for growth rates of *C. perfringens* and *C. botulinum*

The experiments on cured chicken and cured beef (Juneja *et al.*, 2001; Juneja and Marks, 2002) give results for all 9 parameters that are statistically indistinguishable. The cooked ground beef data (Huang, 2003) provides maximum likelihood estimates that are distinct, but apparently largely because the analysis attempts to estimate 9 parameters from only 6 growth curves, each giving a μ and t_m estimate, but with no replicate information available at each temperature. The estimates of s_m, c_{mt}, and s_t obtained from these data appear to be anomalously low.[36] With the variance-covariance matrix forced to be identical to that obtained from the cured chicken and cured beef experiments, the maximum and minimum temperatures, $T_{\min}$ (12.5 °C), $T_{\max}$ (53.5 °C), and the shape parameters X_m and X_t for the Ratkowsky curves all agree with the cured beef and cured chicken ones, although there appears to be faster growth (by a factor of 1.9) and shorter times to start division (about 1.6-fold shorter). The difference in growth rate and time to start division are expected because of differences in growth media, so we adopted the analysis with variance-covariance matrix forced to be identical with the cured chicken and cured beef analyses. The broth data (Juneja *et al*., 1999) have statistically different $T_{\min}$ and $T_{\max}$ (13.6 to 54.1 °C). The Ratkowsky growth curve has the same shape as for beef and chicken, but a different amplitude; but the Ratkowsky curve for $1/t_m$ has a different shape. It seems plausible that the curve shape for growth is universal (for these *C. perfringens* strains), with growth rates dependent on experimental conditions; but the curve shape for $1/t_m$ (1/time-to-division) likely depends on activation methods (Figure 3.4 plots the Ratkowsky growth-rate versus temperature curves with parameter values estimated from the data).

It was judged that the most representative estimates for parameters for use in this risk assessment are those corresponding to the cooked cured beef and cooked cured chicken experiments, modified as described below. The parameter estimates for cooked ground beef are similar, but with higher growth rate and shorter delay period, as would be expected for conditions that are probably close to ideal for the *C. perfringens* strains used. The parameter values estimated for cooked cured beef and cooked cured chicken are given in Table 3.23:[37]

[36] This may partly be because at least some of the data are averages of up to three experiments, but such averaging cannot be the whole explanation.

[37] Jointly estimated with those for cooked ground beef, with only the amplitudes of the Ratkowsky curves allowed to differ for the cooked ground beef.

Table 3.23 Maximum likelihood estimates for growth parameters for *C. perfringens* in cooked cured beef and cooked cured chicken.

T_{min} (°C)	12.5
T_{max} (°C)	53.5
A_m (per hour)	2.084
X_m	0.250
A_t (per hour)	0.455
X_t	0.193
s_m	0.347
c_m	0.046
s_t	0.362

Note: parameters are defined in Sections 3.11.1and 3.11.2

The uncertainties in these parameters are given in the standard-deviation/correlation matrix shown in Table 3.24.

Table 3.24 Standard deviations (diagonal) and correlation coefficients (off-diagonal) for the parameter estimates of Table 3.23.

T_{min} (°C)	0.211	0	0	0	0	0	0	0	0
T_{max} (°C)	-0.050	0.912	0	0	0	0	0	0	0
A_m (per hour)	0.217	0.116	0.128	0	0	0	0	0	0
X_m	0.150	0.226	-0.157	0.005	0	0	0	0	0
A_t (per hour)	-0.073	-0.280	-0.341	0.004	0.046	0	0	0	0
X_t	0.293	0.601	0.164	0.091	-0.692	0.026	0	0	0
s_m	0.027	0.015	0.058	0.047	-0.006	0.020	0.040	0	0
c_m	-0.092	-0.044	0.057	-0.023	0.165	-0.127	0.502	0.026	0
s_t	-0.031	-0.017	-0.020	-0.009	0.228	-0.082	0.154	0.552	0.050

Note: parameters are defined in Sections 3.11.1and 3.11.2

The *C. botulinum* data (Juneja and Marks, 1999) give (T_{min}, T_{max}) as 8.2 to 50.03 °C, where additional constraints based on no observed growth for 11 weeks at 11 °C and 50 °C have been applied (in the likelihood estimation) at these temperatures, by specifying t_m > 504 hours in both cases (using the Ratkowsky curve prediction). It is likely that the Ratkowsky curve shape is not

ideal at either end of the range of temperatures, so this strong constraint at the top end may distort the estimated curve away from the data. [38]

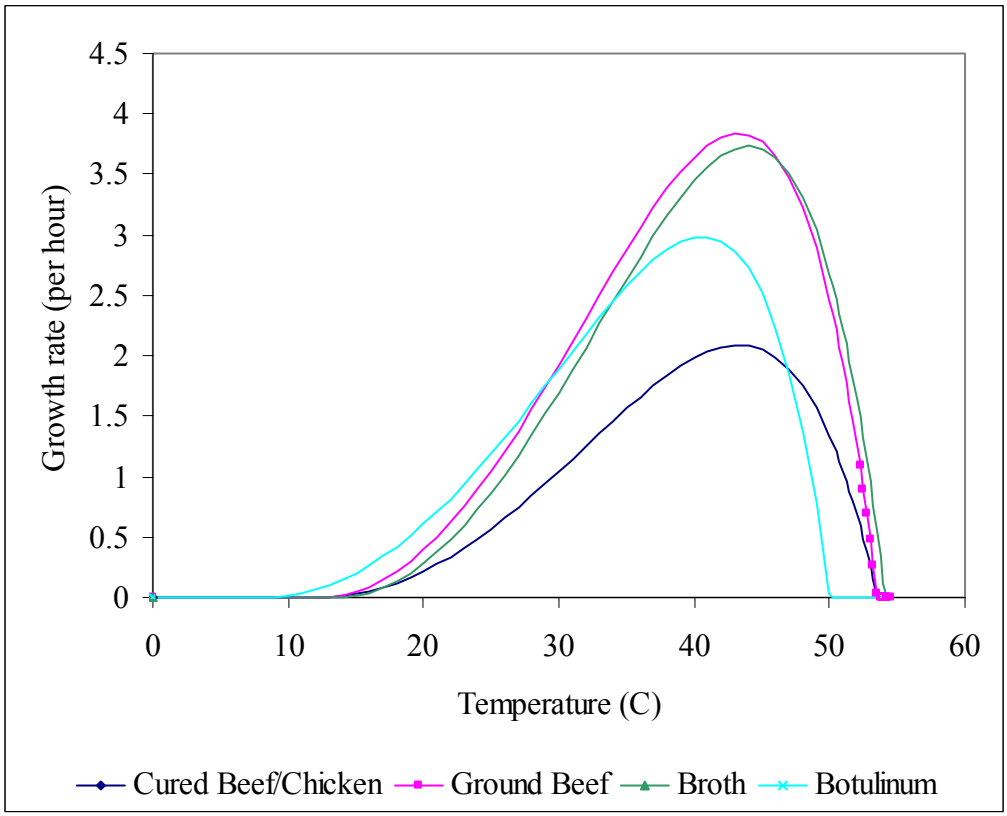


Figure 3.4 Average growth rates of *C. perfringens* in the three media indicated, and of *C. botulinum* in a laboratory medium, and how these rates are estimated to vary with temperature.

3.11.4. Comparison with published growth rates.

The results of Section 3.11.3 apply strictly to just the experiments analyzed. Those experiments were performed on a mixture of three strains of *C. perfringens*, under tightly controlled conditions. The variations between them may therefore underestimate the variations to be expected between growth conditions and strains in RTE and partially cooked foods. In an attempt to evaluate any bias in the results, and to identify any major additional variability, a literature review of growth rates was conducted, to construct a compilation of 174 reported measurements of generation times for *C. perfringens* within meat foods. This compilation

[38] L. Huang has pointed out (personal communication, 2004) that the estimated upper temperature limit for *C. perfringens* may be too high, based on his unpublished laboratory observations. The maximum temperature for which data are reported in the literature is 50 °C (at which temperature growth still occurred), and no limits on growth rate was identified for higher temperatures. Thus for *C. perfringens* the estimated T_{max} is an extrapolation based on the Ratkowsky curve, which may have the wrong shape near T_{max}. For *C. botulinum*, the estimation procedure incorporated a published stringent bound on growth rate at 50 °C. The qualitative feature of a small range of temperatures around and above 50 °C where the growth rate of *C. perfringens* substantially exceeds that of *C. botulinum*, or where *C. perfringens* can grow but *C. botulinum* cannot, is solidly based in observations.

includes almost all measurements that could be identified.[39] The measurements generally were for cooked meat, but include some measurements on raw meat. However, no results were included that resulted from experiments in liquid media or only on the surface of meat. The strains used were identified as:

1362
5 strain composite (NCTC 8679, 8238, 8239, R42, PS44)
8 strain composite (NCTC 8238, 10240, 8797, 8798, 8239; ATCC 3624; S-40, S-45)
8-strain composite (NCTC 8238, 10240, 8798, 8239, 9851; ATCC 3624; S-40, S-45)
ATCC 3624
F2985/50
FD-1
FD-1041
NCTC 8238
NCTC 8239
NCTC 8797
NCTC 8798
S40
S45

The measurements were at temperatures varying from 12 °C to 51 °C. Some of the estimates obtained have considerable uncertainty, since they were obtained from just two points, and/or were obtained by digitizing graphs in the papers.

To compare with the results of Section 3.11.3, the ratio of observed to predicted generation time was constructed, where the "predicted" value is that obtained using the parameters given in Table 3.1. Figure 3.5 shows the distribution of the logarithm of observed to predicted generation times on a normal scale. There are exactly 3 outliers where the model predicts growth rates much lower than observed (generation times much longer). All three are at low temperatures.

- 12 °C, Solberg and Elkind (1970). The observed generation time is 580 minutes, estimated from Figure 5 of the paper, with a model estimate of zero growth (this is shown on Figure 3.5 with a generation time arbitrarily set to 50,000 minutes). This is the only available measurement at such low temperatures (although there are several reports of no growth at 10 °C).

- 15 °C, Juneja *et al.* (1994b). The observed generation time is 43.2 minutes (strain NCTC 8238), with a model estimate of 1660 minutes.

- 15 °C, Juneja *et al.* (1994b). The observed generation time is 43.2 minutes (strain NCTC 8239), with a model estimate of 1660 minutes.

Four other measurements at 15 °C were located in the literature, three of them by Juneja *et al.* (1994b) with the same strains, one by Solberg and Elkind (1970), where the model also

[39] One reference, Naik & Duncan (1977), was obtained too late for inclusion. Smith (1963) includes a graph showing generation times for 5 unidentified strains at 5 °C temperature intervals from 20 °C to 50 °C that was recognized too late for inclusion.

underestimates growth rate (1660 minutes generation time) but not so drastically. The next higher temperature measurement located in the literature is 20 °C.

There are 6 cases where the model predicts growth rates substantially (>1.6-fold, but see below about bias) larger than observed. They are not listed here because such overestimates are conservative for the risk assessment (leading to overestimates of risk).

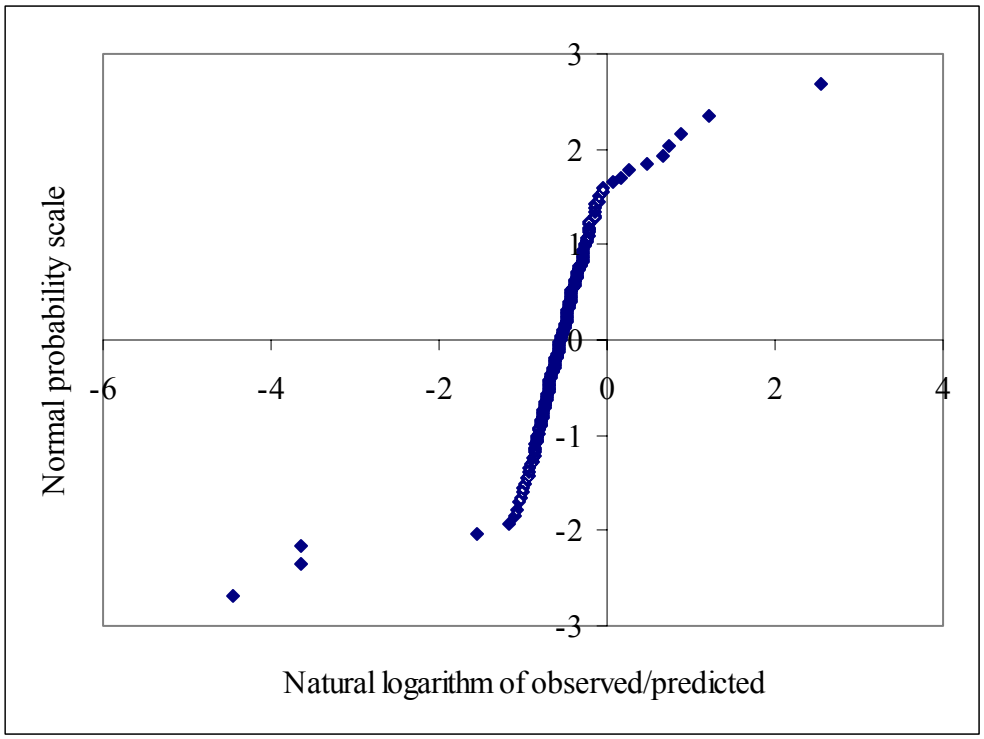


Figure 3.5 Empirical distribution of natural logarithm of observed/predicted ratio of generation times for *C. perfringens* (the most extreme outlier on the left is placed arbitrarily; predicted growth rate is zero, but growth was observed).

The remaining 165 observed/predicted ratios form a lognormal distribution (p=0.55, Shapiro-Wilk statistic; and they look almost exactly straight on a normal probability plot). The median observed/expected generation time is 0.575, so the model generally underestimates published growth rates by about a factor of 1.739. The standard deviation of ln(observed/predicted) is 0.27 (1.3-fold), which is smaller than the similar standard deviation (s_m = 0.35 ± 0.04, 1.4-fold) estimated for the between-experiment variation in the analysis of experiments in Section 3.11.3 (see Table 3.23 and Table 3.24).

The model appears to estimate generation time (growth rate) well, with the following reservations and modifications:
1. The values for growth rates obtained in Section 3.11.3 may be biased to underestimate growth rates. It was considered that the compendium of all published data is more likely to be representative of the distribution of strains and conditions to be expected in meat and spices entering the RTE and partially cooked food chain than the selected experiments analyzed in

Section 3.11.3 (since they were selected by their availability and for the quality of data available for analysis, not their representativeness). All modeled growth rates are therefore increased by a factor of 1.739 to agree with the median of published data[40] (omitting outliers[41]). This should be conservative, although it may not be correct. It is possible that many reported experiments were performed with strains selected to be the fastest growing available, so published generation times may systematically be lower than would be expected for representative selections of strains and conditions.

2. The between-experiment variability in logarithm of growth rate (s_m, Section 3.11.3) estimated in the model fit is large enough to represent the between-situation (between conditions, between strains) variation seen in the published estimates of growth rates. No adjustment to this variability was made.

3. The model may underestimate growth rates at low temperatures, below about 20 °C; this underestimation may come about because of the imposed shape of the Ratkowsky curve — the same underestimation is apparent in the analysis of the experiments of Section 3.11.3. The model predicts no growth below 12.5 °C, but growth has been observed at 12 °C (Solberg and Elkind, 1970). There are very few published data allowing estimates of growth rates below 20 °C.

No similar comparison could be made of estimates of the delay time before exponential growth occurs after heat shock to spores, since there are few such estimates available in the literature. There is some evidence (Juneja and Marks, 2002) that growth rate and delay time are inversely proportional within individual experiments, although the between-experiment variations in growth rates and delay times are practically uncorrelated (see Table 3.24). In view of this evidence, the delay time estimated by the model is similarly decreased by the same factor (median 1.739) as the growth rate is increased. This has very little effect on the modeling performed in this risk assessment, except for the estimates for hot-holding, where it may result in a conservative bias (towards overestimates of illnesses).

The between-experiment variation of delay time is assumed to be adequately represented by the estimates of Table 3.24. A complete accounting for variability would explicitly take account of the likely probabilistic nature of initial cell divisions. However, the measured between-experiment variation incorporates such stochastic variation corresponding to the spore densities used in the experiments. Such variability is probably spore-density dependent (the relative variation increasing at lower spore densities), and most experiments have been with spore densities of around 100 CFU/g. It appears that the major contribution to risk estimates comes from initial spore densities that are lower than 100 CFU/g, so variability of delay times may be underestimated by the between-experiment variation. The extrapolation between spore densities used in growth experiments and those occurring in naturally contaminated servings may thus result in an underestimate in variability in the growth achieved in hot-holding situations[42]. Moreover, the modeling does not incorporate any spore-density-dependent variation of the

[40] This adjustment was added to the model as a lognormal distribution with median 1.739 and an standard error (estimated from the data) of a factor of 1.02.

[41] Outliers were identified initially by eye from Figure 3.5, then confirmed by noting that inclusion of any of them reduced the Shapiro-Wilk statistic (testing for departure from a lognormal distribution) to less than 0.10.

[42] The delay time does not affect any other part of the model for RTE and partially cooked foods, since it is not explicitly used elsewhere.

variability, as would be expected if the initial cell divisions are probabilistic in nature.

3.11.5. Modifications of growth rate by environmental factors

It is expected that the growth rate of *C. perfringens* is influenced by factors other than the temperature. As an example, as the salt and nitrite content of RTE foods increases, it is expected that *C. perfringens* growth is slowed. Similarly, a more acidic environment (low pH) is expected to slow *C. perfringens* growth. Low water activity is expected to slow or halt *C. perfringens* growth. Expectations aside, the challenge for this analysis is to quantify the influences of these physical/chemical factors on *C. perfringens* growth rates.

3.11.5.1. Presence of oxygen.

There is substantial evidence that the presence of oxygen influences the growth of *C. perfringens* in foods (Juneja *et al*., 1994a; Hintlian and Hotchkiss, 1987). Exposure to atmospheric levels of oxygen strongly inhibits the growth of this anaerobic bacterium. However, the manufacturing heat treatment drives off much of the oxygen and thereby provides an acceptable atmosphere for *C. perfringens* to grow. Many RTE foods are cooked in, or rapidly placed in, casings or packagings that help maintain an anaerobic environment. The presence of oxygen was therefore not incorporated into the growth model.

3.11.5.2. Salt and Nitrite effect on growth rate

The presence of nitrites and salt in an RTE food commodity is considered inhibitory of *C. perfringens* growth at levels of 3% salt or greater (see Appendix A). For foods containing nitrite but salt concentrations less than 3%, slower *C. perfringens* growth may occur. For instance, in the range of 1–3% salt, *C. perfringens* growth was slowed in cured and uncured turkey emulsion (Kalinowski *et al.*, 2003), and inhibition by salt (0–2%) of *C. perfringens* growth in a broth mixture including sodium pyrophosphate was also apparent (Juneja *et al.*, 1996b).

To estimate the effect of low salt concentrations in food on the growth of *C. perfringens* the reported data of Kalinowski *et al.* (2003) and Juneja *et al.* (1996b) were examined. The primary growth model was fitted to the data of Kalinowski *et al.* (2003, tables 4 and 5) in cured (156 μg/ml sodium nitrite) and uncured turkey with 1% salt, and a relative growth rate at 2% and 3% salt and 43.3 °C estimated based on the single log(CFU/g) data points published for these salt concentrations and temperature (no growth was observed in the cured turkey at 3% salt). These point estimates of relative growth rate were: 2% — 0.69; 3% — 0.17. Juneja *et al*. (1996b) performed 90 experiments with 45 combinations of conditions according to a partial factorial design for growth of *C. perfringens* in a broth with 0–3% salt, pH 5.5–7, sodium pyrophosphate 0–0.3%, at five temperatures in the range 12–42 °C. They fitted Gompertz models and estimated kinetic parameters from the Gompertz parameter estimates. The published data on exponential growth rate (EGR) were compared with the estimated growth rates at corresponding temperatures from the primary model (Section 3.11.3), and the logarithm of the ratio of these two fitted with a model that included linear and quadratic terms in salt concentration, pH, and pyrophosphate concentration, products in pairs of temperature, salt concentration, pH, and pyrophosphate concentration, and a normal error term (corresponding to the quadratic models of Juneja *et al.*, 1996b, but with all temperature-only terms omitted, since the temperature effect is modeled by the primary growth model). All terms except the linear and quadratic pyrophosphate

term, the temperature-pyrophosphate interaction term, and the quadratic salt term, were non-significant and dropped. The effect of salt could thus be estimated as

$$\ln(R) = k - \lambda S^2 \qquad \textbf{(3.26)}$$

where the terms are

- R ratio of EGR to growth rate μ predicted by the primary model of Section 3.11.3,
- S salt percentage in the broth,
- k a constant accounting for different units of measurement and different conditions for the experiment, and
- λ a coefficient measuring the effect of salt.

The estimated value of λ is 0.179 ± 0.064 (uncertainty standard error; the profile likelihood is very well modeled by a normal distribution) per (salt %)^2, and this value gives estimates for the ratios of the effects at 2% and 3% relative to 1% of 0.58 and 0.24 respectively, consistent (taking account of the uncertainty) with the observations of Kalinowski *et al*. (2003), suggesting that the effect is relatively independent of the growth substrate (ground turkey versus a laboratory broth medium). This estimate for λ is used in the risk assessment, and applied to all foods based on their salt content.

Low concentrations of nitrite appear to affect growth rates independently of salt content, although few data were located to measure the effect quantitatively. Kalinowski *et al*. (2003, tables 4 and 5) report growth curves from spores in cured (156 μg/ml sodium nitrite) and uncured turkey emulsion at 26.7, 32.2, 37.8, 43.3, and 48.9 °C, both at 1% salt content. Growth at the two lower temperatures was substantially suppressed; although some initial growth occurred, the concentration never increased 10-fold, and the measurements are consistent with zero growth. At temperatures closer to the optimum growth temperature, growth rates were reduced by 30–50%

To take some account of the effect of nitrite, the ratio of growth rates for the three higher temperatures was evaluated to be 0.582 ± 0.042 (uncertainty standard error; the profile likelihood is very well modeled by a normal distribution; in the simulation, a normal distribution is used, truncated below at zero). This factor is applied to the estimated growth rates of *C. perfringens* in all Category 1 foods (nitrite-containing) at all salt concentrations and at all temperatures, since it is not known whether the apparent suppression of growth by larger factors occurring at 1% salt and larger temperature deviations from optimum growth conditions would also occur at other salt concentrations.

3.11.5.3. The effect of salt and nitrite on the length of delay time

Few data were identified to estimate the delay time before growth in the presence of combined salt and nitrite in food. In their study, Juneja *et al.* (1996b, see Section 3.11.5.2) evaluated the effect of salt, temperature, sodium pyrophosphate, and pH in a laboratory broth medium. In the statistical analysis of a model for lag phase duration, salt alone appeared to have no main effect, although it did appear to be significant in various interaction terms (lag phase duration was estimated by fitting Gompertz curves to experimental growth data). However, the delay time in broth is significantly longer than that in food-like meat media, so the application of these results to RTE and partially cooked foods is questionable. In view of this probable lack of applicability

to RTE and partially cooked foods, and because the lag phase duration affects only the modeling of hot-holding in this risk assessment, it is here assumed that salt has no effect on the delay time.

Riha and Solberg (1975) estimated the lag phase of heat-resistant strain *C. perfringens* strain NCTC 8797 in laboratory media that contained nitrite only (Table 3.25).

Table 3.25 Mean lag phase and generation time of *C. perfringens* NCTC 8797 at 43 °C. (Riha and Solberg, 1975).

Nitrite concentration (ppm)	No. of lag experiments	Lag phase duration (hrs)	No. of generation experiments	Generation time (min)
0	10	7.8	9	23.9
100	8	10.2	7	25.4
150	4	9.5	8	23.2
175	4	9.8	4	30.3
200	4	-[a]	1	16.2

a. No growth observed for 60 hours.

These data suggest little effect of nitrite alone on lag phase duration. Kalinowski *et al.* (2003) report growth curves from spores in cured (156 μg/ml sodium nitrite) and uncured turkey emulsion at 26.7, 32.2, 37.8, 43.3, and 48.9 °C, both at 1% salt content. When these data are fitted using the primary growth model of Section 3.11.1, there is no significant difference between the delay times in cured and uncured turkey.

Labbe and Duncan (1970) showed that the length of the lag phase of the same *C. perfringens* strain was increased in the presence of 200 ppm nitrite (Table 20). Riha and Solberg (1975) performed experiments in filter sterilized media and suggested that the long lag times were attributable to inhibition by oxygen, as lag times in autoclaved media were about half those in filter-sterilized media. Unfortunately, autoclaving nitrite has been shown to result in a product that is more inhibitory to *C. perfringens* than non-autoclaved nitrite (Perigo and Roberts, 1968; Riha and Solberg, 1973). Therefore, the data on autoclaved nitrite could not be reliably incorporated into the growth model.

Table 3.26 Lag phase of *C. perfringens* NCTC 8798 at 45°C (Labbe and Duncan, 1970).

Nitrite concentration (ppm)	Lag phase duration (mins)
0	~35
100	~45
200	>105[a]
[a] Final sample.	

The available data on nitrite are thus equivocal; however, the only available data indicating an increase in delay time are in laboratory media, and have not been analyzed taking account of all the uncertainties in measurements. For this risk assessment, no change in delay time will be modeled for nitrite (as previously noted, in this risk assessment changes in the delay time affect only the modeling of hot-holding).

3.11.5.4. The effect of pH

Juneja *et al.* (1996b) showed significant effects of pH on lag phase duration and generation time (both estimated from Gompertz fits to experimental growth curves) for *C. perfringens* growing in a laboratory broth medium containing salt and sodium pyrophosphate. An analysis of their published estimates of exponential growth rates (see Section 3.11.5.2) showed no significant effect of pH. No further information was located that would allow estimates of the effect of pH. Since there appears to be no effect of pH (for a reasonable range of values) on exponential growth rates (the closest match to the growth rate parameter used in the primary model used here), this risk assessment does not model any effect. Delay times may be affected in laboratory broth media, but the relevance of that finding to food-like meat media is not clear since delay times differ between these two media types. In view of the lack of reliable observations, and the lack of any pH measurements for the food servings used in the risk assessment, this risk assessment does not model any effect of pH on delay times (with the other assumptions used in the risk assessment, this affects only the modeling of hot-holding).

3.11.5.5. Water activity

Water activity refers to the water available for biological processes. Water activity values for meat foods compiled from the literature (Table 3.27) are all above 0.95. Kang *et al*. (1969) grew heat-activated *C. perfringens* spores in laboratory media with varying water activity. The water activity levels were controlled by the addition of three solutes (glycerol, sucrose, and sodium chloride) in separate experiments. In a test that could not distinguish germination alone from germination plus growth, spores germinated and grew approximately equally over 24 to 48 hours in glycerol adjusted water activities from 0.95 to 0.995, with some germination at water activities down to 0.94. In sucrose or sodium chloride adjusted media, germination and growth was demonstrated over the somewhat narrower range from a water activity of 0.96 upwards.

In other experiments that followed the vegetative cell growth curves from *C. perfringens* heat-activated spores, Kang *et al.* (1969) demonstrated growth in water activities of 0.97 and above, and consistently declining concentrations at 0.93 or lower water activities. A water activity of 0.95 gave growth in glycerol adjusted media, but declining concentrations in sucrose and sodium chloride adjusted media. The growth curves indicate longer delay times in sucrose and sodium chloride adjusted media at the lower water activities, possibly combined with slightly reduced growth rates. In glycerol, there were slightly reduced growth rates or slightly longer delay times or both at lower water activities, but the experimental measurements are inadequate to distinguish these.

Generally, these data suggest that vegetative cell growth rate is not substantially affected at water activities at or above 0.97, but that die-off of organisms begins to occur at or below a water activity of 0.93, with media-dependent results at 0.95 water activity. It is unclear whether low water activities levels kill heat activated spores, or return them to an inactive state. For this risk

assessment, it is assumed that water activity levels at or below 0.93 suppress growth completely, but no lethality from such low water activities occurs. Above 0.93, it is assumed that growth rates and delay times are unaffected, despite the observation of somewhat longer delay times and/or lower growth rates. The assumption of no effect is justified because the observations could be explained solely by slightly longer delay times, yet such delay times are media-dependent and appear smaller in food-like meat media compared with laboratory liquid media. Water activity values for foods compiled from the literature (Table 3.27) are all above 0.95, and these are assumed representative of foods retained in the risk assessment (low water activity foods are screened from the risk assessment by the procedures adopted in Appendix A) so in this risk assessment no adjustment for water activity is applied.

Table 3.27 Water activity values of meat items (Chirife and Ferro Fontan, 1982; Alzamora and Chirife, 1983; Taormina *et al*., 2003; Fett, 1973).

Sample	Chirife and Ferro Fontan, 1982	Alzamora and Chirife, 1983	Taormina *et al*., 2003	Fett, 1973
Beef	0.98-0.99			
Beef Corned		0.972, 0.979		
Roast beef		>0.982		
Bologna, raw			0.965, 0.965	
Bologna, cooked			0.966, 0.952	
Pork	0.99			
Pork sausage				
Measurement method 1				0.99, 0.97
Measurement method 2				0.973, 0.973
Ham, cooked		0.971		
Ham, deviled		0.971, 0.970, 0.975, 0.977		
Ham, chunked raw			0.973, 0.977	
Ham, chunked cooked			0.964, 0.967	
Ham, whole muscle raw			0.979, 0.985	
Ham, whole muscle cooked			0.972, 0.978	
Chicken, boned		0.982		

3.11.5.6. The maximum vegetative cell density

In evaluation of the experiments of growth rates of vegetative cells derived from heat-shocked spores (Sections 3.11.2 and 3.11.3) the maximum cell density was assumed to be identical for all growth conditions within each set of experiments described by the various authors. The estimated values obtained for the maximum vegetative cell densities were 9.9-$\log_{10}$ (experiments of Juneja *et al.*, 1999) using a broth medium; 7.6-$\log_{10}$ (experiments of Juneja *et al.*, 2001) in cooked cured beef; 8.07-$\log_{10}$ (experiments of Juneja and Marks, 2002) in cooked cured chicken; and 8.03-$\log_{10}$ (experiments of Huang, 2003) in cooked ground beef. No formal analysis was performed of the variability between these values, nor was any attempt made to account for potential differences between the experiments at different temperatures reported in each study.[43] It is expected that different foods, with different meat fractions, could have substantially different maximum possible *C. perfringens* vegetative cell densities, but little information was identified in the literature that would allow testing of such a hypothesis. To encompass the differences observed in the laboratory experiments performed on meat media (the high value measured in broth was discounted), it was assumed that the maximum cell density in all foods is 8-$\log_{10}$, with a variability of 0.5 on the $\log_{10}$ scale. The effect of this assumption is tested in the sensitivity analysis.

3.12. Growth during chilling, stabilization and secondary cooking steps — the factor G_c

The amount of growth allowed during chilling, stabilization, and secondary cooking steps is the proposed control variable for regulations, and so must be modeled as an input to the risk assessment in some fashion. A fully realistic evaluation of the effect of different regulations would require knowledge of a mapping between the regulatory level of growth allowed, and the distribution of the amount of growth achieved in practice in all RTE and partially cooked foods. We do not have that mapping, nor do we have the information needed to model it — we do not have, for example, the extensive information on the cooling curves that would be used in the industry under various regulatory regimes (indeed, we are unable to say what is the current distribution of growths achieved under the current regulatory regime).

Given these circumstances, we opt for an approach that can provide some information, although not necessarily the exact information desired. In the implementation of the model, the option is provided of specifying any variability distribution for growth. Thus it is possible to specify a single value for the growth experienced by all RTE and partially cooked foods (using a point distribution), or a distribution of values corresponding to the possible range of values that would be achieved in practice for a given regulation. The results we present correspond to using fixed values of growth (G_c chosen to be a fixed value, typically with $\log_{10}G_c$ equal to 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, or 3.5), with the full realization that they do not correspond exactly to any regulatory regime.

3.13. Storage and transport phases of the distribution system for RTE foods

Once an RTE product is manufactured and has been stabilized, it is distributed to the final consumer for preparation and consumption. Nevertheless, distributing RTE products from a relatively small number of producers to a very large number of consumers results in possibly

[43] The maximum cell density appeared to be homogeneous between temperatures, except for the 50 °C experiment in Huang (2003), where the maximum cell density tested as significantly lower than at lower temperatures; however, this difference was ignored in the analysis.

long periods of storage. Typically, the product must move from the manufacturing plant to a retail store; then move to a consumer's refrigerator. Some degree of spontaneous germination of spores remaining in the products is expected and the data used to assess this are described in this section. Additionally, during the period between manufacture and preparation, the product may be stored at some temperature(s) that could allow growth, retard growth or cause cell death. These temperatures and the associated times are also discussed in this section.

3.13.1. Spontaneous germination of spores during storage and transport — the fraction g_s

Spores that remain in RTE or partially cooked products after chilling may spontaneously germinate during storage of the products. For simplicity, conservatism, and because this is expected to be a minor contributor to risks, it is assumed in the model that all spontaneous germination takes place at the beginning of any such storage.

Section 3.9.5 summarized the available evidence on germination of spores without heat activation. As noted there, even under frozen storage a visible fraction of spores germinated after 1 or 2 months (Ahmed and Walker, 1971). Most reported results were, however, under conditions that were presumably more favorable to germination than typical storage conditions for RTE and partially cooked foods.

To encompass the measurements described in Section 3.9.5, but taking account of the harsher expected conditions, the fraction g_s of type A, CPE-positive strains germinating in storage is modeled in the same way as for the fraction germinating under favorable conditions using a triangular distribution ranging from 0 to 5%, with mode 2.5%. A sensitivity analysis is performed on these parameters and distribution shape to determine the effect of this set of assumptions. The fraction germinating was also assumed independent of the temperature, duration, or any other conditions of storage.

3.13.2. Survival or growth of *C. perfringens* during storage and transport — the factor G_s

C. perfringens is inhibited from growing below about 10 °C, but lower temperatures can be lethal. Because standard RTE food chilling practices typically attain temperatures below 5 °C, and storage of RTE and partially cooked products is usually at temperatures below 10 °C (see Section 3.13.3), the lethal effect of low temperatures is included in this model for temperatures below T_{min}. Above T_{min}, the expected growth rates for such higher temperatures are applied (Section 3.13.2.4). The factor G_s of Equation (3.3) is obtained as the product of two factors, one for each period of storage (Section 3.2). The factor for each period is obtained by applying the respective growth or death rate for the corresponding temperature and time (Section 3.13.3).

Available evidence indicates that *C. perfringens* exposed to low temperatures cannot multiply; but rather the cold may kill *C. perfringens* vegetative cells. The exact mechanisms responsible for cold shock lethality are not clear, although freezing of bacterial cell membrane lipids is likely critical (Leder, 1972). Low temperatures could therefore reduce the concentration of *C. perfringens* vegetative cells within an RTE or partially cooked commodity. To evaluate the effect of cold on *C. perfringens* survivability in foods, the following factors were evaluated: (1) cooling during the bacterial growth phase, (2) duration and temperature, and (3) food composition.

The effect of cold shock following bacterial growth
Bacterial growth may be inhibited due to injury and/or death of bacterial cells following chilling in a medium. Vegetative *C. perfringens* cells that are growing exponentially are more susceptible to killing by cold than those that are not in this growth stage. Traci and Duncan (1974) reported that 96% of exponentially growing *C. perfringens* cells were killed upon cold shock at 4 °C. Moreover, 95% of the remaining cells were killed following 90 minutes exposure at 4 °C. In contrast, a greater number of cells in stationary phase remained viable following cold shock.

C. perfringens are likely to experience a several hour cooling process plus a stabilization process at the manufacturing plant (although more rapid cooling processes are in use in some cases). Bacteria exposed to these conditions are not likely to be in exponential phase and may be less susceptible to cold lethality than exponential phase cells.

Duration and temperature of storage
Both the duration and the temperature to which *C. perfringens* are exposed affect the bacteria's survivability. There are indications that freezing temperatures can be less detrimental to *C. perfringens* vegetative cells than refrigeration temperatures (Barnes *et al.*, 1963; Strong *et al*., 1966). It also appears that most killing of *C. perfringens* by cold occurs rapidly, affecting the most susceptible cells and leaving more cold-resistant cells. Blast freezing is typically used to freeze foods such as those listed in Category 3. Data from Barnes *et al.* (1963) suggest blast freezing may result in as much as a 1-$\log_{10}$ reduction in the number of *C. perfringens* vegetative cells. However, the methodology used in this experiment was not reported in sufficient detail to discern whether it was similar to the blast freezing protocols used by industry. Consequently, for this risk assessment, the affect of blast freezing of *C. perfringens* vegetative cells in foods was not modeled.

Food composition
The composition of a product may affect cold lethality of *C. perfringens* vegetative cells. The data of Kalinowski *et al*. (2003) suggests that the presence of nitrite in ground turkey might increase the effect of cold lethality. However, other factors may also account for the differences, as discussed below, and for this risk assessment, lethality due to refrigeration is modeled similarly for all food compositions.

3.13.2.1. Selection of studies on the lethal effect of low temperatures

A number of studies were analyzed to provide evidence of the magnitude of the lethal effect cold temperatures have on *C. perfringens* vegetative cells in foods (Table 3.28). Only studies that examined survival in food matrices were used for evaluation purposes. In all the studies examined, concentrations of *C. perfringens* decreased during storage in a way that was consistent with a regular exponential decrease with time (although other possibilities cannot be ruled out). In cases where cells were subjected to very rapid cooling just before the storage period began, there appeared to be an initial additional killing of cells — the zero-storage-time measured concentration in such cases was ignored in the analyses.

Taormina *et al*. (2003) measured concentrations of *C. perfringens* (an initially equal mixture of spores of 5 strains; ATCC 3264 [CPE-negative], ATCC 12916 [CPE-positive], FD1041 [CPE-positive], and two strains isolated from meat product blends with unknown CPE status) through

simulated commercial cooking, chilling, and storage in bologna (cured), ground cured chunked ham with emulsion, and ground cured whole-muscle ham. Storage was at 4.4 °C for 14 days, with concentration measurements immediately after completion of chilling (temperature 7.2 °C) and at 2 days, 7 days, and 14 days. There was no initial killing effect from rapid cooling in this case.

Barnes *et al*. (1963) inoculated about 10^5 vegetative cells of *C. perfringens* strain F2985/50 into raw beef blocks pre-sterilized by radiation and kept at 1 °C in impermeable bags. The vegetative cells were prepared by dilution in RCM broth from a culture grown in Robertson's cooked meat for 24 or 48 hours, so were probably in stationary phase. Storage was at −5 °C or −20 °C for 26 weeks, with measurements immediately after blast freezing and at 3, 5, 8, 12, and 26 weeks. There was an initial killing effect from the blast freezing, but the analysis here omits pre-blast-freezing measurements.

Kalinowski *et al*. (2003) inoculated approximately 100 spores/g of a mixed spore culture (strains NCTC 8239, NCTC 8798, NCTC 8449, and ATCC 13124 into raw cured or uncured turkey breast emulsion in vacuum sealed pouches. The pouches were cooked to 73.9 °C in flowing steam, cooled and held at 42 °C for 2 hours, then held at 0.6, 4.4, or 10 °C for 7 days, with sampling daily for 4 days and on the final day. The effect of cold shock was not measured. As discussed below, the vegetative cells (germinated from the spores) were probably in exponential phase.

Juneja *et al*. (1994a) inoculated approximately 1000 CFU/g of centrifuged and re-suspended stationary phase cell culture of strain NCTC 8239 into cooked ground beef in filter stomacher bags. Half the bags were vacuum packed in plastic barrier bags to maintain anaerobic conditions. Storage was at temperatures of 4, 8, and 12 °C, with measurements at days 0, 4, 8, 16, 24, 32, and 40. The effect of cold shock was not measured. Other temperature and time conditions resulting in growth are not analyzed here. There was no apparent distinction between aerobic and anaerobic conditions, and both were included in the analysis

Juneja *et al*. (1994b) inoculated approximately 1000 CFU/g of centrifuged and re-suspended stationary phase cell culture of strains NCTC 8238 and NCTC 8239 into cooked ground turkey in filter stomacher bags. Half the bags were vacuum packed in plastic barrier bags to maintain anaerobic conditions. Storage was at a temperature of 4 °C, with measurements at days 0, 6, 12, 18, 24, and 30. Results were reported for anaerobic conditions for NCTC 8238, and for both strains for aerobic conditions. Both aerobic and anaerobic results are included, since there was no apparent distinction. The effect of cold shock was not measured. Other temperature and time conditions resulting in growth are not analyzed here.

Strong and Canada (1964), in separate experiments, cultured five strains (8799F 1546/52, 214D, 65,108, and 142A) of *C. perfringens* in chicken gravy for 6 hours at 37 °C, sealed 1 ml samples in glass tubing, and froze those samples at −17.7 °C. Samples were enumerated at 1, 2, 3, 10, 20, 30, 60, 90, 120, 150, and 180 days, but only enumerations at 1, 10, 30, 90, and 180 days are reported and analyzed here.

Three other studies reported in Table 3.28 were omitted from the analysis, either because too few data were reported above detection limits or because only non-food media were used.

Table 3.28 Measurements on survival of *C. perfringens* vegetative cells under freezing and refrigeration conditions.

Reference	strain	Storage time (days)	Media
Taormina *et al*., 2003	CPE-positive: FD1041, ATCC12916; CPE-negative: ATCC 3624; and two CPE unknown strains	0–14	Ground bologna, chunked ham, and whole-muscle ham, all w/ nitrite
Barnes *et al.*, 1963	Heat-resistant, F2985/50[b,c]	0–182	Raw beef blocks
Kalinowski *et al*., 2003	Heat-resistant, NCTC 8239, 8798, 8449 and ATCC 13124[f]	0–7	Cured and uncured turkey
Juneja *et al*., 1994a	Heat-resistant, NCTC 8239	0–40	Cooked ground beef
Juneja *et al*., 1994b	Heat-resistant NCTC 8238 and 8239	0–32	Cooked ground beef
Stiles and Ng, 1979[a]	Heat-resistant, NCTC 8339-H	0–30	Sliced ham
Strong and Canada, 1964	Type A, 8799F 1546/52[b,d], 214D[b,d], 65[d],108[d], 142A[d]	0– 180	Chicken gravy
Raj and Liston, 1961[a]	*C. perfringens*	0– 393	Lab media and fish homogenate
Solberg and Elkind,1970[e]	Heat-resistant, S-80	3–83	Distilled water
Traci and Duncan, 1974[e]	Heat-resistant, NCTC 8798	0–0.04	Lab media

a. Too few data above detection limits for analysis.
b. *C. perfringens* strains isolated from food implicated in food poisoning.
c. *C. perfringens* grown for 24-48 hrs in Robertson's cooked meat before dilution in RCM broth and inoculation into meat, suggesting stationary phase cells
d. *C. perfringens* grown for 6 hrs at 37°C in chicken gravy prior to freezing, suggesting exponential to late exponential phase cells.
e. Data not used, as cold lethality studies were conducted in water and lab media.
f. Vegetative cells were likely exponentially growing.

3.13.2.2. Analysis of selected studies for lethality at low temperatures

Concentrations of vegetative cells were assumed to decrease exponentially at temperatures lower than T_{min} (Section 3.11.1) during studies of cold lethality. No formal test of this assumption was

performed, but all available data appeared to be consistent with it when any effect of initial cold shock was omitted from consideration. The measured concentrations were modeled by

$$\log_{10}(C) = c_c - a_c t + \varepsilon \qquad \textbf{(3.27)}$$

where the terms are

- C the concentration of vegetative cells of *C. perfringens*,
- c_c a constant corresponding to the concentration of cells at time zero (after the effects of any cold shock,
- t the time of storage at the low temperature,
- a_c the rate of decline ($\log_{10}$ reduction/day) of concentration, and
- ε a normally distributed random term.

Parameters (c_c, a_c, and the standard deviation of ε) and their uncertainties were estimated using likelihood methods.[44] Where multiple experiments using the same experimental protocol were reported in the same study, it was assumed that the standard deviation of ε was the same in each such experiment. Where the experimenter(s) performed replicates of experiments and reported only standard deviations for each measurement (rather than the results of each replicate), the variance of ε was estimated as the sum of the reported variance (square of reported standard deviation) and an experiment-wide variance (for the only such study, Taormina *et al.*, 2003, the additional experiment-wide variance was estimated to be zero).

Table 3.29 Summary of rates of decline ($\log_{10}$ reduction/day) of *C. perfringens* concentrations during cold storage.

Source	Temperature	Product	Slope[a] ($\log_{10}$ reduction/day)	SE
Taormina *et al.*, 2003	4.4	Bologna	0.074	0.018
	4.4	Cured chunk ham	0.089	0.032
	4.4	Cured whole ham	0.040	0.012
Barnes *et al*., 1963	-5	Raw beef blocks	0.005	0.001
	-20	Raw beef blocks	0.0015	0.0012
	1	Raw beef blocks	0.041	0.003
	5	Raw beef blocks	0.036	0.006
	10	Raw beef blocks	0.031	0.006
	15	Raw beef blocks	0.037	0.006
Strong and Canada, 1964	-17.7	Chicken Gravy	0.002	0.002
	-17.7	Chicken Gravy	0.010	0.002
	-17.7	Chicken Gravy	0.014	0.002
	-17.7	Chicken Gravy	0.012	0.002

[44] The analyses reported here are performed in the file CP_cold_storage.xls accompanying this risk assessment.

	-17.7	Chicken Gravy	0.010	0.002
Juneja *et al.*, 1994a	8	Cooked ground beef (Anaerobic)	0.039	0.008
	8	Cooked ground beef (Aerobic)	0.025	0.008
	12	Cooked ground beef (Anaerobic)	0.052	0.008
	12	Cooked ground beef (Aerobic)	0.030	0.008
	4	Cooked ground beef (Anaerobic)	0.048	0.008
	4	Cooked ground beef (Aerobic)	0.030	0.008
Juneja *et al*., 1994b	4	Cooked ground beef (Anaerobic)	0.057	0.012
	4	Cooked ground beef (Aerobic)	0.048	0.012
	4	Cooked ground beef (Aerobic)	0.037	0.012
Kalinowski *et al*., 2003	0.6	Cooked cured turkey	0.201	0.058
	0.4	Cooked cured turkey	0.233	0.058
	10	Cooked cured turkey	0.153	0.058
	0.6	Cooked uncured turkey	0.088	0.058
	0.4	Cooked uncured turkey	0.100	0.058
	10	Cooked uncured turkey	0.120	0.058

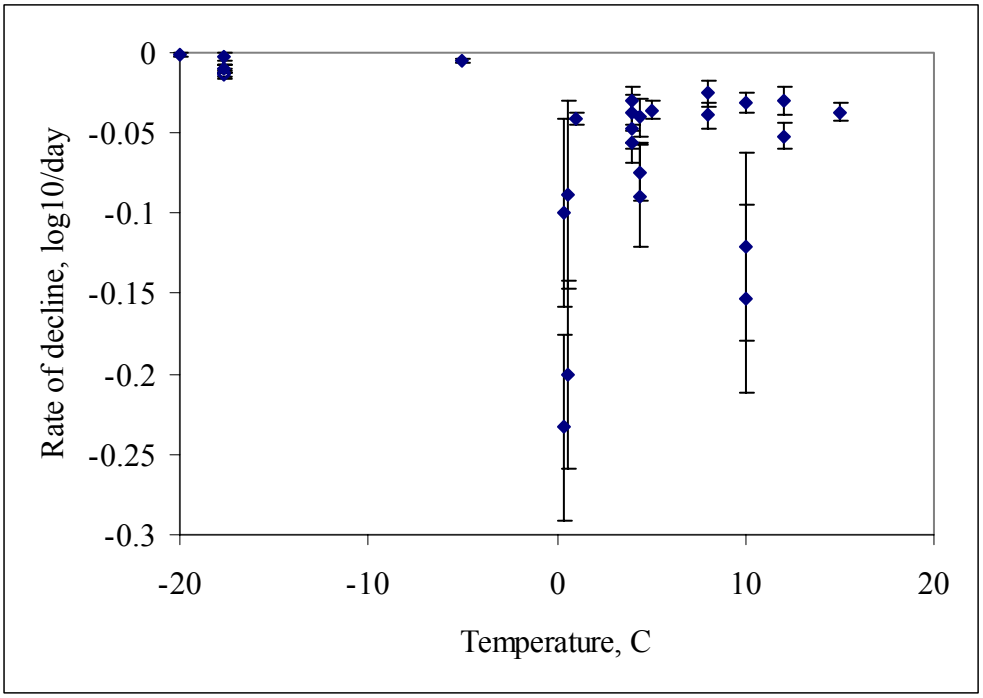
[a] The slope of the plot of base 10 logarithm of concentration against time.

Table 3.29 and

Figure 3.6 summarize the rate at which *C. perfringens* vegetative cell concentrations decline with time during cold storage. There is no apparent variation with temperature above 0 °C (

Figure 3.6), nor below 0 °C, nor with any identified characteristics of the food. The data from Kalinowski *et al*. (2003) stand out as higher than others.

Figure 3.6 Rates of decline of *C. perfringens* concentrations during cold storage (± standard errors).



It is possible that Kalinowski *et al*. (2003) used exponentially growing *C. perfringens*. These authors heat treated 3 mm thin meat samples in pouches to 73.9°C then held the samples at 42 °C for two hours prior to cold shock. Inoculated *C. perfringens* spores would likely have germinated and the product temperature would have equilibrated quickly to 42°C due to the width of the sample. Over two hours at near optimal growth temperature, *C. perfringens* may have entered exponential growth. Additionally, a large differential between the initial temperature and the cold temperature may have increased the lethality of the cold shock (Traci and Duncan, 1974). Kalinowski *et al.* (2003) employed a cold shock differential of 32°C. Substantial initial lethality was observed (and was omitted from the analysis), but the effect on the subsequent decline rate of survivors is not clear.

Despite the discrepancy of the results of Kalinowski *et al*. (2003), it is plausible that similar conditions apply to some RTE and partially cooked foods, so these data were included in the analysis. The variability seen in Figure 3.6 is assumed to be representative of that to be seen in RTE and partially cooked foods, and is modeled by separate lognormal distributions for temperatures above 0 °C and below 0 °C. The parameters for these lognormal distributions, and their uncertainties (which are assumed to be adequately represented by a multinormal distributions[45]), were obtained using likelihood methods from the data of Table 3.29 and are shown in Table 3.30.

[45] The multinormal uncertainty distributions can result in estimates for the standard deviations of the lognormal distribution that are negative. This occurs less than 0.001% of the time for temperatures above 0 °C and less than

Table 3.30 Parameters for the variability and uncertainty distributions for the decline rate of *C. perfringens* cells in refrigerated storage.

Temperatures above zero centigrade			
Arithmetic scale		Natural logarithmic scale	
		Mean	SE
Median (log_{10} reduction/day)	0.056	-2.89	0.13
GSD	1.72	0.54	0.11
		Correlation	0.20
Temperatures below zero centigrade			
Arithmetic scale		Natural logarithmic scale	
		Mean	SE
Median (log_{10} reduction/day)	0.0089	-4.72	0.17
GSD	1.40	0.33	0.18
		Correlation	-0.21

3.13.2.3. Further assumptions for modeling cold lethality

The measurements on refrigerated storage indicate gradual decline in concentrations of vegetative cells at a temperature as high as 15 °C in one case (Barnes *et al.*, 1963), although the analysis performed in this risk assessment indicates that growth can occur at temperatures down to about 12.5 °C (Section 3.11.3) and growth has been observed as low as 12 °C (Solberg and Elkind, 1970). In this risk assessment, it is assumed that the cutoff point for growth is T_{min} (the value of which is included in the uncertainty analysis, but is close to 12.5 °C, see Sections 3.11.2 and 3.11.3). Below that temperature, *C. perfringens* vegetative cells are assumed to die on average, and above that temperature they are assumed to grow on average.

Spores appear to be not greatly affected by refrigeration and freezing temperatures (Barnes *et al.*, 1963; Solberg and Elkind, 1970; Canada *et al.*, 1964), although some declines in spore concentrations are apparent. In this risk assessment, spores are assumed to be completely unaffected by storage at any temperature encountered in practice, so that the lethality factor l_s in Equation (3.2) is assumed to be unity.

Data used to estimate the effect of freezing temperature require thawing of the meat to measure the *C. perfringens* levels. The combined effect of freezing storage and thawing are therefore reflected in the data analyzed here. It is unknown if the thawing methods used by the researchers

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4% of the time for temperatures below 0 °C, and in such cases the standard deviation is set to zero. This approximation was considered adequate, because the uncertainty in death rates during cold storage contributes so little to the overall uncertainty.

reflect typical thawing methods that might be used by consumers. Moreover, it is unknown whether the freezing methods used in practice will affect *C. perfringens* vegetative cell levels — sufficient cold shock clearly does kill cells, but the degree of cold shock occurring in practical production of RTE and partially cooked foods is not known. Any immediate effects of freezing methods have been eliminated from the analyses performed here, and it is assumed in the risk assessment that they have no effect.

3.13.2.4. Growth during storage

If temperatures in storage rise above T_{min} (Section 3.11.1), vegetative cells will start growing. This process is modeled in the risk assessment by assuming that vegetative cells in RTE and partially cooked foods are ready to enter the exponential phase of growth with no delay period, and applying the growth rates obtained in Section 3.11 for the duration of storage.

3.13.3. Duration and temperature of post-manufacturing storage

The period between manufacturing to consumption of food is assumed to include two storage periods, one between manufacturer and retailer, the second between retailer and final consumption. The times and temperatures of storage vary among RTE and partially cooked products, and is discussed by food category in what follows.[46] Food categories were defined in Section 3.4 and in more detail in Appendix A — briefly the categories are: (1) foods with 2.2%–3% salt in the presence of nitrites; (2) foods unlikely to be reheated before consumption; (3) foods likely to be reheated before immediate consumption; and (4) foods served hot but not necessarily prepared for immediate consumption.

Category 1 and 2 foods.

The FDA/FSIS *Listeria monocytogenes* risk assessment (FDA/FSIS, 2003) provides estimated distributions for storage times and temperatures for RTE deli meats and hot dogs stored between their manufacture and arrival at a retail outlet (Table III-12 on page 52), as well as between the retail outlet and preparation or consumption. These distributions are a combination of estimates from available data and expert opinion. The same distributions are used here where no better information is available, since the previously published distributions have had some public scrutiny.

Between manufacturer and retailer, the storage time for each product is assumed to be uniformly distributed between 10 and 30 days. This is the same assumption used in the *Listeria monocytogenes* risk assessment. No uncertainty is assigned to this variability distribution. The storage temperature for each product, reached at the end of the manufacturing (heating and stabilization), is assumed to be represented by temperatures observed for packaged lunch meat immediately after removal from retail display cases in the Audits International/FDA (1999) survey. The observed empirical distribution is used in this risk assessment (Figure 3.7).[47] The data were reported to 1 Fahrenheit degree and accumulated to counts of measurements at each degree. There is an extreme bias towards even Fahrenheit temperatures in the raw data;

[46] The data and analyses reported in this section are included in the worksheet CP_time_temps.xls accompanying this risk assessment.

[47] The distribution assumed in the Listeria monocytogenes risk assessment was a uniform distribution between 1 and 5 °C.

however, these data have been used as reported in order to preserve correlations (see below). No uncertainty has been assigned to this distribution.

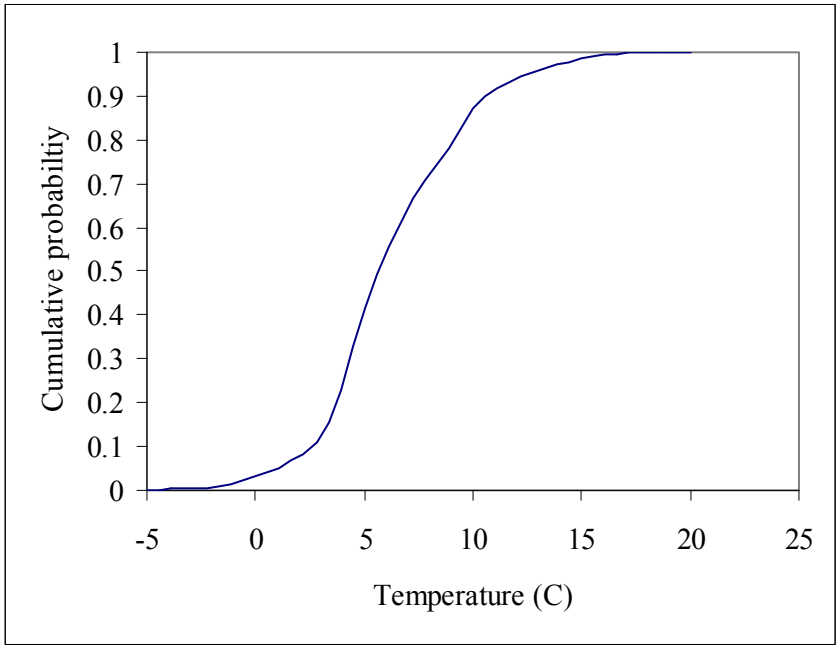

Figure 3.7 Cumulative distribution for the temperature of lunch meat immediately upon removal from its retail display case (based on Audits International/FDA, 1999); these temperatures are assumed to represent storage temperatures for Categories 1 and 2 foods.

For the storage period between retailer and preparation or consumption, data from Audits International/FDA (1999) were used to estimate a distribution of product temperatures, and survey data collected by the American Meat Institute (2001) to estimate a distribution for storage times. Storage temperature is assumed to be represented by the home refrigerator temperatures measured in the Audits International/FDA (1999) survey — the temperature of semi-soft dairy product was measured 24 hours after it was placed in the home refrigerator. This empirical temperature distribution (Figure 3.8) is used as the variability distribution for this risk assessment. Again, the data were reported to 1 Fahrenheit degree and accumulated to counts of measurements at each degree. There is an extreme bias towards even Fahrenheit temperatures in the raw data; however, these data have been used as reported in order to preserve correlations (see below). No uncertainty has been assigned to this variability distribution.

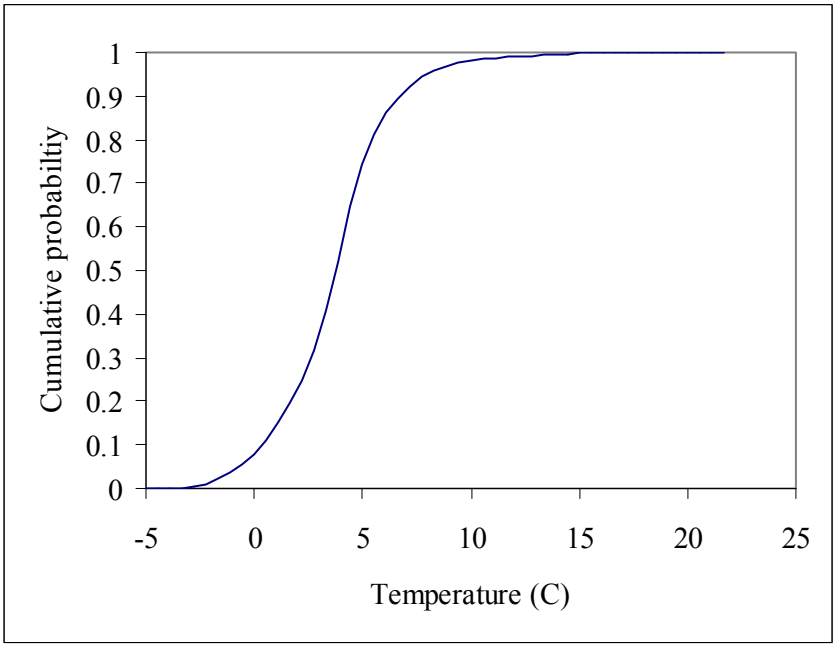

Figure 3.8 Empirical temperature distribution for home refrigeration temperature (based on Audits International/FDA, 1999); assumed representative of post-retail storage temperatures for Categories 1 and 2 foods.

The storage temperatures between manufacturer and retail (pre-retail), and between retail and final preparation or consumption (post-retail), may be correlated (*e.g.* by any effect of ambient temperature on these storage temperatures). In the Audits International/FDA (1999) data (Figure 3.7 and Figure 3.8), there is a slight but significant positive correlation (Pearson correlation coefficient 0.156, $p<0.01$) between the 933 paired measurements available. The 40 unpaired pre-retail and 6 unpaired post-retail measurements are not distinct in distribution from the 933 paired samples ($p > 0.1$ in both cases by both Kolmogorov-Smirnov and Kuiper tests), and their ranges are entirely within those of the paired measurements. To incorporate the correlation, the empirical distributions of paired samples were sampled simultaneously (selecting both measurements at once; so unpaired measurements are not used).

The American Meat Institute survey of 1000 persons (American Meat Institute, 2001) requested information on the average time in storage of prepackaged deli meats and prepackaged hot dogs, reporting numbers of respondents in ranges of periods. The averages so obtained are here assumed to correspond to between-household variation, and the empirical cumulative distribution (Figure 3.9) is used in this risk assessment by interpolating linearly into it.

To incorporate the expected additional intra-household serving-to-serving variation in storage times, a lognormal intra-household distribution was assumed, with a median equal to a random sample from the empirical cumulative distribution (the same as was done in the *Listeria monocytogenes* risk assessment, FDA/FSIS 2003, although there are no available data to justify the selection of a lognormal distribution here). To estimate the standard deviation of the lognormal, and its uncertainty, further data obtained from a pilot questionnaire administered to

callers to a USDA hotline were assumed representative. A response to a question on the storage time of the last-bought hot dogs was obtained from 29 callers, and the likelihood of the values of storage time that they provided (assuming the distributions just described) used to estimate an uncertainty distribution for the standard deviation of the lognormal. A good approximation to the likelihood was obtained by expressing the uncertainty distribution for the standard deviation (the logarithm of the geometric standard deviation) as a mixture of two normal distributions censored to the left at zero. The probability density for the standard deviation (σ) of the lognormal (specifically, the standard deviation of the underlying normal distribution) is thus estimated as proportional to:

$$\frac{\beta}{q_1}\exp\left(-\frac{1}{2}\left(\frac{\sigma-\sigma_1}{q_1}\right)^2\right)+\frac{1-\beta}{q_2}\exp\left(-\frac{1}{2}\left(\frac{\sigma-\sigma_2}{q_2}\right)^2\right) \qquad \sigma \geq 0 \qquad \textbf{(3.1.28)}$$

where the estimated values are:

σ_1 = 0.0071
σ_1 = 0.4349
q_1 = 0.0769
q_2 = 0.3358
β = 0.3134

No uncertainty is assigned to the resulting distributional estimates for household storage time.

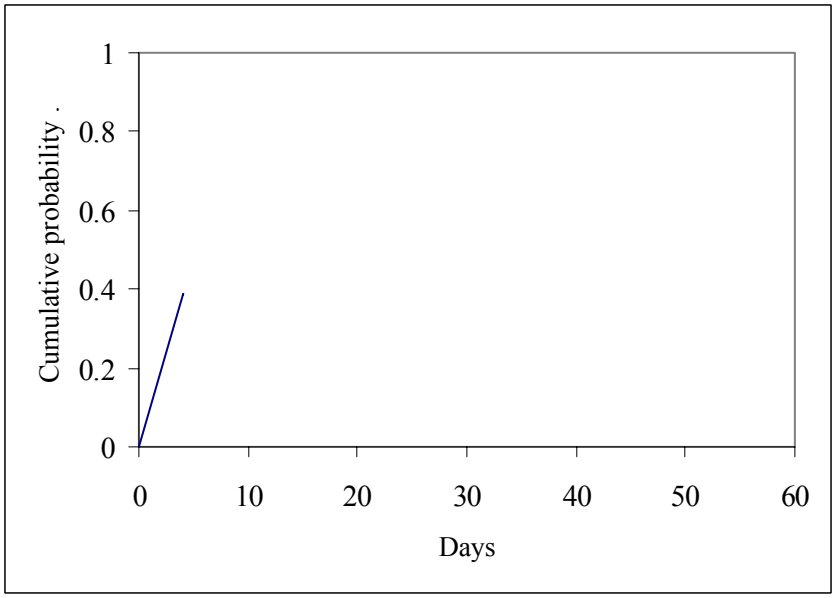

Figure 3.9 Cumulative frequency distribution for average home storage time (American Meat Institute, 2001).

Category 3 and 4 foods.

Category 3 and 4 foods are assumed to be sold frozen. Storage temperatures between manufacturing and retail, and post-retail, were estimated from the Audits International/FDA (1999) survey. It is assumed that the retail storage temperatures of frozen entrées, measured in this survey as the temperature of a frozen entrée immediately after removal from a retail display case, are representative of storage temperatures between manufacturing and retail. For post-retail storage, the temperatures of home freezers, measured in this survey as the temperature of ice cream 24 hours after being placed in the freezer, are assumed to be representative.

The empirical distributions for these temperatures are used in the risk assessment as variability distributions (Figure 3.10 and Figure 3.11). The data were reported to 1 Fahrenheit degree and accumulated to counts of measurements at each degree. There is an extreme bias towards even Fahrenheit temperatures in the raw data; however, these data have been used as reported in order to preserve correlations (see below). No uncertainty has been assigned to these variability distributions.

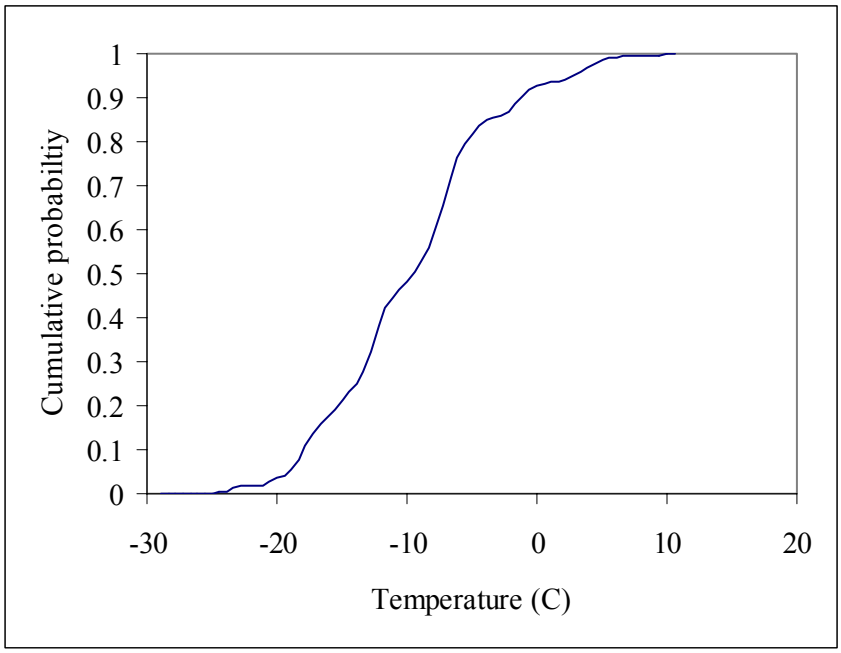

Figure 3.10 Empirical distribution for retail storage temperatures of frozen entrées (based on Audits International/FDA, 1999).

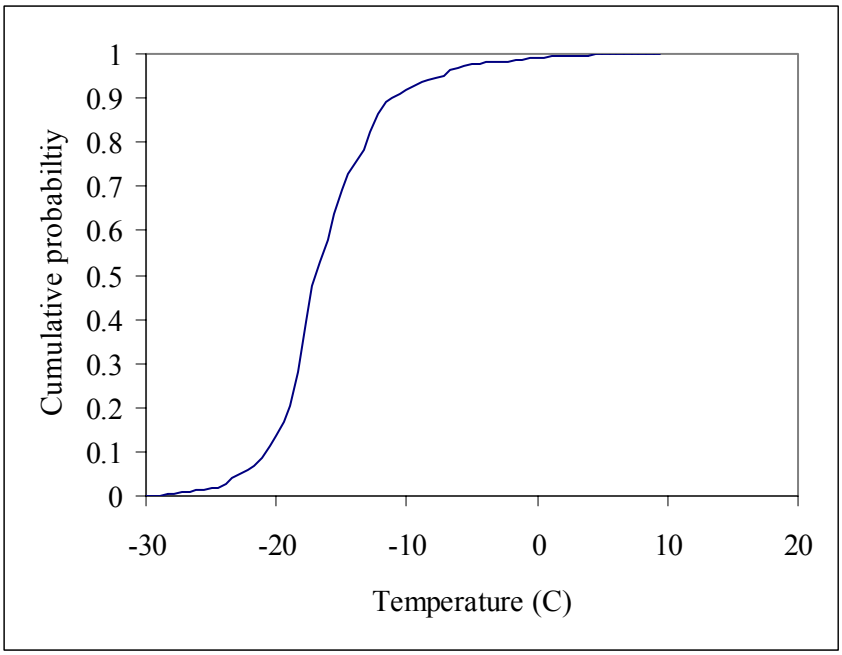

Figure 3.11 Empirical distribution for home freezer temperatures (based on Audits International/FDA, 1999).

The storage temperatures between manufacturer and retail (pre-retail), and post-retail, may be correlated (*e.g.* by any effect of ambient temperature on these storage temperatures). In the Audits International/FDA (1999) data (Figure 3.10 and Figure 3.11), there is a slight but significant positive correlation (Pearson correlation coefficient 0.217, $p<0.01$) between the 888 paired measurements available. The 34 unpaired pre-retail measurements are not distinct in distribution from the 888 paired measurements ($p >0.1$), and their range is entirely within that of the paired measurements. The 52 unpaired post-retail measurements are distinct in distribution ($p < 0.02$ by Kolmogorov-Smirnov test) from the 888 paired measurements (Figure 3.12). To incorporate the correlation, the empirical distributions for paired pre- and post-retail temperatures were sampled simultaneously (selecting both measurements at once). To account for the small difference in the unpaired post-retail measurements, with probability 52/(888+52) the post-retail temperature initially selected is replaced with a random sample from the 52 unpaired post-retail measurements.

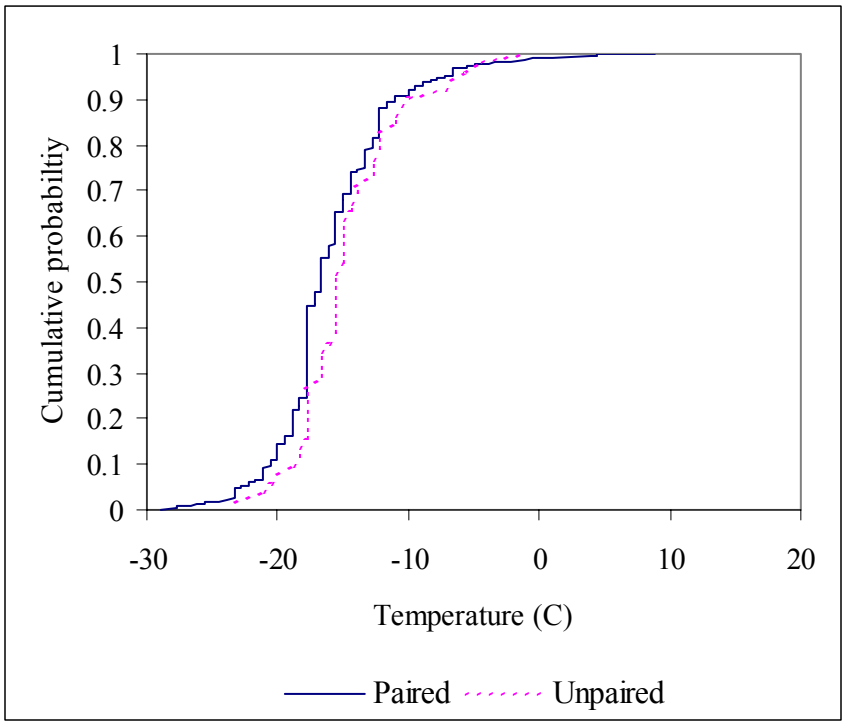

Figure 3.12 Difference between distributions of post-retail storage temperatures for paired (pre- and post-retail) and unpaired (post-retail only) measurements for storage temperatures for Category 3 and 4 foods.

No measurements of duration of storage after manufacture and prior to preparation of foods in categories 3 and 4 have been identified. In their absence, the manufacturing to retail and post-retail times are assumed to be the same as for categories 1 and 2. A sensitivity analysis is performed to evaluate the importance of this assumption.

3.14. Re-heating and hot-holding of RTE foods

RTE foods in Categories 3 and 4 are assumed to be reheated before consumption. During such reheating the number of *C. perfringens* vegetative cells may initially increase, so long as the temperature of the food remains below 53.5 °C. As the temperature rises above 53.5 °C, destruction of some to all vegetative cells will occur. The net effect is controlled by the timing and temperature of reheating, with longer times at higher temperatures causing more lethality.

Reheating may also contribute to an increase in vegetative cells if the product is hot-held at too low a temperature after reheating, if the reheated RTE product is cooled from its cooking temperature into a range of temperatures that allow for *C. perfringens* growth. The hazard of reheating is that the holding period after reheating allows for substantial multiplication of any surviving vegetative cells, or of newly germinated spores, before the food is consumed. In this risk assessment, it is assumed that for hot-held foods the reheating is to a sufficiently high temperature that all vegetative cells are killed and spores are activated, so the hazard arises from subsequent holding at lower temperatures allowing the germinated spores to grow.

3.14.1. Evaluation of experimental data on death of *C. perfringens* vegetative cells during heating

The destruction of vegetative cells at high temperatures is generally characterized by D-values. At a fixed temperature, and under specified conditions, the D-value is the length of time taken for the concentration of vegetative cells to decrease by a factor of 10 (1-$\log_{10}$) on the portion of the survival versus time curve that is exponential.[48] Measurements of the destruction of many pathogens at high temperatures demonstrate that over a small temperature range the logarithm of the D-value itself decreases linearly with temperature (and such behavior agrees with simple analogies with chemical reaction rates) if other conditions are held invariant. The rate of decrease of the D-value with temperature is measured by a z-value, the temperature change projected[49] to change the base 10 logarithm of the D-value by unity (that is, to reduce the D-value ten-fold).

Experimental evidence on D-values and z-values for *C. perfringens* vegetative cells were collected and analyzed (Table 3.31).[50] Roy *et al.* (1981) measured D-values and z-values for two strains (NCTC 8238 and 8798) in late exponential growth or early stationary phase after cultivation at four fixed temperatures, or after cultivation at temperatures that were increased linearly with time from 20 °C to 50 °C. In all cases, both cultivation and testing was in autoclaved ground beef (17% or 22% fat). Juneja and Marmer (1998) measured D-values and z-values for a mixture of three strains (NCTC 8238, 8239, and 10240) cultivated at 37°C in fluid thyoglycolate medium (FTM) to stationary phase and then mixed with autoclaved 90% lean ground beef and chicken (with fat poured off while the meat was hot). Smith *et al*. (1981) examined D-values in a heat-resistant strain (S-45) cultivated in FTM to maximum stationary phase and then tested in FTM at fixed temperatures of 60°C and 65.5°C.

Examination of the D-values and their variation with temperature indicated that they could be classified into two classes. The first are those obtained after cultivation of *C. perfringens* vegetative cells at constant temperatures of 37 to 45 °C, followed by determination of D-value at a temperatures 15 °C or more higher than the cultivation temperature, involving a substantial heat shock (Figure 3.13). The second are those obtained after cultivation of *C. perfringens* vegetative cells at temperatures higher than 45 °C or with the temperature increasing at a constant rate before determination of the D-value, so that heat shock was minimized (Figure 3.14).

[48] At short times, there is often a rapid drop in survival before a steady exponential decline; and at later times the curve may "tail" in a non-exponential fashion. The former may be due to the rapid increase in temperature used in some experiments killing some susceptible fraction of the population. The latter may be attributable to some fraction of particularly hardy organisms, especially in cases where multiple strains are tested together.

[49] The actual temperature range used for the measurement may be less than that required to reduce the D-value ten-fold. The z-value is more generally the negative of the inverse of the slope of the log(D-value) versus temperature curve.

[50] All calculations reported in this section were carried out in the workbook CP_D_values.xls accompanying this risk assessment.

Table 3.31 Summary of available data on D-values (in minutes) for *C. perfringens.*

	Temperature, °C							
	55	57	57.5	59	60	61	62.5	65.6
Conditions[a]	D-values in minutes[b]							
Juneja and Marmer, 1998, mixed NCTC 8238, 8239, and 10240								
Lean Beef, cultivation temp. 37 C	21.6		10.2		5.3		1.6	
Turkey, cultivation temp. 37 C	17.5		9.1		4.2		1.3	
Roy *et al*., 1981, NCTC 8238								
Beef, cultivation temp. 37 C		7.3		2.3				
Beef, cultivation temp. 41 C		10.2		3.0				
Beef, cultivation temp. 45 C		17.2		4.1				
Beef, cultivation temp. 49 C				6.9				
Beef, cultivation ramp 4 C/hr				7.6				
Beef, cultivation ramp 6 C/hr	122.0	17.0		11.9	3.7	3.7		
Beef, cultivation ramp 7.5 C/hr				6.8				
Roy *et al*., 1981, NCTC 8798								
Beef, cultivation temp. 37 C		11.0		3.1				
Beef, cultivation temp. 41 C		13.7		4.4				
Beef, cultivation temp. 45 C		24.3		5.2				
Beef, cultivation temp. 49 C				10.6				
Beef, cultivation ramp 4 C/hr				11.0				
Beef, cultivation ramp 6 C/hr	179.0	21.0		8.4		2.3		
Beef, cultivation ramp 7.5 C/hr				7.6				
Smith *et al*., 1981, S-45								
FTM, cultivation temp. 37 C					5.4			0.65

a. cultivation temp.: cultivated at a fixed temperature lower than the test temperature;
cultivation ramp: cultivated in a rising temperature, generally terminating at the test temperature.

b. Geometric means of multiple values where multiple experiments were made under the same condition. The D-value is the length of time taken for the concentration of vegetative cells to decrease by a factor of 10 (see text).

For this risk assessment, these two classifications were used to derive z-values for each situation, which were assumed to apply to microwave cooking (large heat shock) or oven cooking respectively (lesser heat shock). The D-values (from Table 3.31) shown in Figure 3.13 and Figure 3.14 were separately fitted with exponentially declining curves according to the model

$$\log_{10} D_{ij} = \alpha - \beta\left(T_j - T_0\right) + \varepsilon_{ij} + \theta_i \qquad \textbf{(3.29)}$$

where D_{ij} is the geometric mean measured D-value at temperature T_j in experiment i, α and β are parameters (the latter being the inverse of the z-value), T_0 a convenient reference temperature, ε_{ij} a random experimental error, and θ_i a random fluctuation from experiment to experiment. The

random experimental error was assumed to be normal with standard deviation σ, and the random fluctuation was also assumed to be normal with a standard deviation θ. The loglikelihood for the observations is then (up to a constant)

$$J = \sum_i \left\{ -(n_i - 1)\ln\sigma - \frac{1}{2}\ln\left(\sigma^2 + n_i\theta^2\right) + \frac{1}{2\sigma^2}\left[\sum_j s_{ij}^2 - \frac{\left(\theta\sum_j s_{ij}\right)^2}{\sigma^2 + n_i\theta^2} \right] \right\} \qquad \textbf{(3.30)}$$

where $s_{ij} = \log_{10} D_{ij} - \alpha + \beta\left(T_j - T_0\right)$

and n_i is the number of temperatures for which a D-value was measured in experiment i.

The parameters α, β, σ, and θ were estimated by maximizing the expression (3.30), and the uncertainties of α, β, and θ approximated by the usual normal approximation to the likelihood function (with variance-covariance matrix equal to the inverse of the information matrix), treating σ as a nuisance parameter (re-optimizing on σ while computing the information matrix for α, β, and θ). The reference temperature T_0 was selected to make the correlations between the uncertainty estimates for α and β small, to improve the normal approximations for these uncertainties.

Table 3.32 shows maximum likelihood estimates for α, β, and θ for the two situations examined (with substantial heat shock, and with less heat shock), and Table 3.33 summarizes the multinormal uncertainty distributions obtained for these parameters. The maximum likelihood estimate for θ with less heat shock is zero, and it is relatively close to zero (approximately 2.4 standard deviations away) in the case of substantial heat shock. In both cases, in the Monte Carlo analysis, the multinormal distribution is re-sampled until θ is positive.

Table 3.32 Maximum likelihood estimates for the parameters α, β, and θ.

	Substantial heat shock	With less heat shock
α	0.7507	1.0693
β, per °C	0.1585	0.2755
θ	0.0889	0

Table 3.33 Standard deviations (main diagonal) and correlation coefficients (off diagonal) for the parameters α, β, and θ.

With substantial heat shock			
	α	β, per °C	θ
α	0.0419		
β, per °C	-0.0085	0.0139	
θ	0.0197	0.3787	0.0544
With less heat shock			
	α	β, per °C	θ
α	0.0331		
β, per °C	0.0195	0.0189	
θ	-0.0016	-0.0035	0.0371

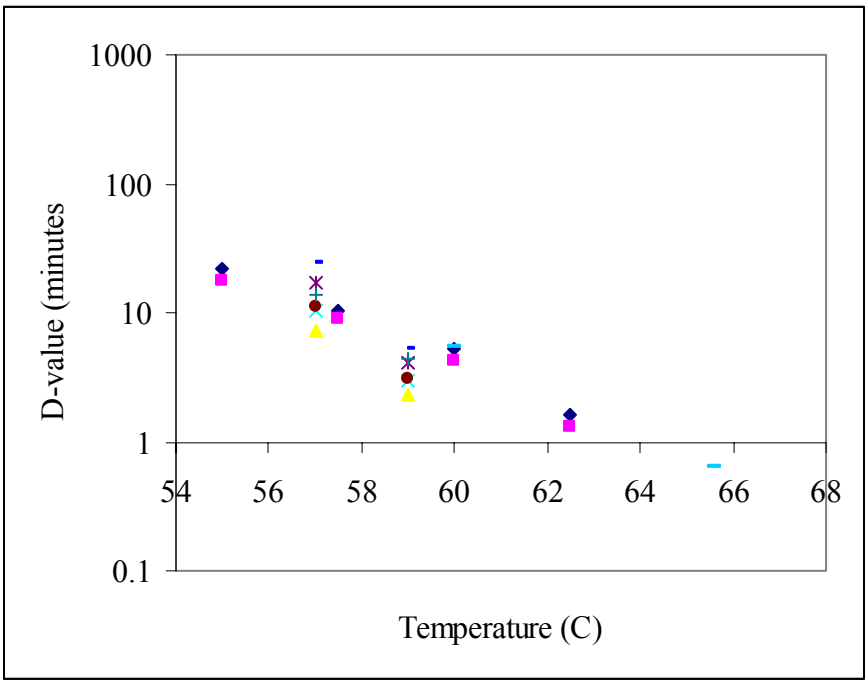

Figure 3.13 D-values where the cells were subjected to substantial heat shock.

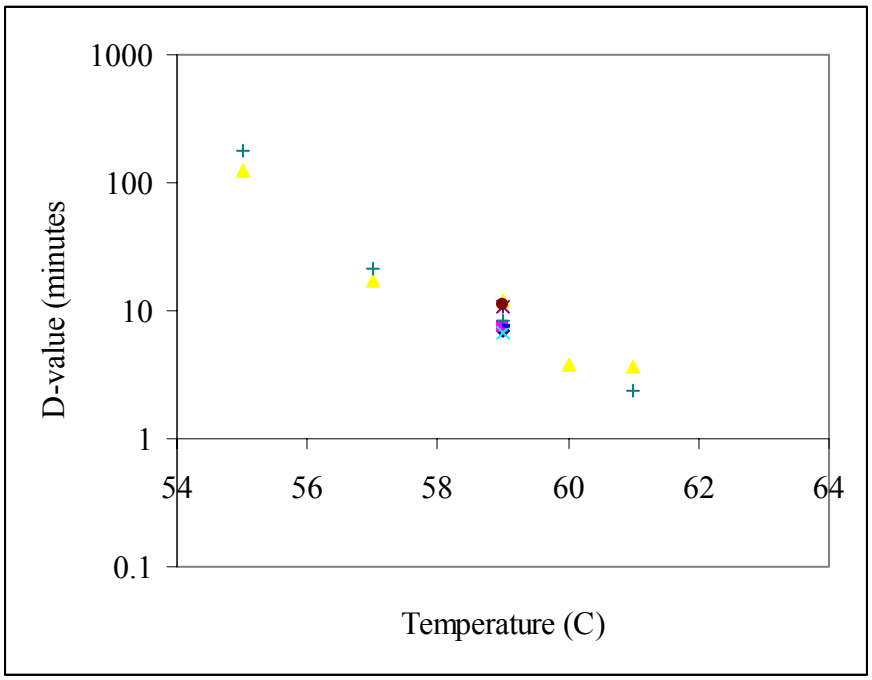

Figure 3.14 D-values obtained under conditions with less heat-shock.

3.14.2. Re-heating times and temperatures.

A total of 3387 cooking temperatures for foods were measured by 608 of 979 participants in a nationwide survey conducted by Audits International/FDA (1999). Those temperature measurements are here assumed representative as a basis for estimating re-heating temperatures for Category 1, 3 and 4 foods. A total of 288 measurements were made on commercially pre-cooked foods, here considered representative of RTE foods, by 224 participants. A performance check on 7% of the participants in the study indicated that temperature measurements were made by 56% immediately after cooking was considered finished, within 1 to 2 minutes by 37%, within 3 to 5 minutes by 5%, and after more than 5 minutes by 2%. Thus some recorded temperatures can be expected to be somewhat lower than the final cooking temperature. The empirical distribution of the measurements on commercially pre-cooked foods (Figure 3.15) shows substantial bunching of recorded measurements at 10 °F intervals (at Fahrenheit temperatures divisible by 10), considered here to be an observational artifact,[51] and a practically uniform distribution with some deviation from uniformity at upper and lower temperatures. In view of the likelihood for measuring temperatures that were lower than final cooking temperature, the bottom tail of the distribution was disregarded; and the upper tail was disregarded as being unimportant in this risk assessment (at the upper temperatures, total destruction of *C. perfringens* vegetative cells would occur very rapidly, Section 3.14.1).[52] The distribution of cooking temperatures used in the risk assessment for all foods in categories 1, 3 (except 3b) and 4, is uniform between 41.5 °C and 87.5 °C (Figure 3.15), values estimated by eye to ensure a match with the majority of the empirical distribution. This interpolation of the

[51] The same type of bunching would be expected if cooking was terminated automatically by temperature probes set at such 10 °F intervals, but that is considered less likely.

[52] No sensitivity analysis was performed to evaluate the effects of this treatment of data. Informally, cooking procedures have trivial effects on the results, so these modifications should have negligible effect.

measurements was preferred to using the empirical distribution itself in order to smooth the measurement artifacts (bunching of observations at 10 °F intervals). The uncertainty of this distribution was considered small enough to ignore, so no uncertainty is assigned to it.

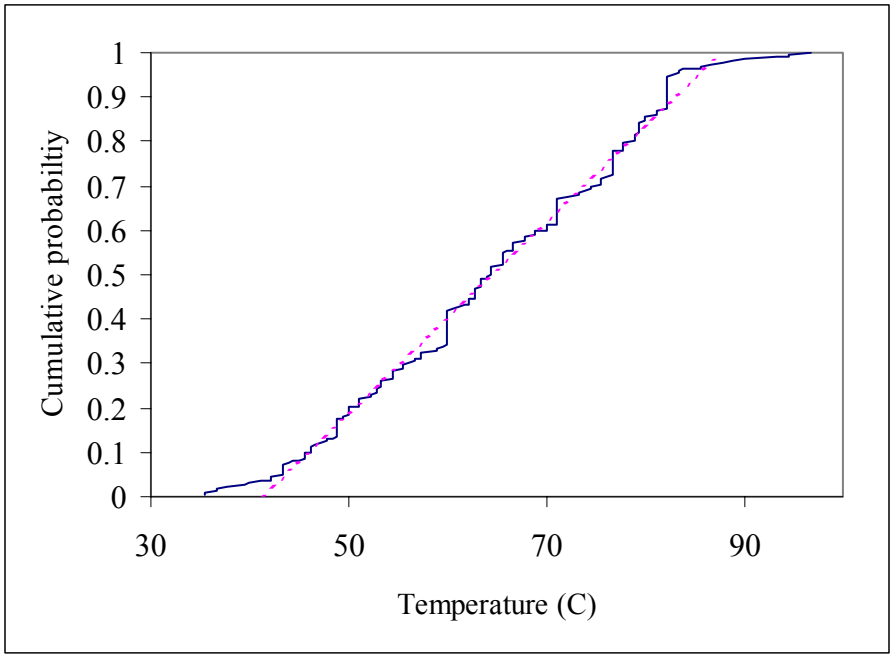

Figure 3.15 Empirical cumulative distribution (black, solid) of measurements of re-heating temperatures for commercially pre-cooked foods, and the uniform distribution used in the risk assessment (mauve, dotted).

Partially cooked foods are assigned to Category 3b, and are likely to be heated more thoroughly than RTE foods. The only partially cooked food codes explicitly identified in the CSFII database (USDA, 2000) were described as "chicken patty, fillet, or tenders, breaded, cooked" and "chicken or turkey cake, patty, or croquette." Of the available categories in the Audits International/FDA (1999) survey of cooking temperatures (Beef/Pork/Lamb, Commercially Pre-Cooked, Fish and Seafood, Ground Beef, Poultry, Re-Heated Leftovers, Starch/Dairy/Protein, and Vegetables), the categories Poultry, Ground Beef, and Beef/Lamb/Pork are most likely to represent the temperatures to which partially cooked foods are heated. The distribution of cooking temperatures for these categories considered separately are almost identical (Figure 3.16), and they were combined to represent the cooking temperatures of partially cooked foods. The empirical distribution of the measurements shows substantial bunching of recorded values at 10 °F intervals (at Fahrenheit temperatures divisible by 10), and this bunching is again considered here to be an observational artifact. To remove the effect of such bunching, the empirical distribution was interpolated by a smooth curve that corresponds to a density function initially linearly increasing, and subsequently declining exponentially (Figure 3.17).

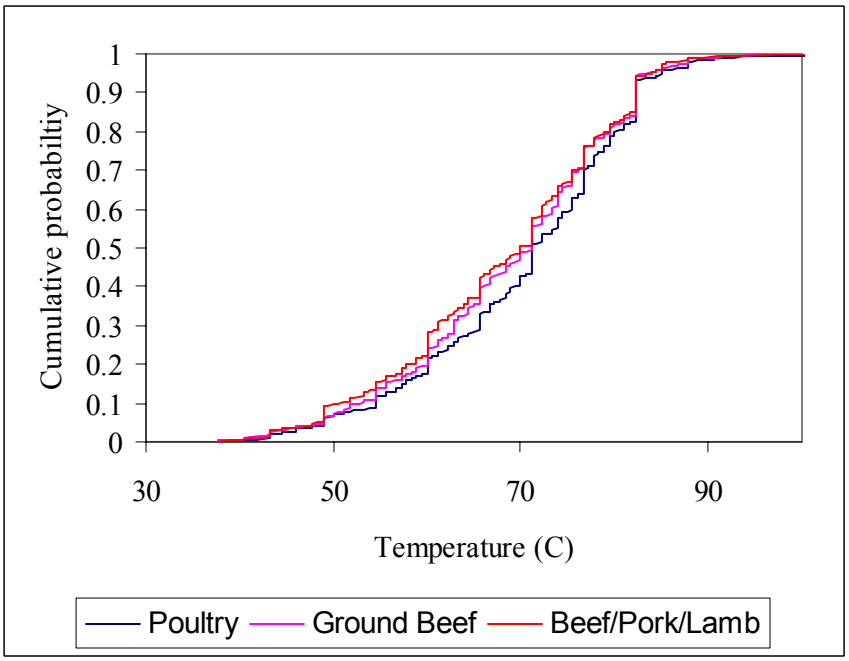

Figure 3.16 Cumulative distributions of cooking temperatures for poultry, ground beef, and beef/pork/lamb categories (Audits International/FDA, 1999).

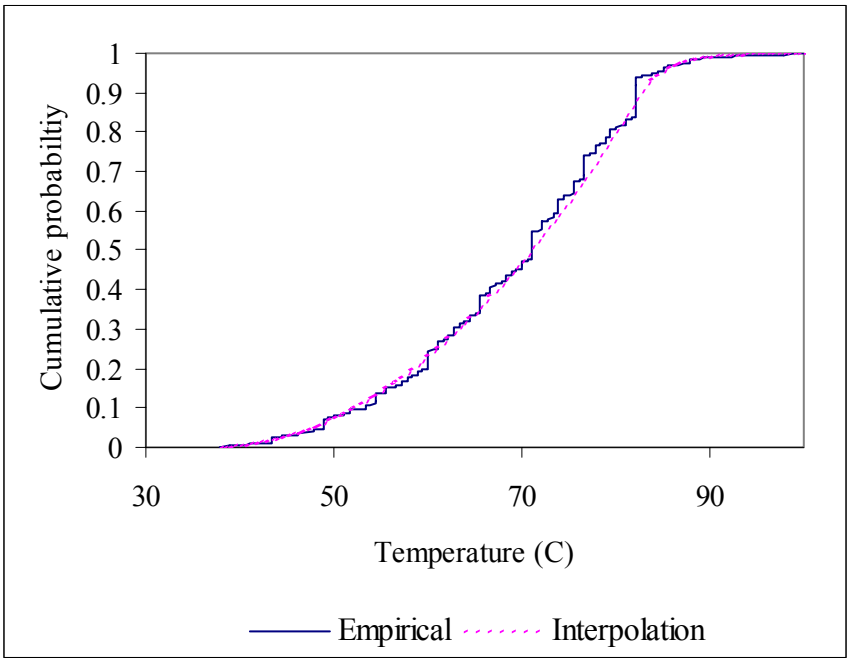

Figure 3.17 Cumulative distribution for cooking temperature for combined Audits International/FDA (1999) categories used to represent partially cooked foods, and the smooth interpolation used in this risk assessment.

The density function used is:

$$\begin{aligned} p(T) &= \alpha(T - T_l) && T_l \le T \le T_u \\ &= \alpha(T_u - T_l)\exp\left(-(T - T_u)/T_f\right) && T \ge T_u \end{aligned} \qquad \textbf{(3.1.31)}$$

$$\text{where } \alpha = \frac{2}{(T_u - T_l)(2T_f + T_u - T_l)}$$

with values:

T_l = 36.73 °C
T_u = 82.22 °C
T_f = 2.941 °C

The uncertainty of this distribution was considered small enough to ignore, so no uncertainty is assigned to it.

Category 3 and 4 foods are all assumed to be reheated before consumption. Independent of the reheat temperature is the time the product takes to reach that temperature, and the time after preparation and before consumption. No survey data were identified that provide information on the times for which foods are heated, or the time before consumption. For the risk assessment it is assumed that 50% of RTE and partially cooked food is heated rapidly, as in a microwave oven, reaching the final temperature in a time that varies from 1 to 10 minutes. This variability is initially modeled as a uniform distribution. The other 50% of RTE and partially cooked foods are assumed to be cooked as in an oven, with cooking times varying from 10 to 30 minutes, again modeled as a uniform distribution. All these parameters are subject to a sensitivity analysis to determine their effect on the risk assessment results. During cooking, temperature of the food is assumed to rise linearly to the final cook temperature at the end of the cooking time. These two assumptions for heating times are categorized as "microwave" and "oven" heating in what follows, but are clearly oversimplifications of what happens during cooking (for example, any method of heating is likely to differentially heat different parts of the food); however, we located no experimental data that would allow taking more complex heating patterns into account. The insensitivity of the results to heating times (Sections 6.6.9 and 6.6.10) suggests that any effects on the risk assessment would be small.

Some of the foods assigned to Category 1 are customarily eaten cold (*e.g.* ham and cheese sandwich, with lettuce and spread, [not grilled]), while others are occasionally eaten cold (*e.g.* hot dogs, which make up a major fraction of Category 1 RTE foods). The availability of data on the fraction of these foods eaten cold prompted the splitting of Category 1 into Categories 1a (hot dogs or frankfurters) and Category 1b (others), and the fractions of each eaten cold are evaluated in the next two sections.

3.14.2.1. The fraction of Category 1a foods eaten cold

The USDA hotline questionnaire obtained some information on eating of hot dogs cold, directly from the package. However, the available results are ambiguous, although they indicate that between 14 and 46 of 223 persons in the families of the 84 people responding ate hot dogs cold under some (unspecified) circumstances.

The American Meat Institute (AMI) survey of 1000 persons (American Meat Institute, 2001) obtained information on the fraction of hot dogs eaten cold, and this information is here used to

estimate the fraction of hot dogs so eaten. This fraction is applied to all food servings in Category 1a (Section A.4.1), which consists exactly of those foods described as frankfurters or hot dogs. Among the AMI survey respondents (all were at least 18 years old), 134 indicated that they sometimes ate hot dogs raw, 97 indicated that other members of their household sometimes did so, and 657 indicated that they (and by implication other members of the household also) always reheated them. This was treated as a binomial observation ((134+67)/(134+67+657) = 231/858) of the probability for a hot dog to be eaten by a person who might eat them raw. Of the 134 persons who ate them raw, 133 persons indicated what fraction of the time they ate them raw, within ranges specified in the questionnaire (Table 3.34). These observations were assumed to provide multinomial samples of the corresponding fractions.

Table 3.34 The fraction of time that respondents ate hot dogs raw.

Don't know/refused	1
9% or less of the time	64
10% to 24% of the time	21
25% to 49% of the time	18
50% to 74% of the time	22
75% to 99% of the time	4
100% of the time	4

The probability density for the fraction of time that hot dogs are eaten raw by a person who might eat them raw was assumed to decrease monotonically and to linearly interpolate between values at 10%, 25%, 50%, 75% and 100% of the time (corresponding to the ranges specified in the questionnaire), with an additional finite probability for such a person to always eat them raw. Figure 3.18 shows the agreement between sample and MLE estimates for these fractions. The overall probability for a Category 1a serving to be eaten raw is then the product of the mean of this distribution and the probability for a hot dog to be eaten by a person who might eat them raw. Evaluating the distribution and the latter probability using likelihood methods[53] gave a maximum likelihood estimate for the fraction of hot dogs eaten raw as 0.0670, and an uncertainty that is accurately represented by a lognormal distribution with median 0.0670 and geometric standard deviation 1.120.

[53] The calculations are performed in the spreadsheet CP_Hot_dog_raw.xls accompanying this risk assessment.

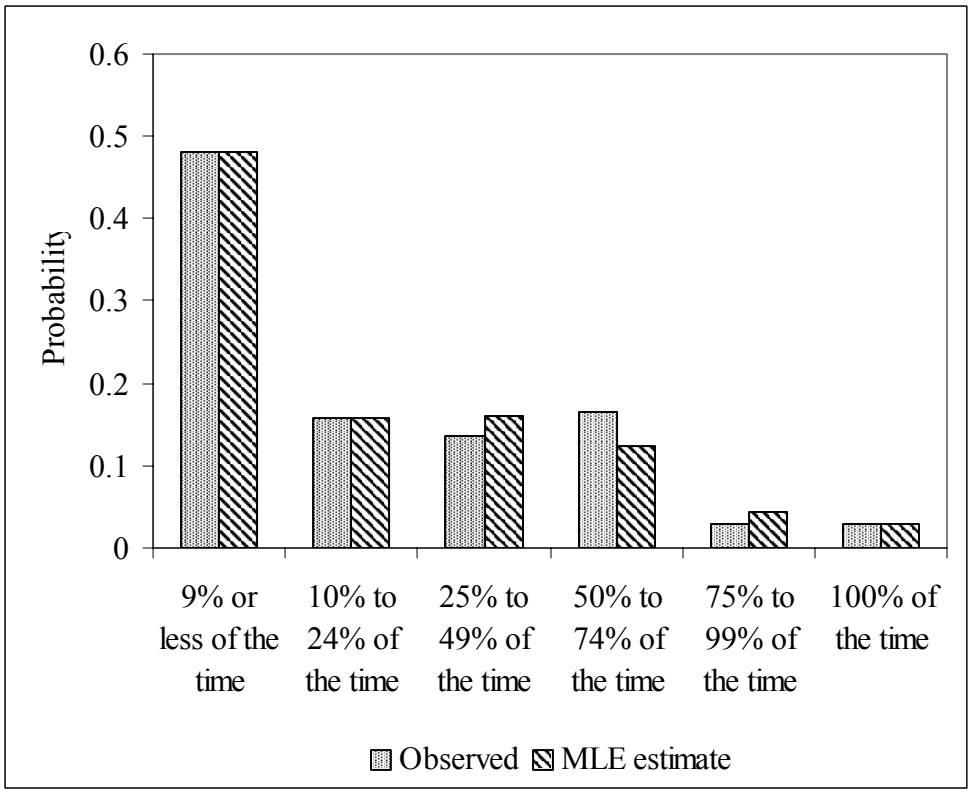

Figure 3.18 Observed and modeled fraction of the time that hot dogs are eaten raw by those who ever eat them raw.

3.14.2.2. The fraction of Category 1b foods eaten cold

No data were located that allows an estimate of the fraction of Category 1b foods that are eaten cold. They are considered sufficiently different from Category 1a foods (hot dogs) that extrapolation between these categories is not justified. It is here assumed that 20% of Category 1b foods are eaten without heating, and sensitivity analysis used to evaluate the importance of this assumption.

3.14.3. Spore germination during re-heating — the factor g_p

Spores in RTE products may germinate during the reheating step and, therefore, become vegetative cells that can grow during the hot-holding period. In principle, the number of spores that germinate during reheating should be added to the number of vegetative cells that survive reheating and this total number of vegetative cells would then be capable of multiplying during any hot-holding. For this risk assessment, it was assumed that the number of vegetative cells that survive re-heating prior to hot-holding its zero, so only the number of spores that germinate during re-heating is used.

Individual spores within a population will germinate differently relative to the majority of spores. Specifically, some spores within a population are known as 'superdormant.' These spores tend not to germinate under conditions that normally allow for germination (Gould, 1969). It is possible that the remaining spores following the initial lethality (heating) step at the manufacturing plant will not react to heat treatment as the initial spore population. However, for

this risk assessment, it will be assumed all spores react equally to heat treatment. FSIS is unaware of any data that could be used to estimate the population of superdormant spores and the percentage that would germinate due to a second heating. The factor g_p in Equation (3.2) is therefore evaluated using the general analysis of the fraction of spores that germinate on re-heating, in Section 3.9.4.

3.14.4. Hot-holding temperature and time

Many RTE products are consumed immediately after reheating, but Category 4 foods are frequently prepared in restaurants or institutions in advance of consumption. Many are frozen products that require reheating before consumption. Such products will be held after reheating for variable times at variable temperatures. Category 1 foods, such as hot dogs, may be similarly handled. The intent of hot-holding is to maintain the product at temperatures above 53.5 °C so that *Clostridium perfringens* growth will not occur; or at least to limit the time product spends in the optimal temperature range for *C. perfringens* growth.

Survey data on temperatures during hot-holding were collected incidentally during an FDA survey on compliance with the 1997 FDA Food Code (FDA, 1997). This survey was national in scope, and designed to be reasonably representative of the industry segments (institutional food service establishments, restaurants, and retail food stores) examined. However, while sampling of the chosen institutions was random within each geographic region that was the responsibility of individual FDA specialists, it was not in proportion to food consumption, so may be biased for the purposes of this risk assessment. Nevertheless, these data are used as though representative on a per-serving basis. A total of 1270 observations of food holding temperatures were recorded during (non-regulatory) evaluation of whether hot-holding temperatures were in or out of compliance with 1997 FDA Food Code requirements for a temperature exceeding 60 °C (140 °F).

The distribution[54] of all 1270 measurements was found to be close to normal (Figure 3.19),[55] with a mean of 63.8 °C (147 °F) and a standard deviation of 13.3 °C (24 °F), but includes many measurements on foods that are not the subject of this risk assessment.

[54] The raw data (censored to remove identifiers) and analyses described in this section are available in the workbook CP_time_temps.xls accompanying this risk assessment.
[55] A formal test rejects normality with high probability.

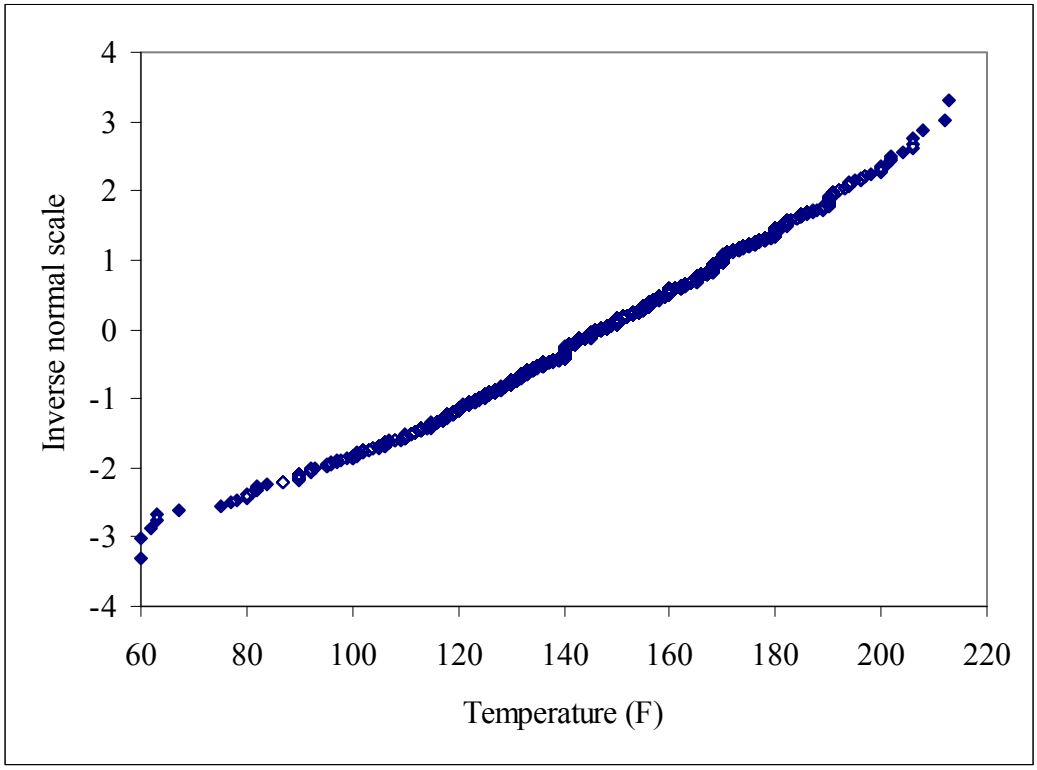

Figure 3.19 Distribution of all hot-holding temperatures found in the FDA survey (FDA, 2000) on a normal scale.

Examination of subsets of the measurements corresponding to potentially meat-containing foods that may have been RTE or partially cooked of categories 1 (n=57), 4a (n=14), 4c (n=27), and 4d (n=72) showed that distributions of measured hot-holding temperatures were roughly consistent with normal.[56] The distributions for categories 4a and 4c were indistinguishable, but those for categories 1, 4a+4c, and 4d were distinct (Figure 3.20)

[56] Formal tests showed marginal normality for Category 4a, but the measurements in the other three categories were indistinguishable from normal.

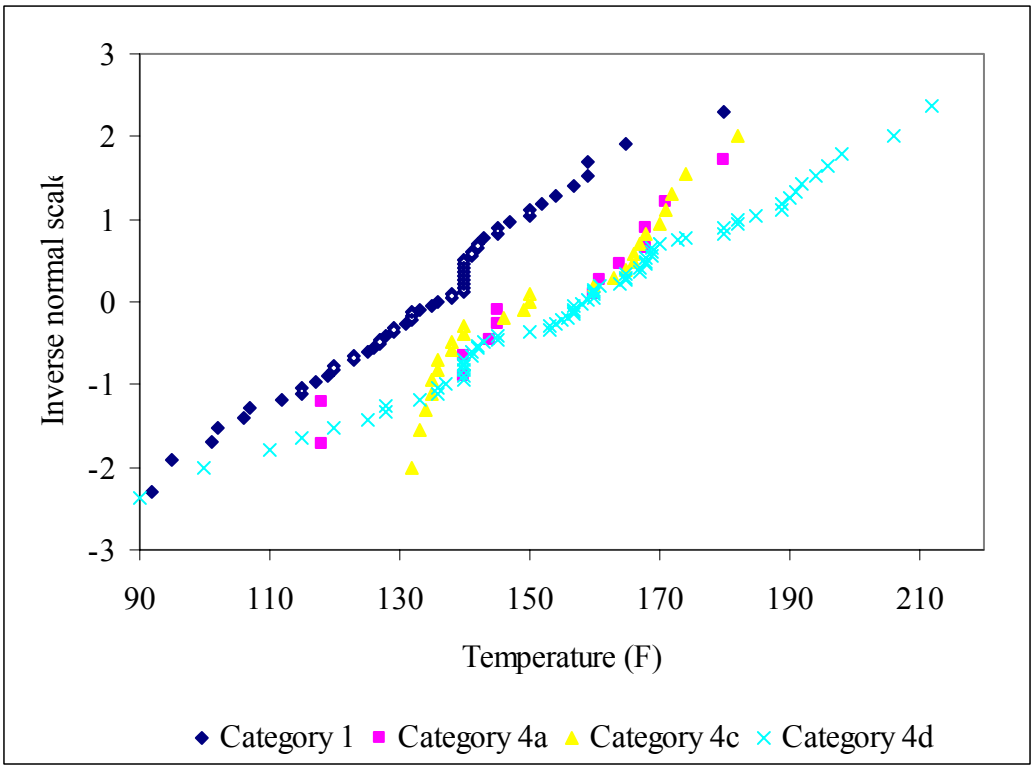

Figure 3.20 Observed distribution of hot-holding temperatures for foods of Categories 1 and 4 (based on FDA, 2000).

Based on these observations, hot-holding temperatures for foods of categories 1, 4a+4c, and 4d were assumed to vary normally with means and standard deviations given in Table 3.35. Uncertainties in these means and standard deviations were estimated using likelihood methods with the assumption that the measurements are representative. The uncertainties are assumed to be normal with parameters also given in Table 3.35 as standard deviations and correlation coefficients.

Table 3.35 Parameters of distributions for hot-holding times. All in °C (except correlations).

	Mean	SD
Category 1	56.27	9.53
	SD (diagonals)/correlation (off axis)	
	1.27	
	0.23	1.03
Category 4a +4c	66.75	9.23
	SD (diagonals)/correlation (off axis)	
	1.45	
	0.27	1.18
Category 4d	69.81	13.34
	SD (diagonals)/correlation (off axis)	
	1.58	
	0.21	1.23

No data on the duration of hot-holding was located. The 1997 FDA Food Code calls for a maximum holding time of 4 hours, and holding for substantially longer periods is unlikely since food held for such long periods would likely become unpalatable. Shorter periods of holding seem more likely than longer periods. To evaluate the effect of hot-holding period, it is initially assumed that the period varies from 0.5 to 5 hours, with a probability density that decreases linearly to zero at 5 hours. The effect of this assumption is tested by sensitivity analyses.

3.14.5. Growth of *C. perfringens* vegetative cells during hot-holding

Vegetative cells already present in the food, or spores newly germinating during re-heating, may proliferate in hot-held food and present a hazard. For this risk assessment, it is assumed that hot-held food is initially heated sufficiently hot to activate spores and kill all vegetative cells present. Subsequently growth is assumed to proceed as detailed in Section 3.11.

3.15. Numbers of servings

3.15.1. Total number of servings of RTE and partially cooked foods

Two estimates have been made of the total number of servings represented by the foods selected from the CSFII survey (USDA, 2000) for inclusion in this risk assessment, and which contain RTE and partially-cooked foods.

First, the total number of person-days in the 4-year CSFII survey used as a basis for obtaining food serving data is 42,269 (21,662 day 1 samples and 20,607 2-day samples). There are 26,548 food servings in the sub-set of servings that are sampled for the risk assessment. This implies 0.628 servings per person-day. The population of the U.S. is about 281,000,000 (in 2000, U.S. Census Bureau, 2003) so that a country-year is (281,000,000 people × 365.25 days) or

103,000,000,000 person-days. The total estimated servings in the country for one year is then 64,600,000,000.

Second, each survey person has either one or two days worth of food consumption data and a weighting factor to account for variable probabilities that that person would be selected for interview in the survey. The number of food servings reported to be eaten by a sample person (and selected for use in this risk assessment) was divided by the number of days for which that person was surveyed to give the individual's servings per day (of the servings selected in this risk assessment). This value was multiplied by the person's single day sampling weight, all of these values were added together, and the sum was divided by the sum of all the sampling weights to give a weighted average servings per day of 0.677 for the sampled population (again, this refers to the servings selected in this risk assessment). Multiplying this value by the U.S. population (281,000,000, from the 2000 census) and the days per year gives a total national, annual number of servings of foods selected in this risk assessment of 69,600,000,000.

These second estimate is preferred because it uses the weighting factors for inclusion within the sample; and the difference (about 7%) from the first estimate indicates that the relative uncertainty in this number contributes a small fraction of the total uncertainty in the risk assessment.

Some fraction of the foods selected from the CSFII survey will not be RTE or partially cooked. No survey information has been identified that could be used to estimate this fraction. It will be assumed for this risk assessment that 80% of the servings selected (that is, 55.7 billion servings) represent RTE or partially cooked foods. The same fraction is applied to all categories of food.

3.15.2. Fraction of servings that are hot-held

No survey information has been identified that allow estimation of the fraction of RTE and partially cooked food that is hot-held after re-heating. For this risk assessment, it is assumed that 1% of Category 1 and 4 servings are so treated.

Appendices for Chapter 3

Appendix 3.1 Fitting gamma concentration distributions to observed counts

Observational studies on concentrations of vegetative cells of *C. perfringens* in meat samples are generally conducted by sampling the meat, homogenizing and diluting the sample, plating the result diluted mixture on suitable agar, incubating under suitable conditions, and counting the resultant colonies of bacterial cells (sometimes with additional safeguards such as confirmation that the colony consists of *C. perfringens*). The procedure is often performed with duplicates of the diluted sample (applying to multiple agar plates), or with replicates of the original meat sample (a second sample put through an identical sequence of homogenization, dilution, and plating), or both. Thus the data available from such sampling consists ultimately of the quantity of meat that was effectively plated, together with a count of the colonies[57] associated with that quantity of meat, which count is taken to equal the number of CFUs in the quantity of meat that was plated.[58]

Suppose the effective quantity of meat plated from a particular sample is m (mass; this may be the sum of the effective quantities applied to multiple plates), and the concentration of viable vegetative cells in the sample is x (CFU per unit mass). The expected number of viable cells plated is then simply mx, and the probability g(r,x,m) to see a particular number r of colonies from that particular sample is just Poisson distributed:

$$g(r,x,m) = \frac{(xm)^r \exp(-xm)}{r!} \qquad \textbf{(A3.1.1)}$$

Now if in multiple samples the CFU concentration varies from sample to sample, and the distribution $p(x,a,b)$ of the concentration is gamma distributed:

$$p(x,a,b) = \frac{(x/b)^{a-1} \exp(-x/b)}{b\Gamma(a)} \qquad \textbf{(A3.1.2)}$$

then the probability $P(r,m,a,b)$ to obtain exactly r colonies in any given sample is

$$\begin{aligned} P(r,m,a,b) &= \int_0^\infty dx\, p(x,a,b) g(r,x,m) \\ &= \left(\frac{bm}{1+bm}\right)^r \left(\frac{1}{1+bm}\right)^a \frac{\Gamma(a+r)}{r!\Gamma(a)} \end{aligned} \qquad \textbf{(A3.1.3)}$$

Then for an experiment in which N total samples were measured using a common methodology (same value of m, *i.e.* same sensitivity, for each sample), and exactly k_r of those samples were measured with r colonies of interest (where necessarily $\sum_{r=0}^{\infty} k_r = N$), the loglikelihood J is given by

[57] In some circumstances, particularly with high expected plate counts, plates with zero counts are discarded as being incubation failures.

[58] One could correct for a (fixed) plating inefficiency, but such a correction makes no essential difference to the following discussion. Incorporation of distribution for plating efficiency would be possible, but we have no data to evaluate such a distribution.

$$J = \sum_{r=0}^{\infty} k_r \ln\left(P\left(r,m,a,b\right)N/k_r\right) \qquad \textbf{(A3.1.4)}$$

The normalization adopted here gives $J = 0$ for an exact fit of the probabilities P to the observed fractions k_r/N. Terms with $k_r = 0$ contribute zero to the loglikelihood.

In some cases, the exact values of the r are not known for a given sample, but some information is known. For the data of Kalinowski *et al*. (2003), in one case it was known that 48 colonies were observed on a given sample, of which 5/12 were confirmed as *C. perfringens*. The general case would be that s/S measured colonies, of a total T colonies observed for the sample, are confirmed to be of the type of interest. In that general case, the probability p_r for exactly r colonies of interest is just

$$p_r = \frac{\binom{S}{s}\binom{T-S}{r-s}}{\binom{T}{r}} \qquad \textbf{(A3.1.5)}$$

and the contribution of that particular sample to the loglikelihood may be taken as

$$\ln\left(\sum_{r=s}^{T-S+s} p_r P\left(r,m,a,b\right)\right) \qquad \textbf{(A3.1.6)}$$

(this has no convenient normalization).

For the data of Taormina *et al*. (2003), the published information does not allow an exact specification of the pattern of (r,k_r) pairs, since the published data are consistent with six such patterns. Suppose that there are q such patterns, k^j_r, indexed by j. Then the likelihood for the published result is just

$$\ln\left(\sum_{j=1}^{q}\exp\left(\sum_{r=0}^{\infty} k_r^j \ln\left(P\left(r,m,a,b\right)\right)\right)\right) \qquad \textbf{(A3.1.7)}$$

Again, this has no convenient normalization.

The available data from the studies on raw meat (Section 3.7) varied from study to study. Strong *et al*. (1963) provided only the total number of samples, the number with detections, and the range of estimated concentrations. This allows an approximate calculation of the loglikelihood (approximate[59] since the concentrations are only estimates) by calculating the expected probability for concentrations to be below the bottom of the range of reported concentrations, within that range, and above the end of that range from the gamma distribution (A3.1.2). The probability $P(x_1,x_2)$ for an observation to be within a given range of concentrations x_1 to x_2 is just

$$P\left(x_1,x_2\right) = \int_{x_1}^{x_2} \frac{\left(x/b\right)^{a-1}\exp\left(-x/b\right)}{b\Gamma\left(a\right)}dx = I\left(a,x_2/b\right) - I\left(a,x_1/b\right) \qquad \textbf{(A3.1.8)}$$

where I is the incomplete gamma distribution integral

[59] Approximate also because we are ignoring that the upper end of the concentration range, at least, was not pre-selected but is in fact an order statistic for these data.

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

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

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

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[60] to be added to the loglikelihood of the form

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

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

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

and

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

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

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

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)

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

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 uncertainty distributions for the parameters were obtained by first finding transformations of the

[60] 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.

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[61] distribution with the numerically estimated variance-covariance matrix.

[61] 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[62] 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):

$$\frac{\partial C_s}{\partial t} = -kC_s$$
$$\frac{\partial C_v}{\partial t} = qkC_s + \mu C_v\left(1 - C_v/C_m\right) \qquad \textbf{(A3.2.1)}$$

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

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

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.[63]

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:

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

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

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

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

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

where

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

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

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

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

[63] 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.

$$C_s = C_0\left(1-\int_0^t g(s)ds\right) = C_0\left(1-G(t)\right)$$
$$\text{where} \quad G(t)=\int_0^t g(s)ds \quad \text{so} \quad G(\infty)=1 \qquad \textbf{(A3.2.7)}$$

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

$$y = C_v/C_m$$
$$P(t) = qg(t)C_0/C_m \qquad \textbf{(A3.2.8)}$$

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

$$\frac{\partial y}{\partial t} = P(1-y)^2 + \mu y(1-y) \qquad \textbf{(A3.2.9)}$$

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

$$y = \frac{z}{1+z} \qquad \textbf{(A3.2.10)}$$

where

$$z(t) = \exp\left(M(t)\right)\int_0^t P(s)\exp\left(-M(s)\right)ds \qquad \textbf{(A3.2.11)}$$

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

$$M(t) = \int_0^t \mu(s)ds \qquad \textbf{(A3.2.12)}$$

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:

$$\begin{aligned}\frac{\partial y}{\partial t} &= P\left(1+\left(\beta-2\right)y-\left(\beta-1\right)y^2\right)+\mu y\left(1-y\right)\\ &= P\left(1-y\right)\left(1+\left(\beta-1\right)y\right)+\mu y\left(1-y\right)\end{aligned} \qquad \textbf{(A3.2.13)}$$

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

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

where

$$R\left(t\right)=\int_0^t P\left(s\right)ds \qquad \textbf{(A3.2.15)}$$

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

$$k\left(t\right)=\lambda t^{\alpha-1} \qquad \textbf{(A3.2.16)}$$

but then specialized to $\alpha = 1$, corresponding to an exponential for P, and μ = 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 μ = 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

$$k\left(t\right)=a+bt \qquad \textbf{(A3.2.17)}$$

since then

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

where Φ is the standard normal integral

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

(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)[64] 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.[65]

A3.2.2.2 Model 2

To test for such behavior, the model given by Equation (A3.2.16) was implemented in the form[66]

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

so that

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

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.[67]

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 rate and a peaked shape — should be adequate. The effect of different functional forms for $k(t)$

[64] 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.

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

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

[67] 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.

is easiest to implement using the alternative formulation given at Equation (A3.2.6). Further work therefore used Equation (A3.2.9), with[68]

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

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:

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

where I is the incomplete gamma integral

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

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

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

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

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

Equations (A3.2.4) or (A3.2.9) may then be analytically integrated. For the usually measured[69] (and usually fitted) quantity $C_s + C_v$, the former gives

[68] 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.

[69] 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).

$$\begin{aligned} C_s + C_v &= C_0 && \text{for} \quad t < t_m \\ &= \frac{C_m}{1 + \left(C_m / C_0 - 1\right) \exp\left(-\mu\left(t - \quad_m\right)\right)} && \text{for} \quad t > t_m \\ &= \frac{C_m}{1 + \exp\left(-\mu\left(t - t_m\right) + \ln\left(C_m / \quad_0 - 1\right)\right)} \end{aligned} \qquad \textbf{(A3.2.27)}$$

Equation (A3.2.9) gives a minor modification:

$$\begin{aligned} C_s + C_v &= C_0 && \text{for} \quad t < t_m \\ &= \frac{C_m}{1 + \left(C_m / C_0\right) \exp\left(-\mu\left(t - \quad_m\right)\right)} && \text{for} \quad t > t_m \\ &= \frac{C_m}{1 + \exp\left(-\mu\left(t - t_m\right) + \ln\left(C_m / C_0\right)\right)} \end{aligned} \qquad \textbf{(A3.2.28)}$$

(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[70] with a slight modification of Equation (A3.2.4). If the growth curve is instead given by

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

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

$$\begin{aligned} C_s + C_v &= C_0 && \text{for} \quad t < t_m \\ &= C_m \exp\left(\ln\left(\frac{C_0}{C_m}\right) \exp\left(-\mu\left(t - \quad_m\right)\right)\right) && \text{for} \quad t > t_m \\ &= C_m \exp\left(-\exp\left(-\mu\left(t - t_m\right) + \ln\left(\ln\left(C_m / \quad_m\right)\right)\right)\right) \end{aligned} \qquad \textbf{(A3.2.30)}$$

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

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

where the symbols represent:

[70] 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:

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

where

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

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

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

(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[71]

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:

$$\begin{aligned}\frac{\partial C_s}{\partial t} &= -\frac{C_0}{t_m} h\left(a, t/t_m\right) \\ \frac{\partial C_v}{\partial t} &= \frac{C_0}{t_m} h\left(a, t/t_m\right)\left(1 - C_v/C_m\right)^2 + \mu C_v\left(1 - C_v/C_m\right)\end{aligned} \qquad \textbf{(A3.2.35)}$$

where

$$h\left(a, w\right) = \left(aw\right)^a \exp\left(-aw\right)/\Gamma\left(a\right) \qquad \textbf{(A3.2.36)}$$

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

$$\begin{aligned}\frac{dw}{dt} &= \frac{1}{t_m\left(T\left(t\right)\right)} \\ \frac{dC_s}{dt} &= -\frac{C_0}{t_m} h\left(a, w\right) \\ \frac{dC_v}{dt} &= \frac{C_0}{t_m} h\left(a, w\right)\left(1 - C_v/C_m\right)^2 + \mu C_v\left(1 - C_v/C_m\right)\end{aligned} \qquad \textbf{(A3.2.37)}$$

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 $1/\sqrt{a}$).

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

[71] It is not necessary to model growth from spores under varying temperature conditions in this risk assessment.

w as the fundamental variable — multiply the second and third equation by t_m, and use the first to obtain

$$\begin{aligned}\frac{dw}{dt} &= \frac{1}{t_m\left(T\left(t\right)\right)}\\ \frac{dC_s}{dw} &= -C_0 h\left(a,w\right)\\ \frac{dC_v}{dw} &= C_0 h\left(a,w\right)\left(1-C_v/C_m\right)^2 + \mu t_m C_v\left(1-C_v/C_m\right)\end{aligned} \qquad \textbf{(A3.2.38)}$$

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

$$\begin{aligned}C_s\left(w\right) &= C_0\left(1-I\left(a+1,aw\right)\right)\\ C_v\left(w\right) &= C_m\frac{u\left(w\right)}{1+u\left(w\right)}\end{aligned} \qquad \textbf{(A3.2.39)}$$

where

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

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).

- The fraction of time that hot dogs are eaten cold (Section 3.14.2.1) is adequately represented by the American Meat Institute survey.
- 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 analyze 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*[72]). 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[73] 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 and 2.5×10^8 spores) or in canned beef stew (213 g containing an average of 2.5×10^{10} total

[72] *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.

[73] 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"

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 (≥30 mins at 100 C)[d]	
683, 689, 690, 692	ND[74]	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)[75].

[74] ND: Not Determined

[75] The two cases where observed rates decreased with increasing dose are ascribed here to the randomness of individual 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.

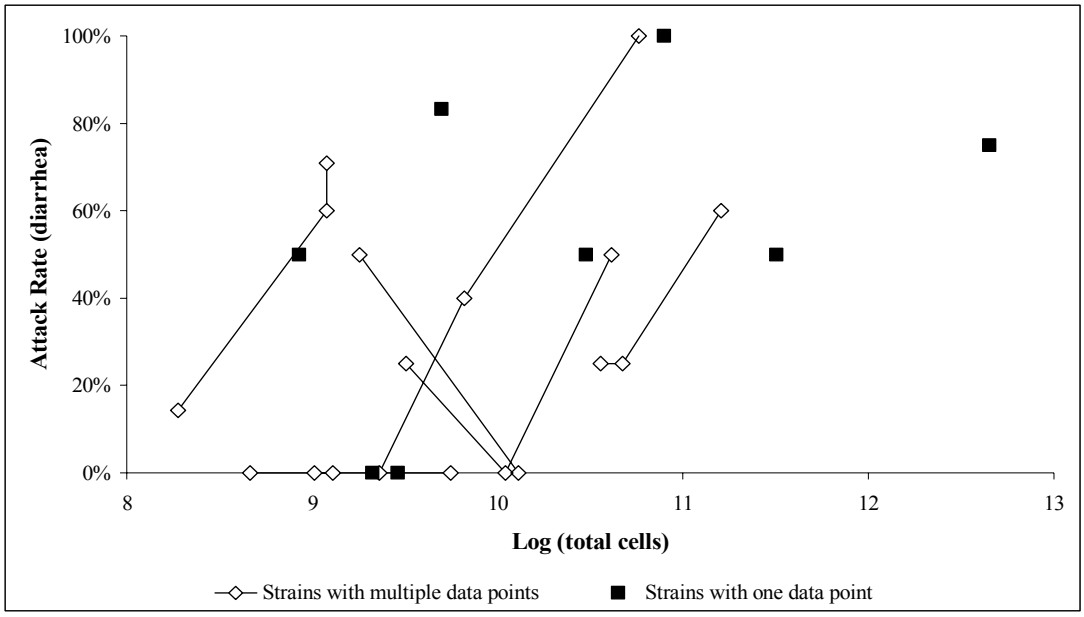

Figure 5.1 Dose-response relationship for *C. perfringens* (total cells).

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.[76] Although spores of this strain are heat resistant (≥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, 9856 were used in the study. For human volunteer studies, only strains 685, 686, and 690

[76] 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.

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:

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

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.[77] 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):

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

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:

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

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).

Fitting the dose-response curve[78] 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

[77] 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.

[78] The calculations are performed in the workbook CP_dose_response.xls accompanying this risk assessment.

multi-dose experiments was acceptable in all cases ($p>0.01$).[79] A likelihood test for equality of the values of potencies k showed that a single value for all the strains tested was highly unlikely ($p\sim10^{-35}$).

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 *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

[79] 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.

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.[80]

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.[81] 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.

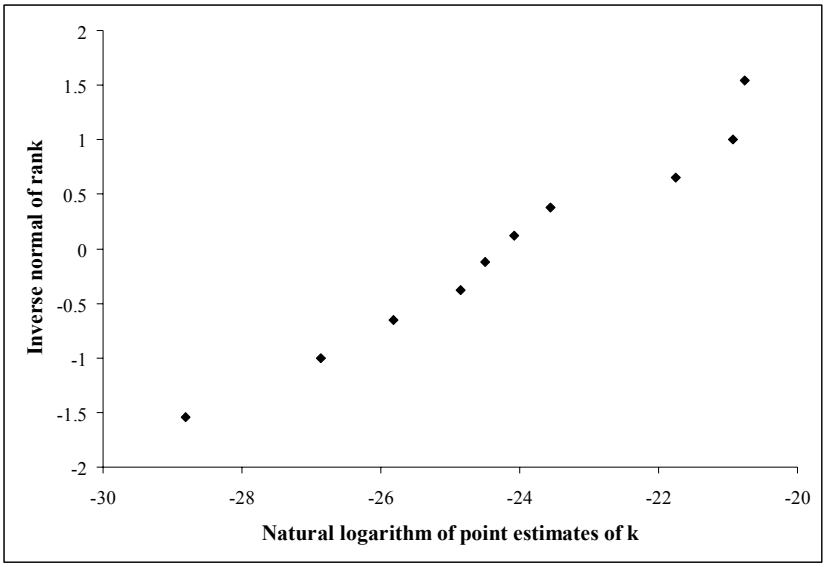

Figure 5.2 Distribution of maximum likelihood estimates for potency (*k*).

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

[81] 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.

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:

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

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:

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

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[82] 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 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[83] 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.

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[82] 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 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.

[83] 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.

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

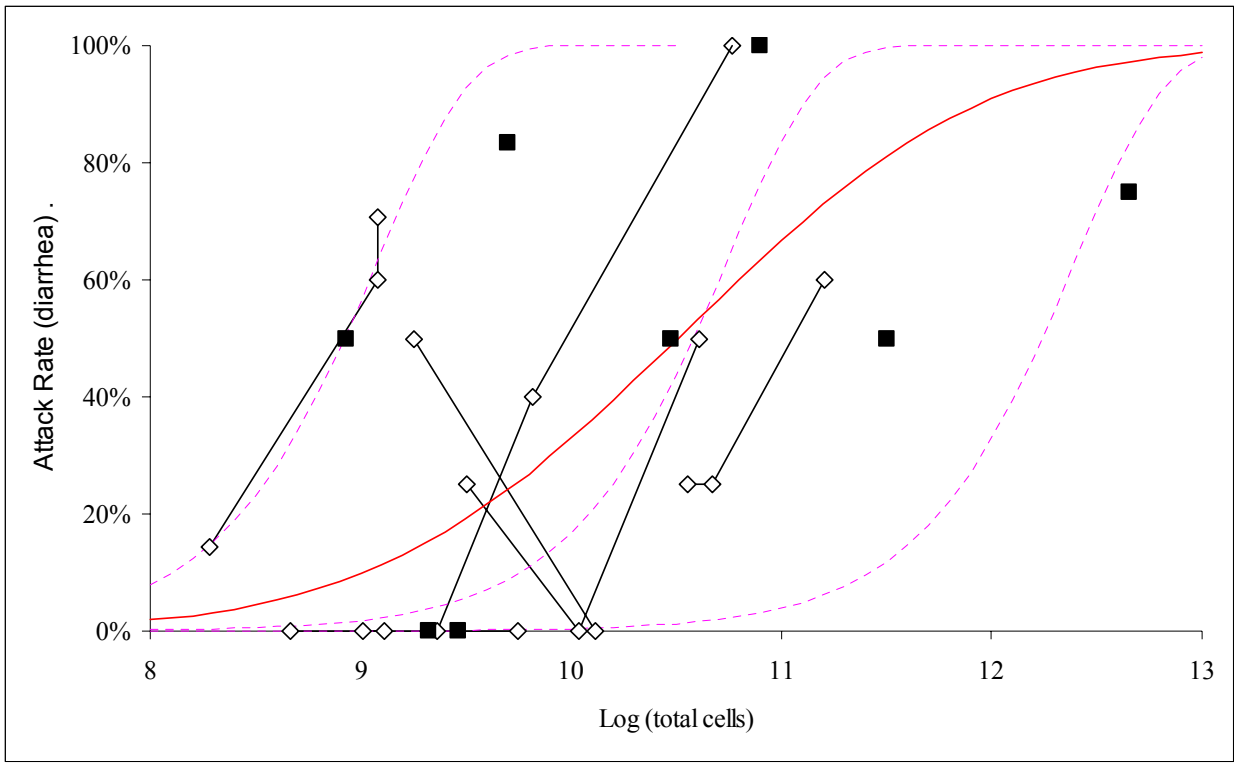


Figure 5.3 Individual strain dose-response curves (dotted, pink) at the median and 95 % confidence limits on the distribution for strains, and the strain-averaged dose-response curve (solid, red), superposed on experimental data.

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,[84]
- 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

[84] A food matrix effect is likely, but probably not discernible in the available data (see footnote 79). A weaker assumption, that any matrix effects are dominated by the between-strain variation, is sufficient here.

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*.

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 1.3 illnesses per million servings up to 2.7 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 74,000 diarrheas per year up to 149,000 per year for 0.5-$\log_{10}$ to 3.5-$\log_{10}$ growth, respectively (using the curve fit to the median estimates).

Table 6.1 Estimates for annual numbers and rate of illnesses.

	Annual number of illnesses (55.7 billion servings)			Rate per million servings		
Growth ($\log_{10}$)	MLE estimate[a]	Median estimate[b]	Curve fit[c]	MLE estimate[a]	Median estimate[b]	Curve fit[c]
0.5	74,000	75,000	74,000	1.33	1.34	1.34
1	82,000	78,000	79,000	1.47	1.40	1.42
1.5	89,000	89,000	86,000	1.59	1.59	1.54
2	97,000	93,000	96,000	1.74	1.67	1.72
2.5	101,000	108,000	108,000	1.82	1.95	1.95
3	117,000	128,000	126,000	2.10	2.29	2.26
3.5	137,000	148,000	149,000	2.46	2.66	2.68

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

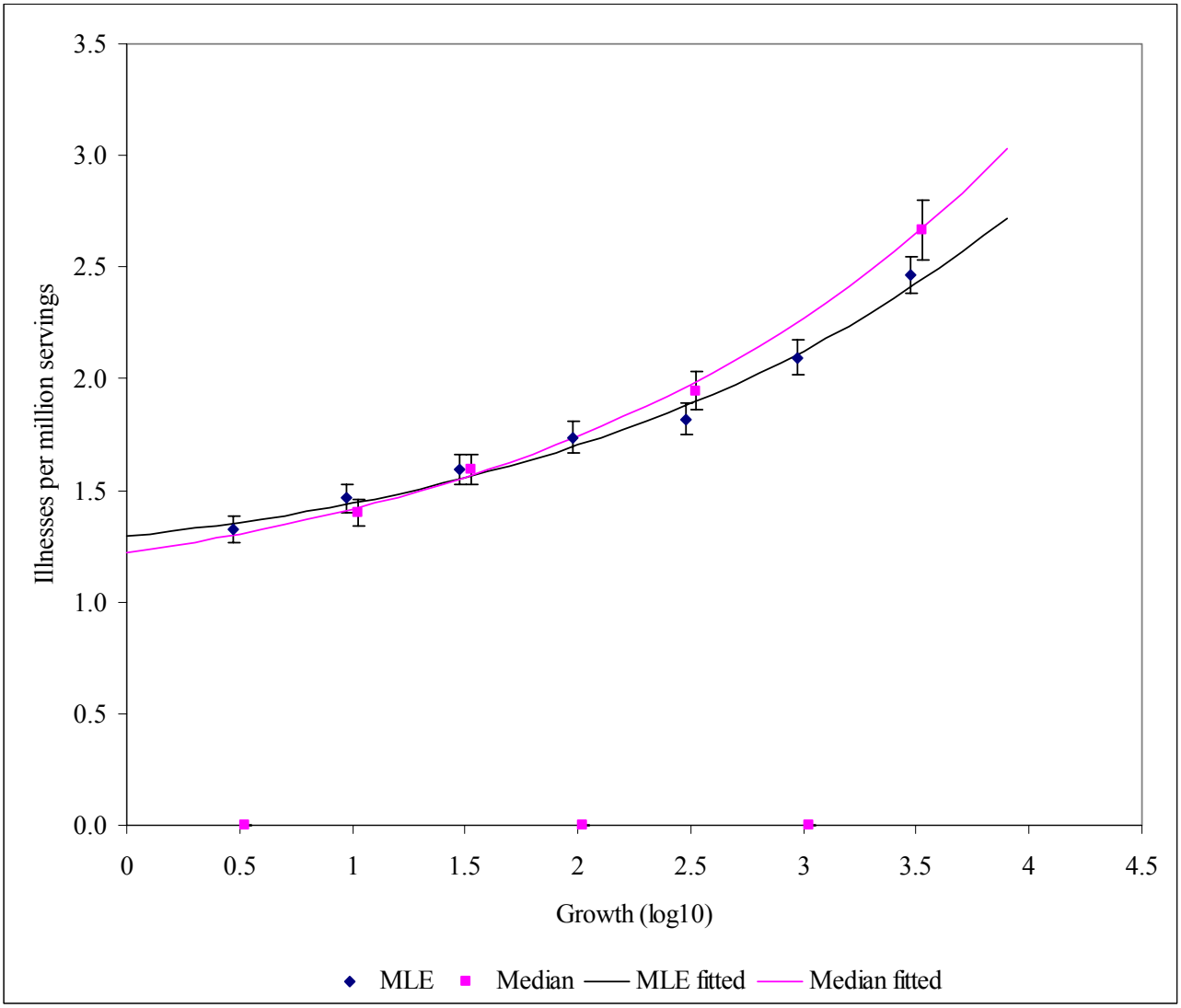
[b] Geometric mean of 600 values for each growth, with each value corresponding to an uncertainty simulation of 30 million servings.

[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 380) 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 79,000 illnesses at 1-$\log_{10}$ growth obtained here by modeling[85] 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).



[85] 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[86] number of serving simulations has 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 very similar 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[87] 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 growth, 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 principal 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.

[86] 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).

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

6.2. Uncertainty estimates

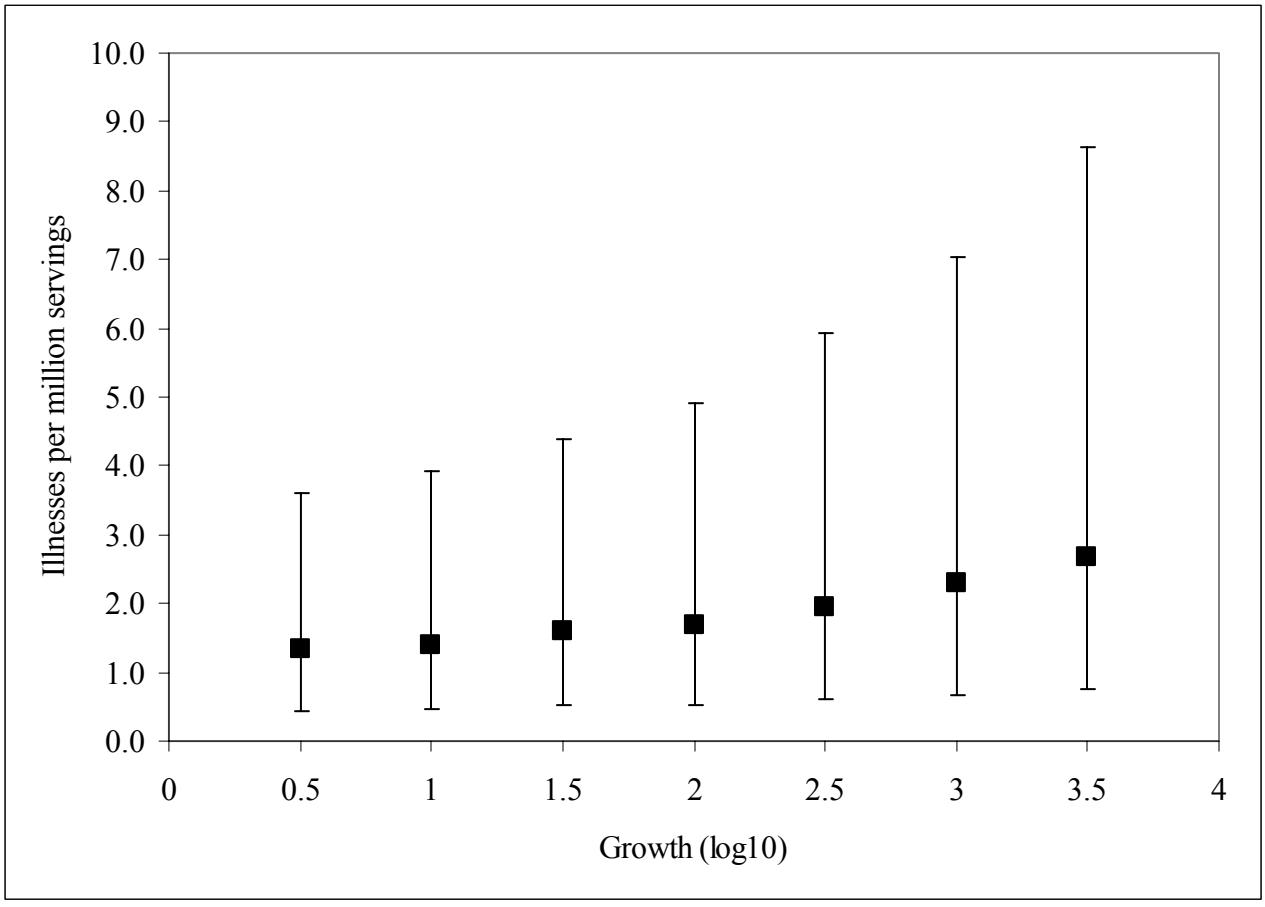
6.2.1. Uncertainty not incorporated in the model

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 limitations of the exposure modeling, and Section 5.4 the further uncertainties of dose-response modeling. To emphasize 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-$\log_{10}$.

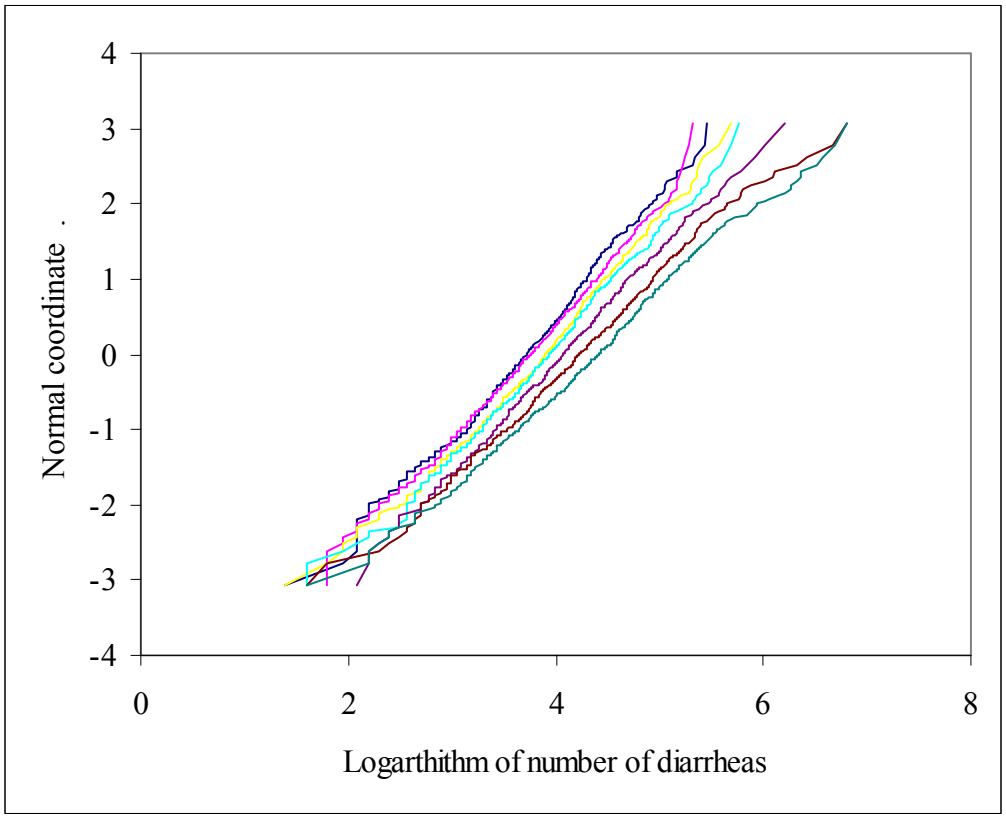
Figure 6.2 Uncertainty estimates for rate of diarrhea for fixed growth during stabilization.



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.[88] 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).

Figure 6.3 Uncertainty distributions at fixed growth during stabilization.



Examination of these uncertainty distributions at different growths during stabilization shows that they have standard deviations that increase slightly with growth during stabilization from about 0.64 (on a natural logarithmic scale) at 1-$\log_{10}$ to 0.72 at 3-$\log_{10}$. 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:

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

where

[88] 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

R_0 = 1.22×10^{-6} per serving,
β = 0.121,
γ = 0.029,
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 a standard deviation that varies with growth g as 0.60 + 0.039g (so e^{ε} varies from about 1.9 to 2.1 for growth from 1-$\log_{10}$ to 3-$\log_{10}$).

One implication is that the uncertainty increases almost directly in proportion to the median rate of illness, so that for all values of growth during stabilization the uncertainty can be practically expressed as the same multiple of the median, specifically a factor about 2.0.[89] In this sense, the uncertainty is practically 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 $\varepsilon = 0.68 \times (-1.2816) = -0.87$ and $0.68 \times 1.2816 = 0.87$ 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*

The following sections provide quantitative estimates for the sources of illness-causing *C. perfringens*, based on the Monte Carlo simulation results with all uncertainty parameters set at their median values. No estimates of uncertainty for these estimates have been made, since these results are not the primary results of the analysis.

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).

[89] 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.

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

	Fraction of all	Normalized fraction
	Not hot-held	
Meat	0.63	0.68
Spices	0.30	0.32
Unknown	0.002	0.002
Germinating spores	0.007	0.007
Total	0.94	1
	Hot-held	
Meat	0.002	0.036
Spices	0.058	0.96
Unknown	0.0002	0.004
Total	0.06	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.

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.

	Fraction by food category[a] observed in the simulation									
Growth	1a	1b	2	3a	3b	3c	3d	4a	4c	4d
0.5	0.15	0.10	0.68	0	0	0	0.0008	0.026	0.0023	0.048
1	0.17	0.08	0.67	0	0	0	0	0.029	0.0007	0.048
1.5	0.16	0.10	0.67	0	0	0	0	0.022	0.0006	0.048
2	0.18	0.11	0.63	0	0.0012	0.0012	0.0017	0.022	0.0029	0.051
2.5	0.19	0.12	0.64	0.0006	0.0017	0	0.0055	0.017	0.0017	0.030
3	0.17	0.13	0.62	0.0014	0.0076	0.0024	0.013	0.025	0.0019	0.034
3.5	0.19	0.12	0.55	0.0057	0.019	0.0069	0.028	0.024	0.0032	0.049

[a] Food categories are defined in Table 3.1.
Note: values less than 0.001 correspond 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 initial 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[90] to the rate is given by

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

where

r is the rate of illnesses, per serving

$r_0 = 0.079 \times 10^{-9}$,

$a = 2.23$,

$b = 0.013$,

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

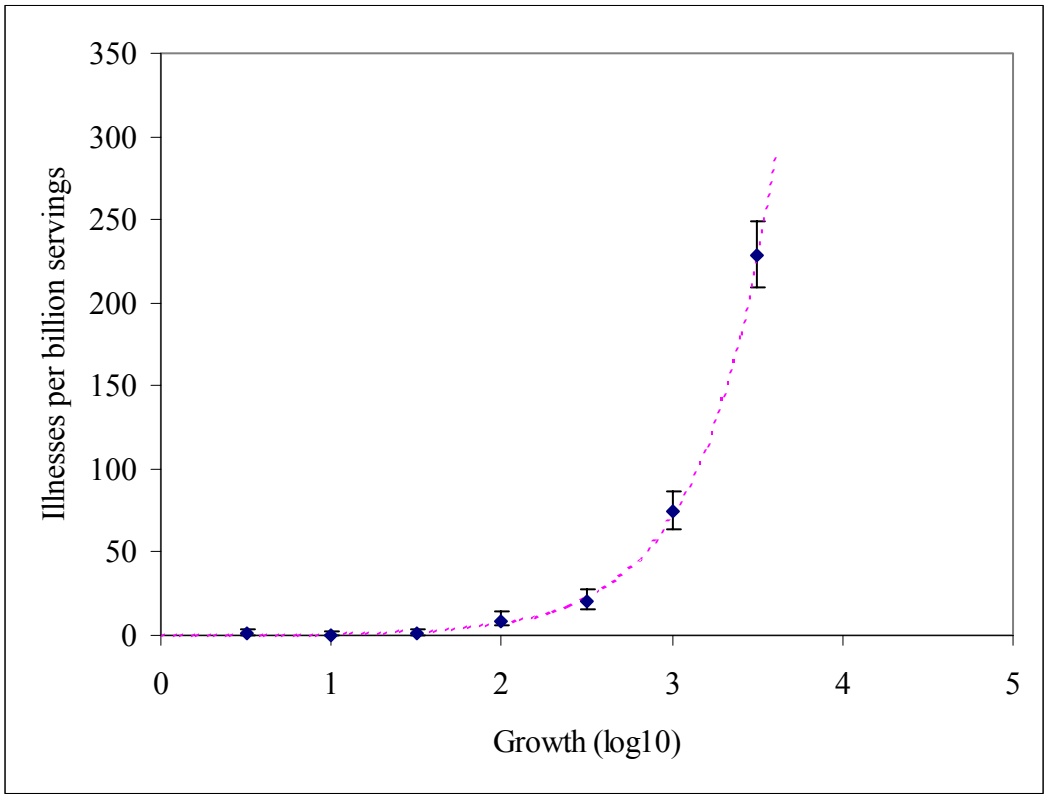
To obtain the estimated number of illnesses per year, replace r_0 in Equation (6.2) with 4.36, giving, for example, an estimated numbers of illnesses due entirely to growth during stabilization shown in Table 6.4.

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

Growth ($\log_{10}$)	Number of illnesses
0.5	13
1	40
1.5	130
2	400
2.5	1300
3	4000
3.5	13000

[90] 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 illnesses is too small to obtain a reliable breakdown by category.

Figure 6.4 Rate of illnesses due entirely to growth of *C. perfringens* during stabilization. Error bars show the numerical precision due to the small number of illnesses simulated, not uncertainties.



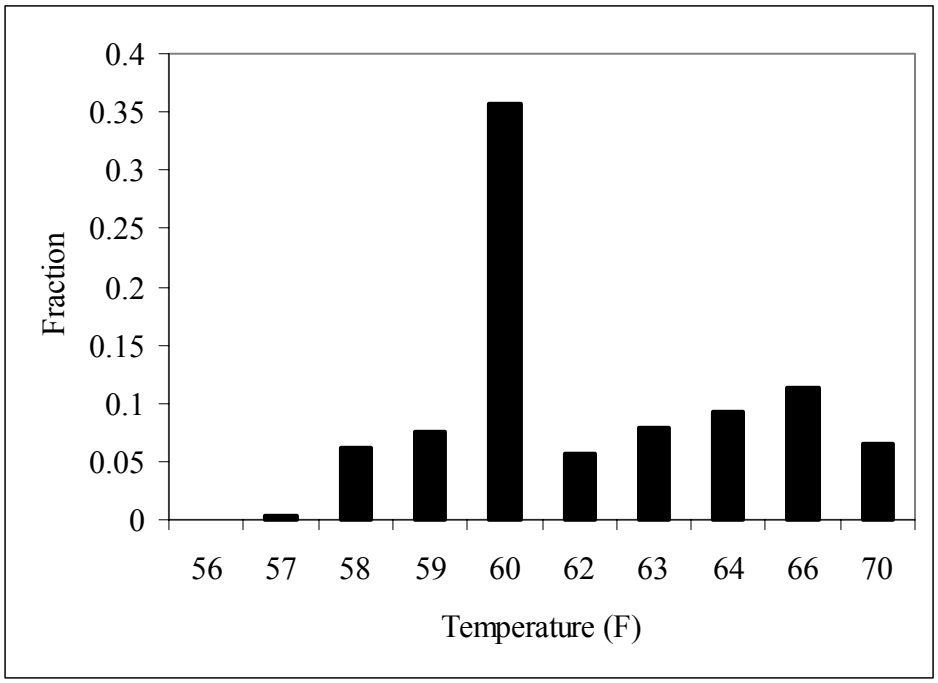
6.3.4. Source by storage temperature

Approximately 90% 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.[91] The large peak at 60 °F (15.6 °C) 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 most illnesses are caused by improper storage, since all the temperatures shown correspond to inadequate refrigeration.

[91] 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 1.4 illnesses per million servings, corresponding to approximately 79,000 illnesses per year in the U.S., at 1-$\log_{10}$ growth during stabilization, through 1.7 illnesses per million servings at 2-$\log_{10}$ growth during stabilization, corresponding to approximately 96,000 illnesses per year, to approximately 2.3 illnesses per million servings, corresponding to approximately 126,000 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 2.4 higher for all growth rates during stabilization, ranging from approximately 179,000 per year at 1-$\log_{10}$ growth, through 228,000 at 2-$\log_{10}$ growth, to 315,000 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 similar at any percentile of the uncertainty distribution, and the relative uncertainty is about the same (a factor of 2 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 1.1 in 10 million servings, corresponding to about 6,000 illnesses of the numbers given above, or about 7.6% 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

underestimates 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 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.[92] 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 contaminated or spoiled. The rate of such illnesses is predicted to be below 1 in a billion servings for 1-$\log_{10}$ of growth during stabilization, rising to about 7 in 100 million servings for 3-$\log_{10}$ of growth (approximately 4,000 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

[92] 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[93]) 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 thermoduric 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

[93] 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 (13.9–21.1 °C)[94] 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 90% 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 10% of the original estimate; at 50% suppression at all temperatures between 57 and 70 °F, the total number of illnesses would be reduced to 55% of the original estimate.[95] 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 sight, taste, or smell, by a fraction of consumers.[96] 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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[94] Temperatures were recorded to the nearest °F, but not all temperatures in this range were seen. A temperature of 56 °F was observed, but no illnesses were predicted for this storage temperature in the seven billion servings simulated.

[95] 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.

[96] 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:

$$p = 1 - \exp\left(-\ln(10) \frac{\ln(C/C_{min})}{\ln(C_{90}/C_{min})} \right) \quad \textbf{(6.3)}$$

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	74,000	20,000	5.9
1	82,000	24,000	6.4
1.5	89,000	25,000	7.1
2	97,000	27,000	7.7
2.5	101,000	34,000	8.7
3	117,000	39,000	9.0
3.5	137,000	46,000	10.1

[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

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

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	< 0.06	t
Mean fraction of spores germinating in RTE production	0.025–0.04	t
Mean fraction of spores germinating with no heat step	0.025–0.04	t
Mean fraction of spores germinating in second heat step	0.06	t
Mean fraction of spores germinating during storage	0.007	t
Mean storage time in manufacture and retail	1.6	n
Fraction of Category 1 foods eaten cold	0.019	t
Fraction of heated foods heated in an oven	~ –0.04	n
Mean microwave heating time	± < 0.04	n
Mean oven heating time	± < 0.06	n
Mean fraction of Category 1 & 4 foods hot-held	0.06	t
Hot-holding time	NE (<0.06)	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 NE: Not evaluated. This is probably small, but would require numerical measurement using on the order of 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 6% of the total, so the sensitivity of the total number of diarrheas to this fraction is less than about 0.06.

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

The fraction of spores that germinate (η, see Section 3.9.4) 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 fraction 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 (6.1), a good estimate for the variation in number N of illnesses with growth during stabilization is

$$N = N_0 \exp\left(\beta g + \gamma g^2\right) \qquad \textbf{(6.5)}$$

where

N is the number of illnesses per year,

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

β = 0.121,

γ = 0.029,

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

Thus if

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

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

$$\frac{x}{N}\frac{\partial N}{\partial x} = f\frac{\beta + 2\gamma g}{\ln 10} \qquad \textbf{(6.7)}$$

At $g = 1$ the term on the right of Equation (6.7) is $0.078f$, and at $g = 3$ it is $0.13f$, and the fraction f is approximately equal to the fraction of illnesses in which the vegetative cells present after the lethality step are due to spores in spices. Section 3.8.3 shows that the concentration of vegetative cells due to spores from spices is proportional to η, while Section 3.5 shows that the concentration of vegetative cells due to spores in meat is independent of η. Table 6.3 shows that only a negligible fraction of illnesses is due to partially cooked foods (Category 3b), hot-holding is predicted to cause only a small fraction (6%, Table 6.2), and only a negligible fraction are due to spores germinating after the stabilization process (Table 6.2), so practically all illnesses are caused by the vegetative cells germinating during stabilization. It is found that $f = 0.32$, practically independent of growth during stabilization.[97] Thus the sensitivity of the number of illnesses to the mean estimate of the fraction of spores germinating during RTE is approximately 0.025 at $g = 1$ to approximately 0.04 at $g = 3$.

[97] 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 fraction (ϕ, see Section 3.9.5) 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; and again about 32% of illnesses (the percentage of illnesses arising from spores in spices) depend directly on this value (Section 3.8.3 shows the concentration of vegetative cells due to spores in spices is inversely proportional to ϕ, whereas the concentration due to spores in meats is independent of ϕ). 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 again about 0.025 to 0.04.

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

This fraction (g_p, see Section 3.9.4) 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 6%, the sensitivity of the total number of servings to the mean value of this parameter is about 0.06.

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

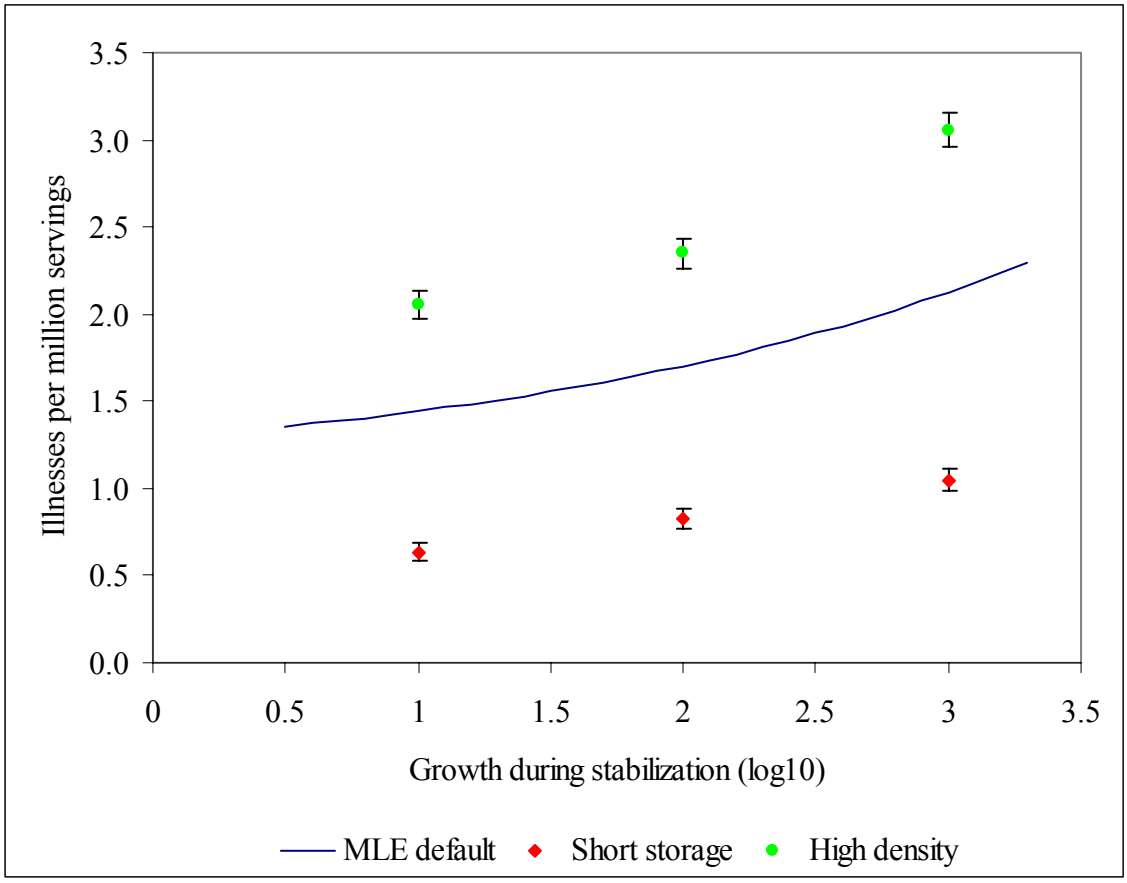
This fraction (g_s, see Section 3.13.1) is a variability distribution with default a triangular distribution (0.0, 0.025, 0.05). The number of illnesses caused by spores germinating in storage is approximately proportional to the mean value of the variability distribution, and the fraction of illnesses due to such germinating spores is about 0.7% (Table 6.2). The sensitivity of the total number of illnesses to the mean value of this parameter is thus about 0.007.

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 0.64 in a million servings at 1-$\log_{10}$ growth, 0.83 in a million at 2-$\log_{10}$ growth, and 1.1 in a million at 3-$\log_{10}$ growth, in each case approximately 0.47 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 –0.76 (ln(0.47)) in the number of illnesses (again, on a logarithmic scale). The sensitivity is thus about –0.76/(–0.47) = 1.6.

Figure 6.6 Approximate variation in MLE of illness rate for sensitive parameters.



6.6.7. The fraction of Category 1b foods that are eaten cold

This parameter is a fixed fraction, with default value 0.2. In the model, Category 1b foods are either eaten cold or hot. Suppose total number of illnesses is N, the number of Category 1b servings is M, the rate of illness, per serving, for cold Category 1b servings is r_1, and for hot Category 1b servings is r_2, and the fraction eaten cold is x. Then

$$N = U + r_1 x M + r_2 (1-x) M \qquad \textbf{(6.8)}$$

where U illnesses are due to other Categories of food. Then

$$\frac{x}{N}\frac{\partial N}{\partial x} = \frac{x(r_1 - r_2)M}{N} = x\left(\frac{n_1}{x} - \frac{n_2}{1-x}\right)\Big/ N \qquad \textbf{(6.9)}$$

where n_1 and n_2 are the numbers of illnesses caused by Category 1b cold and hot foods respectively. Evaluating this expression from the MLE simulation results gives the sensitivity of the number of illnesses to the fraction of Category 1b foods eaten cold as 0.019.

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 — an increase of about 3% in a numerical simulation of 500 million servings, in which the numerical precision in the

simulation is approximately 3% also (at one standard deviation). The logarithmic change in number of illnesses is 0.03 (ln(1.03)), for a logarithmic change in parameter value of –0.69 (ln(0.25/0.5)), giving a sensitivity of about –0.04 (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% at one 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 6%), so the sensitivity to this parameter is approximately equal to 0.06 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 6%) of the total, the sensitivity of total illnesses to this parameter is small (less than 6%). 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 2 in a million servings at 1-$\log_{10}$ growth, 2.3 in a million at 2-$\log_{10}$ growth, and 3.1 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 value for the fraction of RTE and partially cooked servings 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 principal 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 are 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-positive 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-positive 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-positive 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 which after surviving the first heat treatment germinate during the second. The origin of any differences between type A, CPE-positive *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-positive 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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Appendix A Food Categories to be Modeled in the FSIS *C. perfringens* Risk Assessment

A.1 Introduction

The Food Safety and Inspection Service (FSIS) has proposed an RTE rule (FSIS, 2001), a portion of which states that all RTE products, other than thermally processed, commercially sterile products, and processing used to produce partially heat treated products, meet stabilization (*e.g.*, cooling) performance standards to prevent the multiplication of *Clostridium perfringens* (*C. perfringens)*. In an effort to estimate the impact of this rule on the incidence of foodborne illness caused by *C. perfringens* in RTE and partially-cooked foods, a risk assessment was developed. The following document outlines sequentially the procedure adopted by the Agency in selecting and grouping relevant foods for this risk assessment.

A.2 Selection of foods

The most representative available information on foods consumed in the United States was obtained from the Continuing Survey of Food Intakes for Individuals (CSFII 1994–1996, 1998 database, referred to as CSFII, (USDA, 2000)). CFSII was a survey conducted by the Agricultural Research Service (ARS) of the U.S. Department of Agriculture (USDA), initially over the three-year period 1994–1996. During each of those three years, a nationally representative sample of non-institutionalized persons residing in the United States was contacted twice (about 3–10 days apart) and asked about what they had eaten during the previous day (24 hours, midnight to midnight). The 3-year CSFII data set includes information on food and nutrient intakes by 16,103 individuals who provided at least 1 day of dietary data.

The three years of CSFII data from 1994–1996 were augmented by the Supplemental Children's Survey in 1998. This survey was conducted in response to the Food Quality Protection Act of 1996, which required the U. S. Department of Agriculture to provide data from a larger sample of children for use by the Environmental Protection Agency in estimating exposure to pesticide residues in the diets of children. The 1998 supplement adds intake data from 5,559 children where ages ranged from birth through age 9 years to the intake data collected from 4,253 children of the same ages participating in the 1994–96 survey.

The CSFII obtained descriptions and estimates of the quantities for each of the foods and beverages that participants ate or drank. Each food consumed by a person surveyed was assigned a food code and food description that was as specific as possible (*e.g.* it could be a brand name, or a particular ingredient like raw carrot, skin on, or any other descriptive phrase). Each food code has an associated "recipe" that indicates the best available information on the ingredients of that food, and sometimes the cooking and preparation method. However, it should be noted that the CSFII is not designed specifically to obtain information on the ingredients of foods eaten — it is primarily designed to estimate the dietary intake of nutrients. The CSFII contains information on the sodium content of foods (used here to infer salt content), but does not contain information on any nitrite additives.

Using the recipe database of the CSFII, a list of foods that contained meat or poultry was constructed using the following procedures. First, the Recipe Ingredient Dataset, part of the Recipe Database[98] of the CSFII, was searched using the search terms provided in Table A- 1 to find all ingredients containing possible meat and poultry ingredients.

Table A- 1 Search Terms[a] for all Meat and Poultry Ingredients in CSFII.

Piroshki	Ravioli	Opossum	Antelope	Ham	Mountain oysters
Hog	Udder	Crackling	Beaver	Armadillo	Quail
Berliner	Steak	Bear	Ratite	Jerky	Cap(p)icola
Bologna	Buffalo	Venison	Skunk	Zyreicka	Chitterlings
*wurst	Beefalo	Deer	Squirrel	Scrapple	Porcupine
Liver	Peccary	Bison	*burger	Duck	Pastirma
Chorizo	Horse	Rabbit	Meatballs	Cow	Patties
Gyros	Squab	Pheasant	Sremski	Linguisa	Luncheon
Nem-Chua	Game	Dove	Chix	Bacon	Prosciutto
Pastrami	Pigeon	Caribou	Salami	Kidney	Pepperoni
Alessandri	Apenino	Slim Jim	Bouillion	Basturma	Basterna
Wiejskha	Krakowska	Kabanosy	Goralska	Mysliwsa	Kabanosse
White hots	Raccoon	Moose	Brain	Carne	Kabanossy
Feet	Gizzard	Barbeque	Drzewnia	Pate	Krakowska
Turkey	Souse	Poultry	Smokies	Barbecue	Vienna
Link	Dog	Hen	Wieners	Meat	Emu
Basturmi	Patty	Chicken	*furters	Bf	Chick
Ostrich	Goose	Pig	Lamb	Beef	Sausage
Coppa	Head	Veal	Franks	Pork	Goat

a. An asterisk preceding a search term indicates any arbitrary string was considered in connection with the indicated term in the search.

[98] The Recipe Database contains an entry for each unique food code included in CSFII, with the list of food codes corresponding to the list of all unique descriptions of foods described as eaten by participants in CSFII. The Recipe Database entries include ingredients and their amounts, as well as further information not used here.

The list of ingredients thus obtained was searched using the terms listed in Table A- 2 to remove unintentional matches (*e.g.*, meatless bacon, horseradish). The ingredients identified in this second search were examined by hand and those having no meat or poultry products were removed. In addition, food codes 71401000 and 71401030 (respectively French fried potatoes not specified as to fresh or frozen, and prepared from frozen), which are included by the first search because they may contain beef fat, were removed from consideration since any fat used in preparation of these foods would have been subject to temperatures that kill both vegetative cells and spores of *C. perfringens*.

Table A- 2 Search Terms for Meat-Free Ingredients.

meatless	link	substitute	oysters	mock
kidney	patties	milk	barbecue	rolls
imitation	luncheon	cheese	steak sauce	horse
bun	soy	vegetarian	coconut	cocnt
pignolia	seasoning	bar	graham	tea
champagne	egg	head	gooseberry	wheat
substitute	patent	cowpeas	pigeonpea	pigeon pea

Second, the list of ingredients obtained in the first step was merged with the Food Description Database[99] of the CFSII to obtain all the food codes containing them. The Individual Food Intakes Database [100] was then searched with this list of food codes, and those that had been reported as being consumed at least once[101] in the CSFII were compiled. Food codes with descriptions that do not specify the identity of the meat ingredient (*e.g.*, Lima bean soup) were checked against the recipe database to ensure that they were properly identified and, if appropriate, they were eliminated from consideration.

The result was a list of 1,625 food codes describing foods that contain meat or poultry and that are presumed to represent such foods eaten in the U.S. (Appendix B).

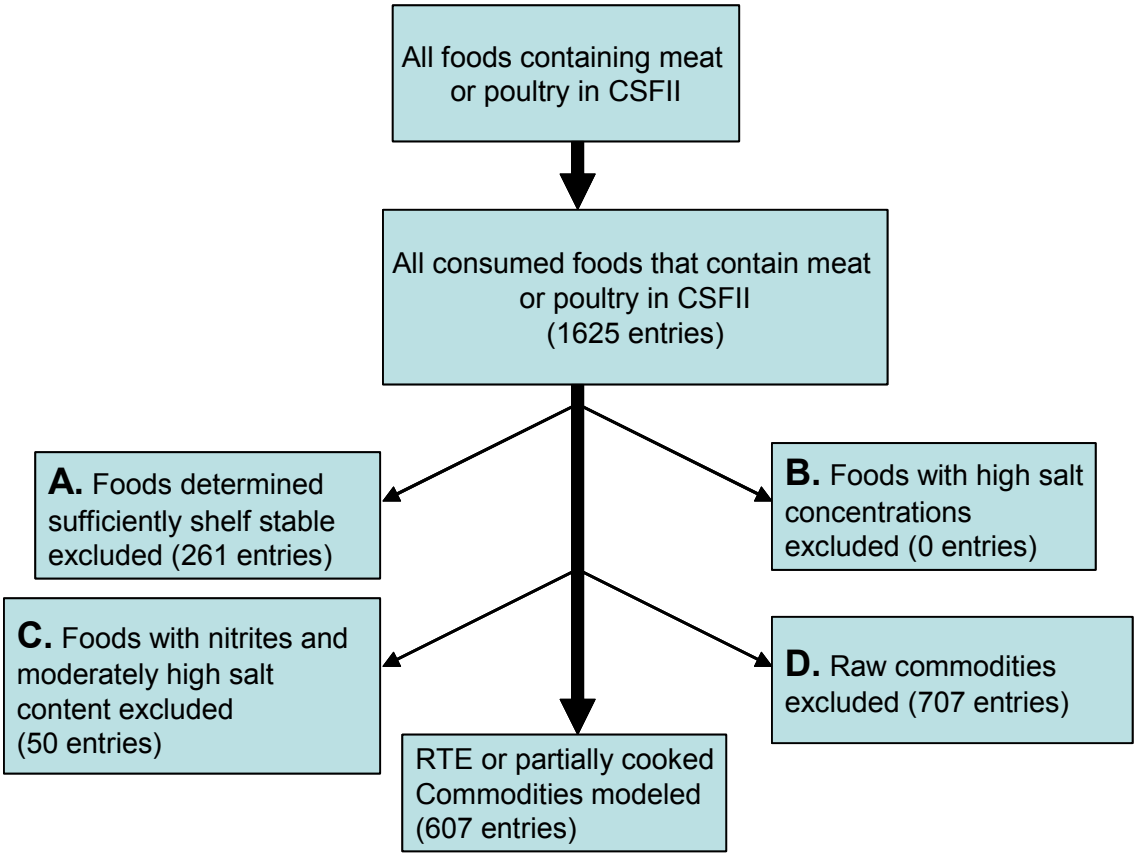
A.3 Exclusion Criteria

The list of 1,625 foods containing meat and/or poultry from the CSFII was modified by excluding those that would not be affected by the proposed rule. This was done by removing from the list raw foods (since the proposed rule affects only RTE and partially cooked foods) and those with characteristics or ingredients that can be expected to inhibit the growth of *C. perfringens* or that are otherwise unlikely to cause human illness from *C. perfringens* (Figure A-1). Food characteristics that make commodities unlikely to cause human illness from *C. perfringens* include those that are: (1) processed in a way that result in shelf stable products, such

[99] In this database, which is a subset of the CSFII, food descriptions are usually generic in nature except for certain breakfast cereals, infant formulas, and candies. Complete and abbreviated descriptions are included. Descriptions for some brand cereals include a name enclosed in parentheses, which denotes the previous name.
[100] The Individual Food Intakes database (a subset of the CSFII, record type 30) contains 598,829 records.
[101] Foods that were not reported to have been consumed were also found using this protocol. This is because foods recorded in pervious CSFII included these commodities and, consequently, food codes describing them were established and remain as part of the database.

as dried meats and foods sold in cans and jars; (2) very high in salt (sodium chloride) content (>8%); or (3) moderately high salt content (3-8%) in combination with nitrites.[102] The identification of foods meeting these criteria for exclusion was done using available food descriptions and characteristics (the CSFII does contains information on sodium content of servings, used here to infer salt content, but not nitrite concentrations). When a food was eliminated it was not reconsidered later with subsequent exclusion criteria even though there is some overlap between the exclusionary groups.

Figure A- 1 Exclusion criteria used for excluding foods from consideration in this risk assessment.



A.3.1 Shelf Stability

Foods that can be stored at room temperature without experiencing growth of *C. perfringens* were eliminated from consideration. Shelf stability is defined in CFR title 9, part 318, Subpart G, 318.300 (u) of the FSIS USDA regulations as "the condition achieved by application of heat, sufficient, alone or in combination with other ingredients and/or treatments, to render the product free of microorganisms capable of growing in the product at non-refrigerated conditions (over 50 °F or 10 °C) at which the product is intended to be held during distribution and storage." The term has been traditionally used by the Agency and is synonymous with the terms "commercial

[102] The Code of Federal Regulations (CFR) Title 9 Chapter III Part 424 subsection 21 states limits of curing regulations for USDA regulated meats. Levels of sodium or potassium nitrite will not exceed 200 part per million (ppm) in the finished product and will reside at lower levels in pork bacon products.

sterility" or "commercially sterile." Dried foods, foods that are retorted during packaging and foods packaged in jars (*e.g.*, baby foods and pickled products) are shelf stable and will be eliminated from consideration because the production methods either eliminate all *C. perfringens* (both vegetative cells and spores) or prohibit the growth of *C. perfringens* as is discussed below.

A.3.2 Dried Foods

Water is necessary for the survival and growth of bacteria including *C. perfringens*. The availability of free water in a food (water that is otherwise not associated with salts, carbohydrates, proteins or other food components and therefore available for use by bacteria) is measured by the water activity (a_w).

In short, studies demonstrate *C. perfringens* growth is optimal at high water activity levels, a_w in the range 0.97–0.995 (Kang *et al*., 1969; Strong *et al.*, 1970). At lower a_w values, within the range 0.93–0.965, the growth rate of *C. perfringens* is decreased (Kang *et al*., 1969; Strong *et al.*, 1970), and depends on a variety of parameters including the solute used, strain, inoculum size, pH, temperature, oxidation-reduction potential, and presence of various nutrients (Craven, 1980).

Based on this information, foods with a_w of less than 0.93 have been assumed to prohibit *C. perfringens* growth. Although CSFII includes some information that might be used in calculation or estimation of a_w, such as the amino acid and salt content, information is insufficient to accurately estimate a_w. Indeed, experimental measurements are necessary to provide reliable quantification of the a_w for foods, so the foods affected by this exclusion have been selected on information independent of the CSFII. Some sausages, salamis, hams, pepperoni, soups, chipped and dried beef products and dried meats have a_w values below this level (Alzamora and Chirife, 1983; Lee and Styliadis, 1996; Holley *et al*., 1988).

A.3.3 Retorted Products

Many commodities packaged in cans and jars have no viable *C. perfringens* bacteria (either vegetative cells or spores) due to retorting. Retorted products are pre-packaged (in cans, jars, or appropriate pouches), hermetically sealed and treated with a post-packaging lethality step that includes heating to 240 °F (116 °C) for a specified period of time (FSIS, 1999). Retorting has been verified and validated as a processing method that is lethal to spores and vegetative cells in production facilities. Due to the lethality achieved, foods processed in this way have been presumed to be free of *C. perfringens* cells or spores.

A.3.4 Non-retorted Shelf Stable Jarred Commodities

Products packaged in jars and cans that are not retorted are generally "hot packed," and pH is adjusted to 4.6 or lower. The temperatures used during hot packing are expected to kill vegetative cells[103]. The low pH of these products is expected to prevent growth of any surviving vegetative cells (21CFR114), and prevent the germination (Craven, 1988; Ahmed and Walker, 1971) and subsequent growth of spores.

[103] "Hot Packed" RTE products use a thermal process schedule that includes times and temperatures determined to be effective by an industry establishment's process authority. Specific times and temperatures are therefore not known, however, as the process is required to be bacterially lethal, it assumed to kill *C. perfringens* vegetative cells.

Growth of *C. perfringens* is optimal between pH 6 and 7. Limited growth may be expected at pH values ≤5.0 and ≥8.3 (Hobbs, 1979; Labbe, 1989). Acidic foods (pH ≤5.0) are generally considered inhospitable for growth of *C. perfringens* (McClane, 2001). Moreover, an acidic pH in foods acts synergistically with other factors, such as the presence of curing salts, to inhibit growth of *C. perfringens* (Labbe, 1989). It is reasonable to assume that RTE and partially cooked meat or poultry products with a pH ≤5.0 are extremely unlikely to support the growth of *C. perfringens* based on the ranges for growth described above, and consequently, foods hot packed and pH adjusted are excluded from the risk assessment.

The 1,625 CSFII meat and poultry containing foods were searched for a variety of terms (Table A- 3) which are assumed to correspond to dried, retorted, or jar packed products, which were determined to be of limited concern for reasons described above. The first 261 entries (rows 1–261) in Appendix B were those foods eliminated due to shelf stable characteristics described in Table A- 3 and are labeled in the Exclusion/Category column as either "ss-c" (shelf stable-canned/jarred) or "ss-d" (shelf stable-dried).

Table A- 3 Search Terms for Shelf Stable Products.

Dried Products		
Dried/Dry	Salami	Cracklings
-beef	-dry	Pastirma
-duck breast	-fermented	Basterna
-not beans	-hard	Basturmi
Ham	Sausage	Basturma
-dry cured	-Alessandri	Jerky
-parma	-Apenino	Bacon Bits
-Serrano	-summer	Pork Rinds (Fried)
-Westfhalia	-fermented	Proschutto
Stick	Slim Jim	Prosciutto
–not drumstick	Pepperoni	Coppa
Bouillon		
Canned/Jarred Products		
Soup	Sauce	Baby
-not home recipe	-spaghetti and meatball	Jar or Canned
-not with game meats	-pasta with meat sauce	
-not mushroom	-not home recipe	
	Stew	Deviled Ham

	-not home recipe	Chipped Beef
Vienna	Dressing	Potted or Roast Beef Spread
Spam	-with bacon	Pickled

A.3.5 Salt

The concentration of salt (sodium chloride) in a food item affects the ability of *C. perfringens* to grow. A review of the published literature identified various studies that examined *C. perfringens* growth in varying concentrations of salt (Table A- 4).

Table A- 4 Effect of salt on *C. perfringens* growth: Summary of studies.

Reference	Inoculum cell type; level	Time (days)	Temperature (°C)	% Salt	Results[a]
Tested in lab media					
Gough and Alford, 1965	Vegetative; unknown	1	37	4	14/18[b] 'good' growth; 4/18 'slight' growth
				6	1/14 'good' growth; 8/14 'slight' growth
				8	1/18 'slight' growth
Mead, 1969	Vegetative; 2-$\log_{10}$ CFU	1, 14	37	6	1 d: 0/4 14 d: 4/4
	7.6-$\log_{10}$ CFU			6	1 d: 3/4 14 d: 4/4
Roberts and Derrick, 1978	Vegetative; unknown	90	35	6	11/21 growth to visible turbidity
				7	1/21 growth to visible turbidity
Tested in a food matrix					
Juneja and Majka, 1995 [b]	Spores; 2.3-$\log_{10}$ CFU/g	0.5	28	3	2-$\log_{10}$ CFU/g growth in beef
Juneja and Marmer, 1996a [b]	Spores; 3-$\log_{10}$ CFU/g	0.75	28	3	2.7-$\log_{10}$ CFU/g growth in turkey

a. Results are indicated in terms of growth as the number of samples in which growth occurred/total number of samples; where not specified, the extent of growth was unspecified — but assumed to be an observed increase over the starting inoculum.
b. Food samples included 0.3% sodium pyrophosphate.

Only at concentrations greater than 8% salt, was growth essentially halted (Gough and Alford, 1965). Consequently, only foods with at least this concentration of salt were considered for elimination. The concentration of salt in each food was calculated using data obtained from the CSFII. A maximum, mean and minimum sodium concentration and serving amount for each food item is provided by CSFII; the minimum was used in the exclusion calculation. To calculate the salt percentage, it was assumed that all sodium present in a particular food was

sodium chloride. The minimum number of grams of sodium reported in the CSFII was then converted into grams of salt and this value was divided by the minimum portion size in grams. Foods found to contain >8% salt, based on this calculation, were eligible for exclusion from this risk assessment. All such commodities had already been eliminated from consideration due to the fact that they qualified as shelf stable.

A.3.6 Salt in the Presence of Nitrites

Nitrites are added to various meat products as preservatives typically in the form of sodium nitrite ($NaNO_2$) or potassium nitrite (KNO_2). Foods with nitrite were considered for exclusion from the final list of food items. The available data (Table A- 5) suggest nitrite and salt are effective at inhibiting *C. perfringens* growth; however, most of the experiments were conducted at temperature below the *C. perfringens* optimum growth temperature (43 and 47°C) and could not be used to predict growth in foods containing salt and nitrite at higher temperature. One study conducted at a higher temperature suggests that a combination of a minimum of 3% salt and 156 ppm ingoing nitrite is effective at inhibiting *C. perfringens* growth (Kalinowski *et al*., 2003). As the level of ingoing nitrite in this study was below the maximum allowed in most products (200 ppm), it was assumed that products known to contain nitrites would have similar nitrite levels to those used by Kalinowski and contributors.

Table A- 5 Effect of combined nitrite and salt on *C. perfringens* growth: Summary of studies.

Reference	Food matrix	Inoculum cell type; level	Time (days)	Temperature (°C)	Nitrite (ppm)[a]	Salt (%)	Result (growth)
Solberg and Elkind, 1970	beef/pork frankfurters	Unclear; 3-log_{10}	3	15	136	2.2	2-log_{10} growth increase
			5	12			
Paradis and Stiles, 1978	bologna	Vegetative; 2–3-log_{10} CFU/g	1	30	Exact nitrite level unspecified	2.4	No growth
Hallerbach and Potter, 1981	beef/pork frankfurters	Spores; 2–3-log_{10} CFU/g	3.1	20	140	2.2	No growth
	Thuringer cervelat sausage		4		156	2.7	
Vareltzis *et al*., 1984	Chicken frankfurters	Spores; 4.7-log_{10} CFU/g	9	20	150	2.6	No growth; ~0.7-log_{10} CFU/g decline
Kalinowski *et al*., 2003	Cooked turkey	Spores; 2-log_{10} CFU/g	0.25	43.3	156	3.0	No growth; post 1 hr, levels fell below 3 CFU/g (detection

							level)

a. Ingoing levels of nitrite.

The PROFILE® ShowCase (2002) includes information on 696 manufacturers and suppliers and lists their products and labeling information. This database was used to develop a list of search terms that are representative of foods containing nitrite. A minimum of two manufacturers' product labels were arbitrarily chosen from those available for each type of food. If the products contained nitrites from all companies checked, all similar products were assumed to also contain nitrites. Table A- 6 indicates the search terms that were used to establish which foods had nitrite. The 50 foods found in rows 262–311 of Appendix B contain a minimum of 3% salt in addition to the nitrite indicated and were excluded from consideration in the risk assessment.

Table A- 6 Search Terms[a] for Foods with Nitrite.

Capicola	Cappicola	Souse	Hot Dogs
Cure	Cured	Ham	Cold Cuts
Corned Beef	Pork -not *chop -not fresh	Bacon -not w burger -not w chicken	Sausage -not fresh
Pastrami	Chorizo	Mortadella	Wieners
Scrapple	*wurst	Salami	Head Cheese
Pizza (cross referenced with recipe data set to establish meat type)		Luncheon	Benedict
Smoked meat products		*furters	Bologna

a. An asterisk proceeding a search term indicates any arbitrary string was considered in connection with the indicated term in the search.

A.3.7 Raw Commodities

This risk assessment addresses RTE and partially cooked foods. Consequently, those foods that can be presumed to have left production plants raw were eliminated from consideration. First, foods consisting of exotic meats, organ meat, or wild game were excluded based on the assumption that these are not commonly available as RTE or partially cooked commodities in the marketplace. Second, foods that include descriptors specifically designating the commodity as raw were excluded (*e.g.* cooked, home recipe). Third, foods were excluded that are not commonly available as RTE or partially cooked based on the PROFILE® ShowCase (2002). The terms used to identify raw foods according to these criteria are listed in Table A- 7.

Table A- 7 Search Terms for Foods Presumed to be Prepared from Raw Meat.

Brain	Head (not headcheese)	Ostrich
Gizzard	Feet (chicken, pig)	Kidney
Liver	Neck	Tail
Back	Tripe	Stomach
Duck	Rabbit	Squirrel
Lamb	Goat	Quail
Caribou	Bison	Dove
Venison	Ratite	Bear
Pheasant	Emu	Deer
Sparerib (Barbecued)	Egg (scrambled with meat)	Egg Casserole
Ground meat/poultry	Egg Casserole	Tartare
Steak	Burger	Oxtail
Bacon	Cooked[a]	Prepared
Mushroom (soup) mixture	Raw	Uncooked[a]
Nonvalue added meats: meats listed either with or without bone, with or without skin, lean or whole, and cooked various ways but without sauces or side dishes.		Home Made
		Home Recipe

a. The apparent contradiction of having both "cooked" and "uncooked" in these search terms is that uncooked may identify raw ingredients directly, while cooked in the CSFII database (USDA, 2000) often indicates that the participant prepared the food from raw ingredients.

The 707 foods excluded using the above terms are found in rows 312 through 1018 in Appendix B and are marked in the Exclusion/Category column with an "R".

A.3.8 Factors Not Employed as Exclusion Criteria

In addition to shelf stability, salt content, nitrites in combination with salt, and raw foods, the effects of added antimicrobials and the availability of oxygen were considered as a means for exclusion of foods. Examination of the scientific evidence, the disparity of industrial product formulations, and the fact that these product formulations are protected from disclosure prohibit the Agency from excluding the possibility of *C. perfringens* growth based on the presence of any allowable antimicrobials or the exclusion of oxygen.

A.4 Food Categories

The 607 foods not excluded based on the preceding methods (rows 1019 through 1625 of Appendix B) were examined for similarities that would allow examination of a number of commodities in tandem. The characteristics that were considered to be most relevant are:

1) foods containing nitrites with between 2.2% and 3% salt,
2) foods unlikely to be reheated prior to consumption,
3) foods likely to be reheated immediately prior to consumption, and
4) foods reheated prior to consumption but not necessarily immediately before consumption ("hot-held").

It was not possible to determine if some of the foods identified in the CSFII were RTE or prepared from raw ingredients. In these instances it was clear that the foods, if RTE, would have been frozen due to commercial availability. Foods of this type are assigned to one of the appropriate categories listed above and the number of servings used in the exposure assessment will be adjusted according to a factor that correlates to the percent of foods that are believed to be RTE.

A.4.1 Category 1: Foods Containing Nitrites and between 2.2% and 3% Salt

The effects of nitrite were previously discussed. Foods were excluded if they contained nitrite in the presence of at least 3% salt. Foods that have between 2.2% and 3% salt are likely to inhibit *C. perfringens* growth (Solberg and Elkind, 1970; Kalinowski *et al*., 2003), although they may not completely prevent growth. Due to the different growth rates anticipated in these foods, they will be modeled as a group. Since information on the fraction of hot dogs (frankfurters) that are eaten cold is available (Section 3.14.2.1), this group was further subdivided into categories 1a (hot dogs or frankfurters) and 1b, based on food description. Foods in this group are marked in Appendix B with a "1a" or "1b" in Column D and encompass the 62 foods in rows 1018–1080.

A.4.2 Category 2: Foods Unlikely to be Reheated for Consumption

RTE meat salads and sandwiches are sold refrigerated with instructions to keep refrigerated and serve cold. Additionally, meats such as cold cuts loose moisture quickly if heated, and therefore are likely to be prepared and served cold. There are 23 foods from the CSFII that are unlikely to be reheated prior to consumption and will thus be modeled as a group to reflect these consumer practices. They are marked with a "2" in Column D, rows 1081–1112 of Appendix B.

A.4.3 Category 3: Foods Likely to be Reheated for Immediate Consumption

It is assumed that foods reported in CSFII as "frozen meals" are not bulk foods and consequently are highly unlikely to be stored above refrigeration temperatures for any extended period of time. Focus group studies conducted for the Food Safety and Inspection Service's (Office of Policy and Program Development) Labeling and Consumer Protection Staff (Cates *et al.*, 2002) have indicated that consumers consider preparation instructions for frozen entrees and dinners "most useful." The study also found that focus group members believe such preparation instructions are product specific, so that consumers are likely to follow the instructions when preparing frozen meals. While the results are qualitative and were not intended to be nationally representative, this suggests that consumers are unlikely to abuse such products in such a way as to facilitate *C. perfringens* spore germination and subsequent cell growth. Additionally, in a Home Food Safety Study (Audits International, 2000) that monitored meal preparation, service, post-meal clean up, and the handling or storage of leftovers in a non-random, non-representative

group of 115 household kitchens, no instances of hot-holding (either proper or improper) were observed in homes. When the in-home results were compared to analogous observations in food service establishments (*i.e.*, hospitals, nursing homes, schools, full service restaurants, and fast food establishments), homes were found to have much higher compliance (68%) with appropriate holding times and temperatures than full service restaurants (37%). Since no observations of hot-holding were made in households, the only temperature abuse observed involved improper cold storage. Audits International suggested this is "logical because homes tend to cook for immediate consumption whereas restaurants tend to hold food, thus increasing their opportunities for a violation." As described, the study conducted by Audits International was not designed to be nationally representative; but lacking any other sources of data, the observations have been used here to indicate likely national characteristics.

Because "frozen meals" are not commonly available in hotel, restaurant or institutional settings where hot-holding is likely to occur (PROFILE® ShowCase, 2002) and, because consumers are reported to follow explicit preparation instructions provided by manufacturers for frozen meals, all frozen meals considered in this risk assessment are modeled as a part of the "foods likely to be reheated for immediate consumption" group. The food list was also surveyed for foods likely to be prepared for immediate consumption. The main trait that qualifies a food as such is a likelihood that food quality would grossly deteriorate if held warm for extended periods. The foods that were reported to be frozen meals in CSFII are denoted with a "3" in Column D, rows 1113–1515 of Appendix B.

A.4.4 Category 4: Foods Served Hot but not Necessarily Prepared for Immediate Consumption

Since 46 out of 46 *C. perfringens* outbreaks studied by CDC had "improper hot-holding" as a contributing factor (CDC 2002), foods that are hot-held are considered of greater risk than those that are not. A list of foods commonly hot-held has been provided to FSIS by US FoodService (Appendix C). These foods are modeled so as to incorporate the distribution of times and temperatures associated with hot-holding in the final food preparation component of the risk assessment model. The 110 foods in this category make up the remainder of the list and are identified with a "4" in Column D, rows 1516–1625 of Appendix B.

A.5 Summary

This appendix describes how foods were chosen to be modeled in the *C. perfringens* risk assessment. The steps involved were:

- A list of all foods consumed in the U.S. that contains meat or poultry was constructed from the information in the CSFII.

- Ready to eat and partially cooked foods on this list that are not likely to either have any *C. perfringens* or support the growth of *C. perfringens* due to food characteristics or ingredients were excluded. Foods that were excluded were those foods that are canned, jarred, very high (>8%) in salt, and moderately high (3-8%) in salt and containing nitrites.

- Foods that are sold raw or uncooked, based on the description in CSFII, were excluded from consideration.

- The remaining foods were grouped into four categories that will be modeled in the *C. perfringens* risk assessment. These categories are: (1) foods with 2.2%–3% salt in the presence of nitrites; (2) foods unlikely to be reheated before consumption; (3) foods likely to be reheated before immediate consumption; and (4) foods served hot but not necessarily prepared for immediate consumption.

Appendix B Food code listing

Meat and poultry containing foods considered for inclusion in the *C. perfringens* risk assessment as described in Appendix A. Food codes and descriptions are from the Consumer Survey of Food Intakes for Individuals (CSFII) 1994-1996, 1998, Section 12.2 "Food Codes and Abbreviated Descriptions". Ordering is by exclusion code or category code and sub-code, then by food code.

Key

Foods Excluded from Risk Assessment	
Reason for exclusion	
ss-d	shelf stable dried
ss-c	shelf stable canned/jarred
N	contains ≥3% salt and nitrites
R	raw
Foods Included in Risk Assessment	
Categories codes	
Category 1	foods containing between 2.2 and 3% salt and nitrites
	a = frankfurter (hot dog)
	b = all others
Category 2	foods unlikely to be reheated for consumption
	(no sub-codes)
Category 3	foods likely to be reheated for immediate consumption
	a = sauce, acid as a component
	b = partially cooked
	c = Mexican spices as an ingredient (higher spore count)
	d = all others
Category 4	foods served hot but not necessarily prepared for immediate consumption
	a = sauce, acid as a component
	c = Mexican spices as an ingredient (higher spore count)
	d = all others

Row	FOOD CODE	Description	%NaCl	Exclusion/ Category code	Sub-code
1	21602000	Beef, dried, chipped, uncooked	8.82	ss-d	
2	21602010	Beef, dried, chipped, cooked in fat	0.14	ss-d	
3	21602100	Beef jerky	5.62	ss-d	
4	22003000	Pork, dehydrated, oriental style	1.74	ss-d	
5	22311450	Ham, prosciutto	6.85	ss-d	
6	22709010	Pork skin, rinds, deep-fried	4.67	ss-d	
7	22820000	Meat stick, baby food	1.39	ss-d	
8	23321900	Venison/deer jerky	7.44	ss-d	
9	24705010	Chicken stick, baby food	1.22	ss-d	
10	25220120	Beef sausage, smoked, stick	4.29	ss-d	
11	25221250	Pepperoni	5.18	ss-d	
12	25221520	Salami, dry or hard	4.72	ss-d	
13	25221810	Thuringer	3.16	ss-d	
14	27113200	Creamed chipped or dried beef	1.52	ss-d	
15	27118130	Stewed dried beef, Puerto Rican style (Tasajo guisado, carne cecina guisada)	5.67	ss-d	
16	28310110	Beef, broth, bouillon, or consomme	0.83	ss-d	
17	28310130	Beef, broth, bouillon, or consomme, dry, not reconstituted	52.06	ss-d	
18	28340110	Chicken, broth, bouillon, or consomme	0.81	ss-d	
19	28340140	Chicken broth, bouillion, or consomme, dry, not reconstituted	47.22	ss-d	
20	28520000	Gravy or sauce, Chinese (soy sauce, stock or bouillon, cornstarch)	1.67	ss-d	
21	58163310	Flavored rice mixture	0.68	ss-d	
22	58421000	Sopa seca (dry soup), Mexican style, NFS	1.11	ss-d	
23	58421060	Sopa seca de arroz (dry rice soup), Mexican style	1.08	ss-d	
24	75649050	Vegetable soup, made from dry mix	1.01	ss-d	
25	25221920	Vienna sausage, chicken, canned	3.48	ss-c	
26	20000070	Meat, baby food, NS as to type, NS as to strained or junior	0.15	ss-c	
27	21002000	Beef, pickled	2.88	ss-c	
28	21401400	Beef, roast, canned	0.15	ss-c	
29	21416150	Corned beef, canned, ready-to-eat	2.55	ss-c	

30	21701010	Beef, baby food, strained	0.21	ss-c
31	21701020	Beef, baby food, junior	0.17	ss-c
32	22311500	Ham, smoked or cured, canned, NS as to fat eaten	3.24	ss-c
33	22311510	Ham, smoked or cured, canned, lean and fat eaten	3.15	ss-c
34	22311520	Ham, smoked or cured, canned, lean only eaten	3.19	ss-c
35	22707020	Pork, pig's feet, pickled	2.35	ss-c
36	22810010	Ham, baby food, strained	0.10	ss-c
37	23410010	Lamb, baby food, strained	0.16	ss-c
38	23420010	Veal, baby food, strained	0.16	ss-c
39	24198540	Chicken, canned, meat only, NS as to light or dark meat	0.34	ss-c
40	24198550	Chicken, canned, meat only, light meat	0.46	ss-c
41	24198560	Chicken, canned, meat only, dark meat	0.48	ss-c
42	24198570	Chicken, canned, meat only, light and dark meat	0.34	ss-c
43	24206000	Turkey, canned	1.19	ss-c
44	24701010	Chicken, baby food, strained	0.12	ss-c
45	24701020	Chicken, baby food, junior	0.13	ss-c
46	24703000	Turkey, baby food, NS as to strained or junior	0.16	ss-c
47	24703010	Turkey, baby food, strained	0.14	ss-c
48	24703020	Turkey, baby food, junior	0.18	ss-c
49	24706010	Turkey stick, baby food	1.23	ss-c
50	25180110	Liver, beef, baby food, strained	0.19	ss-c
51	25221910	Vienna sausage, canned	2.42	ss-c
52	25230530	Ham and pork, luncheon meat, chopped, minced, pressed, spiced, canned	3.39	ss-c
53	25230540	Ham, pork and chicken, luncheon meat, chopped, minced, pressed, spiced, canned	2.40	ss-c
54	25230550	Ham, pork, and chicken, luncheon meat, chopped, minced, pressed, spiced, canned, reduced sodium	2.40	ss-c
55	25240000	Meat spread or potted meat, NFS	3.27	ss-c
56	25240210	Ham, deviled or potted	3.28	ss-c
57	25240310	Roast beef spread	2.57	ss-c

58	27111300	Mexican style beef stew, no potatoes, tomato-based sauce (mixture) (Carne guisada sin papas)	1.87	ss-c
59	27111310	Mexican style beef stew, no potatoes, with chili peppers, tomato-based sauce (mixture) (Carne guisada con	1.41	ss-c
60	27120130	Mexican style pork stew, no potatoes, tomato-based sauce (mixture) (cerdo guisado sin papas)	1.14	ss-c
61	27211200	Beef stew with potatoes, gravy	0.07	ss-c
62	27221150	Mexican style pork stew, with potatoes, tomato-based sauce (mixture) (cerdo guisado con papas)	1.02	ss-c
63	27311310	Beef stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-bas	0.14	ss-c
64	27311320	Beef stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based	0.62	ss-c
65	27311420	Beef stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy	0.61	ss-c
66	27330030	Lamb or mutton stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy),	1.00	ss-c
67	27330210	Lamb or mutton stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy),	1.03	ss-c
68	27341310	Chicken or turkey stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy	0.50	ss-c
69	27341320	Chicken or turkey stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy),	0.52	ss-c
70	27341510	Chicken or turkey stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy	0.23	ss-c
71	27341520	Chicken or turkey stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy),	0.63	ss-c
72	27350030	Seafood stew with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-bas	1.04	ss-c

73	27350310	Seafood stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-	1.05	ss-c
74	27360000	Stew, NFS	0.58	ss-c
75	27360100	Brunswick stew	0.57	ss-c
76	27430400	Lamb or mutton stew with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)),	0.47	ss-c
77	27430410	Lamb or mutton stew with vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), gr	0.96	ss-c
78	27563010	Meat spread or potted meat sandwich	2.00	ss-c
79	27601000	Beef stew, baby food, toddler	0.88	ss-c
80	27610100	Beef and egg noodles, baby food, NS as to strained or junior	0.06	ss-c
81	27610110	Beef and egg noodles, baby food, strained	0.04	ss-c
82	27610120	Beef and egg noodles, baby food, junior	0.04	ss-c
83	27610710	Beef with vegetables, baby food, strained	0.06	ss-c
84	27610730	Beef with vegetables, baby food, toddler	0.45	ss-c
85	27640050	Chicken and rice dinner, baby food, strained	0.04	ss-c
86	27640100	Chicken noodle dinner, baby food, NS as to strained or junior	0.13	ss-c
87	27640110	Chicken noodle dinner, baby food, strained	0.04	ss-c
88	27640120	Chicken noodle dinner, baby food, junior	0.04	ss-c
89	27640810	Chicken, noodles, and vegetables, baby food, toddler	0.47	ss-c
90	27642110	Turkey, rice and vegetables, baby food, strained	0.04	ss-c
91	27642120	Turkey, rice and vegetables, baby food, junior	0.04	ss-c
92	27642130	Turkey, rice, and vegetables, baby food, toddler	0.46	ss-c
93	27642310	Turkey vegetable dinner, baby food, strained	0.08	ss-c
94	27644110	Chicken soup, baby food	0.04	ss-c
95	28310120	Beef, broth, bouillon, or consomme, canned, low sodium	0.08	ss-c

96	28310210	Chili beef soup	1.05	ss-c
97	28310220	Chili beef soup, chunky style	0.82	ss-c
98	28310230	Meatball soup, Mexican style (Sopa de Albondigas)	0.19	ss-c
99	28310320	Beef noodle soup, Puerto Rican style (Sopa de carne y fideos)	1.16	ss-c
100	28310330	Beef and rice noodle soup, Oriental style (Vietnamese Pho Bo)	0.69	ss-c
101	28315100	Beef vegetable soup with potato, stew type	0.92	ss-c
102	28315120	Beef vegetable soup with noodles, stew type, chunky style	0.85	ss-c
103	28315130	Beef vegetable soup with rice, stew type, chunky style	0.85	ss-c
104	28315140	Beef vegetable soup, Mexican style (Sopa / caldo de Res)	0.87	ss-c
105	28315150	Meat and corn hominy soup, Mexican style (Pozole)	0.51	ss-c
106	28316020	Beef and mushroom soup, canned, low sodium	0.06	ss-c
107	28317010	Beef stroganoff soup, chunky style	1.11	ss-c
108	28320110	Pork and rice soup, stew type, chunky style	0.68	ss-c
109	28320120	Pork vegetable soup with noodles, stew type, chunky style	1.17	ss-c
110	28320130	Ham, rice, and potato soup, Puerto Rican style	0.51	ss-c
111	28320150	Pork, vegetable soup with potatoes, stew type	2.83	ss-c
112	28320300	Pork with vegetable (excluding carrots, broccoli and/or dark-green leafy) soup, Oriental Style	0.10	ss-c
113	28321130	Bacon soup, cream of, prepared with water	1.20	ss-c
114	28340150	Mexican style chicken broth soup stock	0.48	ss-c
115	28340160	Chicken broth, canned, less or reduced sodium	0.59	ss-c
116	28340170	Chicken broth, canned, low sodium	0.40	ss-c
117	28340210	Chicken rice soup, Puerto Rican style (Sopa de pollo con arroz)	1.02	ss-c
118	28340220	Chicken soup with noodles and potatoes, Puerto Rican style	0.24	ss-c

119	28340310	Chicken gumbo soup	0.99	ss-c
120	28340510	Chicken noodle soup, chunky style	0.90	ss-c
121	28340530	Chicken soup	1.17	ss-c
122	28340550	Sweet and sour soup	1.42	ss-c
123	28340580	Chicken soup with vegetables (broccoli, carrots, celery, potatoes and onions), Oriental style	0.63	ss-c
124	28340610	Chicken or turkey vegetable soup, stew type	0.90	ss-c
125	28340630	Chicken vegetable soup with rice, stew type, chunky style	0.94	ss-c
126	28340640	Chicken vegetable soup with noodles, stew type, chunky style	0.88	ss-c
127	28340670	Chicken vegetable soup with rice, Mexican style (Sopa / Caldo de Pollo)	0.44	ss-c
128	28340690	Chicken vegetable soup with potato and cheese, chunky style	1.06	ss-c
129	28340750	Hot and sour soup	1.63	ss-c
130	28340800	Chicken soup with vegetables and fruit, Oriental Style	0.34	ss-c
131	28345020	Chicken or turkey soup, cream of, canned, made with milk, reduced sodium	0.52	ss-c
132	28345030	Chicken or turkey soup, cream of, canned, made with water, reduced sodium	0.62	ss-c
133	28345110	Chicken or turkey soup, cream of, NS as to prepared with milk or water	1.05	ss-c
134	28345120	Chicken or turkey soup, cream of, prepared with milk	1.07	ss-c
135	28345130	Chicken or turkey soup, cream of, prepared with water	1.03	ss-c
136	28345140	Chicken or turkey soup, cream of, canned, undiluted	2.00	ss-c
137	28345160	Chicken and mushroom soup, cream of, prepared with milk	1.06	ss-c
138	28345170	Duck soup	0.28	ss-c
139	28350050	Fish chowder	0.58	ss-c
140	28355210	Crab soup, cream of, prepared with milk	0.60	ss-c
141	28355350	Salmon soup, cream style	1.58	ss-c

142	28355450	Seafood soup with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy)	0.27	ss-c
143	28355460	Seafood soup with potatoes and vegetables (excluding carrots, broccoli, and dark-green leafy)	0.26	ss-c
144	28355470	Seafood soup with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes))	0.27	ss-c
145	28355480	Seafood soup with vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes))	0.27	ss-c
146	32300100	Egg drop soup	0.76	ss-c
147	41601010	Bean soup, NFS	0.92	ss-c
148	41601020	Bean with bacon or pork soup	0.96	ss-c
149	41601040	Lima bean soup	0.62	ss-c
150	41601050	Soybean soup, made with milk	0.35	ss-c
151	41601060	Bean soup, with macaroni and meat	0.52	ss-c
152	41601070	Soybean soup, miso broth	1.05	ss-c
153	41601090	Bean soup, with macaroni	0.45	ss-c
154	41601100	Portuguese bean soup	0.37	ss-c
155	41601110	Bean and ham soup, chunky style	0.36	ss-c
156	41601130	Bean soup, mixed beans	0.08	ss-c
157	41601170	Bean and rice soup	0.44	ss-c
158	41602010	Chunky pea and ham soup	1.02	ss-c
159	41602030	Split pea and ham soup	1.02	ss-c
160	41602090	Split pea and ham soup, canned, reduced sodium, prepared with water or ready-to-serve	0.50	ss-c
161	41610100	White bean soup, Puerto Rican style (Sopon de habichuelas blancas)	0.17	ss-c
162	53110100	Cake, plum pudding	0.40	ss-c
163	58127110	Vegetables in pastry	0.35	ss-c
164	58128210	Dressing with oysters	1.49	ss-c
165	58130013	Lasagna with meat, canned	1.43	ss-c
166	58131320	Ravioli, meat-filled, with tomato sauce or meat sauce	1.46	ss-c
167	58131323	Ravioli, meat-filled, with tomato sauce or meat sauce, canned	1.37	ss-c

168	58132310	Spaghetti with tomato sauce and meatballs or spaghetti with meat sauce or spaghetti with meat sauce and m	1.10	ss-c
169	58132313	Pasta with tomato sauce and meat or meatballs, canned	0.92	ss-c
170	58132360	Spaghetti with tomato sauce and meatballs, whole wheat noodles or spaghetti with meat sauce, whole wheat	1.10	ss-c
171	58132710	Spaghetti with tomato sauce and frankfurters or hot dogs	1.03	ss-c
172	58132713	Pasta with tomato sauce and frankfurters or hot dogs, canned	1.16	ss-c
173	58134613	Tortellini, meat-filled, with tomato sauce, canned	0.81	ss-c
174	58146110	Pasta with meat sauce	1.83	ss-c
175	58146120	Pasta with cheese and meat sauce	1.46	ss-c
176	58146200	Pasta, meat-filled, with gravy, canned	1.11	ss-c
177	58147510	Flavored pasta	0.74	ss-c
178	58156210	Rice with vienna sausage, Puerto Rican style (arroz con salchichas)	2.41	ss-c
179	58400000	Soup, NFS	1.01	ss-c
180	58400100	Noodle soup, NFS	1.17	ss-c
181	58400200	Rice soup, NFS	0.99	ss-c
182	58401010	Barley soup	0.75	ss-c
183	58402010	Beef noodle soup	1.00	ss-c
184	58402020	Beef dumpling soup	1.51	ss-c
185	58402030	Beef rice soup	0.60	ss-c
186	58403010	Chicken noodle soup	0.98	ss-c
187	58403020	Chicken noodle soup, canned, undiluted	1.92	ss-c
188	58403030	Chicken noodle soup, canned, low sodium, ready-to-serve	0.08	ss-c
189	58403050	Chicken noodle soup, cream of	1.03	ss-c
190	58403060	Chicken noodle soup, canned, reduced sodium, ready-to-serve	0.49	ss-c
191	58404010	Chicken rice soup	0.86	ss-c
192	58404040	Chicken rice soup, canned, reduced sodium, prepared with water or ready-to-serve	0.43	ss-c
193	58404050	Chicken rice soup, canned, reduced sodium, prepared with milk	0.49	ss-c
194	58404500	Matzo ball soup	0.80	ss-c

195	58404510	Chicken soup with dumplings and potatoes	0.81	ss-c
196	58404520	Chicken soup with dumplings	0.91	ss-c
197	58406010	Turkey noodle soup	0.85	ss-c
198	58407000	Instant soup, NFS	0.58	ss-c
199	58407010	Instant soup, noodle	0.82	ss-c
200	58407040	Instant soup, rice	0.99	ss-c
201	58407050	Instant soup, noodle with egg, shrimp or chicken	0.60	ss-c
202	58408010	Won ton (wonton) soup	0.81	ss-c
203	58408500	Noodle soup with vegetables, Oriental style	0.93	ss-c
204	58421020	Sopa de Fideo Aguada, Mexican style noodle soup	0.34	ss-c
205	58421080	Sopa de tortilla, Mexican style tortilla soup	0.53	ss-c
206	58503000	Macaroni, tomatoes, and beef, baby food, NS as to strained or junior	0.07	ss-c
207	58503010	Macaroni, tomatoes, and beef, baby food, strained	0.10	ss-c
208	58503020	Macaroni, tomatoes, and beef, baby food, junior	0.04	ss-c
209	58503050	Macaroni with beef and tomato sauce, baby food, toddler	0.51	ss-c
210	58508500	Ravioli, meat-filled, with tomato sauce, baby food, toddler	0.82	ss-c
211	58509020	Spaghetti, tomato sauce, and beef, baby food, junior	0.05	ss-c
212	67501000	Apples and chicken, baby food, strained	0.03	ss-c
213	67501100	Apples with ham, baby food, strained	0.02	ss-c
214	67501200	Apples and turkey, baby food, strained	0.03	ss-c
215	71803010	Potato chowder	0.55	ss-c
216	71851010	Plantain soup, Puerto Rican style (Sopa de platano)	1.48	ss-c
217	72308000	Dark-green leafy vegetable soup with meat, Oriental style	0.53	ss-c
218	73501000	Carrot soup, cream of, prepared with milk	0.61	ss-c
219	74404030	Spaghetti sauce with meat, canned, no extra meat added	1.00	ss-c
220	74603010	Tomato beef soup, prepared with water	0.96	ss-c

221	74604010	Tomato beef noodle soup, prepared with water	0.96	ss-c
222	74604100	Tomato beef rice soup, prepared with water	1.23	ss-c
223	75144100	Lettuce, wilted, with bacon dressing	0.20	ss-c
224	75601200	Cabbage soup	0.32	ss-c
225	75601210	Cabbage with meat soup	0.29	ss-c
226	75604020	Corn soup, cream of, prepared with water	0.68	ss-c
227	75607040	Mushroom soup, with meat broth, prepared with water	1.01	ss-c
228	75647000	Seaweed soup	1.27	ss-c
229	75649010	Vegetable soup, prepared with water or ready-to-serve	0.86	ss-c
230	75649020	Vegetable soup, canned, undiluted	1.68	ss-c
231	75651020	Vegetable beef soup, prepared with water	0.83	ss-c
232	75651030	Vegetable beef noodle soup, prepared with water	0.91	ss-c
233	75651050	Vegetable chicken or turkey soup, prepared with water or ready-to-serve	0.98	ss-c
234	75651080	Vegetable beef soup with rice, prepared with water or ready-to-serve	0.83	ss-c
235	75651090	Vegetable chicken soup, canned, prepared with water, low sodium	0.09	ss-c
236	75651110	Vegetable chicken rice soup, prepared with water or ready-to-serve	0.93	ss-c
237	75651120	Vegetable chicken noodle soup, prepared with water or ready-to-serve	0.99	ss-c
238	75651140	Vegetable soup with chicken broth, Mexican style (Sopa Ranchera)	0.33	ss-c
239	75652020	Vegetable beef soup, canned, undiluted	1.60	ss-c
240	75652030	Vegetable beef soup, prepared with milk	0.87	ss-c
241	75656060	Vegetable beef soup, chunky style	0.95	ss-c
242	76601010	Vegetable and bacon, baby food, strained	0.11	ss-c
243	76601020	Vegetable and bacon, baby food, junior	0.11	ss-c
244	76602000	Carrots and beef, baby food, strained	0.15	ss-c
245	76603010	Vegetable and beef, baby food, strained	0.05	ss-c
246	76603020	Vegetable and beef, baby food, junior	0.08	ss-c

247	76604000	Broccoli and chicken, baby food, strained	0.05	ss-c
248	76604500	Sweetpotatoes and chicken, baby food, strained	0.06	ss-c
249	76605010	Vegetable and chicken, baby food, strained	0.06	ss-c
250	76605020	Vegetable and chicken, baby food, junior	0.18	ss-c
251	76607010	Vegetable and ham, baby food, strained	0.03	ss-c
252	76607020	Vegetable and ham, baby food, junior	0.22	ss-c
253	76607030	Potatoes with cheese and ham, baby food, toddler	0.52	ss-c
254	76611010	Vegetable and turkey, baby food, strained	0.05	ss-c
255	76611020	Vegetable and turkey, baby food, junior	0.04	ss-c
256	76611030	Vegetables, turkey, and barley, baby food, strained	0.05	ss-c
257	76611500	Green beans and turkey, baby food, strained	0.03	ss-c
258	77563010	Puerto Rican stew (Sancocho)	0.30	ss-c
259	81302030	Orange sauce (for duck)	0.49	ss-c
260	83101500	Bacon dressing (hot)	0.39	ss-c
261	83101600	Bacon and tomato dressing	2.75	ss-c
262	21601000	Beef, bacon, cooked	5.72	N
263	21601500	Beef, bacon, formed, lean meat added, cooked	5.72	N
264	21603000	Beef, pastrami (beef, smoked, spiced)	3.12	N
265	22107020	Pork chop, smoked or cured, cooked, lean only eaten	3.13	N
266	22300120	Ham, fried, NS as to fat eaten	3.04	N
267	22300130	Ham, fried, lean and fat eaten	3.05	N
268	22300140	Ham, fried, lean only eaten	3.20	N
269	22300170	Ham, breaded or floured, fried, lean only eaten	3.00	N
270	22311000	Ham, smoked or cured, cooked, NS as to fat eaten	3.65	N
271	22311010	Ham, smoked or cured, cooked, lean and fat eaten	3.66	N
272	22311020	Ham, smoked or cured, cooked, lean only eaten	3.36	N

273	22421000	Pork roast, smoked or cured, cooked, NS as to fat eaten	3.52	N
274	22421020	Pork roast, smoked or cured, cooked, lean only eaten	3.37	N
275	22501010	Canadian bacon, cooked	3.93	N
276	22600100	Bacon, NS as to type of meat, cooked	4.02	N
277	22600200	Pork bacon, NS as to fresh, smoked or cured, cooked	4.06	N
278	22601000	Pork bacon, smoked or cured, cooked	4.03	N
279	22601020	Pork bacon, smoked or cured, cooked, lean only eaten	3.92	N
280	22605010	Pork bacon, formed, lean meat added, cooked	5.33	N
281	22621000	Salt pork, cooked	3.25	N
282	22704010	Pork, cracklings, cooked	4.06	N
283	24208500	Turkey bacon, cooked	5.80	N
284	25210110	Frankfurter, wiener, or hot dog, NFS	3.23	N
285	25210230	Frankfurter or hot dog, beef and pork, lowfat	3.19	N
286	25210280	Frankfurter or hot dog, meat and poultry	3.01	N
287	25210310	Frankfurter or hot dog, chicken	3.52	N
288	25210410	Frankfurter or hot dog, turkey	3.66	N
289	25220420	Bologna, Lebanon	3.40	N
290	25220460	Bologna, pork	3.01	N
291	25220510	Capicola	3.63	N
292	25220710	Chorizos	3.13	N
293	25220910	Head cheese	3.19	N
294	25221210	Mortadella	3.17	N
295	25221400	Sausage (not cold cut), NFS	3.29	N
296	25221420	Pork sausage, brown and serve, cooked	3.29	N
297	25221430	Pork sausage, country style, fresh, cooked	3.29	N
298	25221530	Salami, beef	2.99	N
299	25221650	Smoked link sausage, pork	3.81	N
300	25221680	Smoked sausage, pork	3.81	N
301	25230110	Luncheon meat, NFS	3.29	N
302	25230210	Ham, sliced, prepackaged or deli, luncheon meat	3.25	N
303	25230230	Ham, sliced, extra lean, prepackaged or deli, luncheon meat	3.63	N

304	25230410	Ham loaf, luncheon meat	3.28	N
305	25230430	Ham and cheese loaf	3.41	N
306	25230510	Ham, luncheon meat, chopped, minced, pressed, spiced, not canned	3.37	N
307	25230520	Ham, luncheon meat, chopped, minced, pressed, spiced, lowfat, not canned	3.63	N
308	25230610	Luncheon loaf (olive, pickle, or pimiento)	3.65	N
309	25230900	Turkey or chicken breast, prepackaged or deli, luncheon meat	3.65	N
310	27120150	Pork or ham with soy-based sauce (mixture)	3.05	N
311	27520250	Ham on biscuit	3.22	N
312	20000000	Meat, NFS	0.16	R
313	20000200	Ground meat, NFS	0.54	R
314	21000100	Beef, NS as to cut, cooked, NS as to fat eaten	0.97	R
315	21000110	Beef, NS as to cut, cooked, lean and fat eaten	0.98	R
316	21000120	Beef, NS as to cut, cooked, lean only eaten	0.37	R
317	21001000	Steak, NS as to type of meat, cooked, NS as to fat eaten	0.15	R
318	21001010	Steak, NS as to type of meat, cooked, lean and fat eaten	0.15	R
319	21001020	Steak, NS as to type of meat, cooked, lean only eaten	0.43	R
320	21003000	Beef, NS as to cut, fried, NS to fat eaten	0.99	R
321	21101000	Beef steak, NS as to cooking method, NS as to fat eaten	0.97	R
322	21101010	Beef steak, NS as to cooking method, lean and fat eaten	0.97	R
323	21101020	Beef steak, NS as to cooking method, lean only eaten	0.17	R
324	21101110	Beef steak, broiled or baked, NS as to fat eaten	0.20	R
325	21101120	Beef steak, broiled or baked, lean and fat eaten	0.15	R
326	21101130	Beef steak, broiled or baked, lean only eaten	0.44	R
327	21102110	Beef steak, fried, NS as to fat eaten	0.18	R

328	21102120	Beef steak, fried, lean and fat eaten	1.00	R
329	21102130	Beef steak, fried, lean only eaten	0.79	R
330	21103110	Beef steak, breaded or floured, baked or fried, NS as to fat eaten	0.48	R
331	21103120	Beef steak, breaded or floured, baked or fried, lean and fat eaten	0.50	R
332	21103130	Beef steak, breaded or floured, baked or fried, lean only eaten	0.48	R
333	21104110	Beef steak, battered, fried, NS as to fat eaten	0.83	R
334	21104120	Beef steak, battered, fried, lean and fat eaten	0.82	R
335	21104130	Beef steak, battered, fried, lean only eaten	0.39	R
336	21105110	Beef steak, braised, NS as to fat eaten	0.96	R
337	21105120	Beef steak, braised, lean and fat eaten	0.70	R
338	21105130	Beef steak, braised, lean only eaten	0.15	R
339	21301000	Beef, oxtails, cooked	0.59	R
340	21302000	Beef, neck bones, cooked	0.27	R
341	21304000	Beef, shortribs, cooked, NS as to fat eaten	0.25	R
342	21304110	Beef, shortribs, cooked, lean and fat eaten	0.17	R
343	21304120	Beef, shortribs, cooked, lean only eaten	0.15	R
344	21304200	Beef, shortribs, barbecued, with sauce, NS as to fat eaten	0.66	R
345	21304210	Beef, shortribs, barbecued, with sauce, lean and fat eaten	0.66	R
346	21304220	Beef, shortribs, barbecued, with sauce, lean only eaten	1.01	R
347	21305000	Beef, cow head, cooked	0.57	R
348	21401000	Beef, roast, roasted, NS as to fat eaten	0.16	R
349	21401110	Beef, roast, roasted, lean and fat eaten	0.38	R
350	21401120	Beef, roast, roasted, lean only eaten	0.55	R
351	21407000	Beef, pot roast, braised or boiled, NS as to fat eaten	0.57	R
352	21407120	Beef, pot roast, braised or boiled, lean only eaten	0.58	R
353	21410000	Beef, stew meat, cooked, NS as to fat eaten	0.24	R
354	21410120	Beef, stew meat, cooked, lean only eaten	0.34	R

355	21416000	Corned beef, cooked, NS as to fat eaten	2.88	R
356	21416110	Corned beef, cooked, lean and fat eaten	2.88	R
357	21420100	Beef, sandwich steak (flaked, formed, thinly sliced)	0.18	R
358	21500000	Ground beef, raw	0.17	R
359	21500100	Ground beef or patty, cooked, NS as to regular, lean, or extra lean	0.58	R
360	21500110	Ground beef, meatballs, meat only, cooked, NS as to regular, lean, or extra lean	0.45	R
361	21500200	Ground beef or patty, breaded, cooked	1.40	R
362	21501000	Ground beef, regular, cooked	0.43	R
363	21501200	Ground beef, lean, cooked	0.39	R
364	21501300	Ground beef, extra lean, cooked	0.18	R
365	21540100	Ground beef with textured vegetable protein, cooked	1.09	R
366	22000100	Pork, NS as to cut, cooked, NS as to fat eaten	0.97	R
367	22000110	Pork, NS as to cut, cooked, lean and fat eaten	0.98	R
368	22000120	Pork, NS as to cut, cooked, lean only eaten	0.97	R
369	22000200	Pork, NS as to cut, fried, NS as to fat eaten	0.24	R
370	22000210	Pork, NS as to cut, fried, lean and fat eaten	0.98	R
371	22000220	Pork, NS as to cut, fried, lean only eaten	0.23	R
372	22000300	Pork, NS as to cut, breaded or floured, fried, NS as to fat eaten	1.59	R
373	22002000	Pork, ground or patty, cooked	0.56	R
374	22002100	Pork, ground or patty, breaded, cooked	1.22	R
375	22101000	Pork chop, NS as to cooking method, NS as to fat eaten	0.16	R
376	22101010	Pork chop, NS as to cooking method, lean and fat eaten	0.98	R
377	22101020	Pork chop, NS as to cooking method, lean only eaten	0.17	R
378	22101100	Pork chop, broiled or baked, NS as to fat eaten	0.23	R
379	22101110	Pork chop, broiled or baked, lean and fat eaten	0.32	R

380	22101120	Pork chop, broiled or baked, lean only eaten	0.16	R
381	22101130	Pork chop, breaded or floured, broiled or baked, NS as to fat eaten	1.05	R
382	22101140	Pork chop, breaded or floured, broiled or baked, lean and fat eaten	1.05	R
383	22101150	Pork chop, breaded or floured, broiled or baked, lean only eaten	0.88	R
384	22101200	Pork chop, fried, NS as to fat eaten	0.16	R
385	22101210	Pork chop, fried, lean and fat eaten	0.16	R
386	22101220	Pork chop, fried, lean only eaten	0.67	R
387	22101300	Pork chop, breaded or floured, fried, NS as to fat eaten	0.55	R
388	22101310	Pork chop, breaded or floured, fried, lean and fat eaten	1.11	R
389	22101320	Pork chop, breaded or floured, fried, lean only eaten	0.58	R
390	22101400	Pork chop, battered, fried, NS as to fat eaten	1.05	R
391	22101410	Pork chop, battered, fried, lean and fat eaten	0.19	R
392	22101420	Pork chop, battered, fried, lean only eaten	0.72	R
393	22101500	Pork chop, stewed, NS as to fat eaten	0.12	R
394	22101510	Pork chop, stewed, lean and fat eaten	0.12	R
395	22101520	Pork chop, stewed, lean only eaten	0.14	R
396	22107000	Pork chop, smoked or cured, cooked, NS as to fat eaten	2.72	R
397	22107010	Pork chop, smoked or cured, cooked, lean and fat eaten	2.72	R
398	22201000	Pork steak or cutlet, NS as to cooking method, NS as to fat eaten	0.16	R
399	22201020	Pork steak or cutlet, NS as to cooking method, lean only eaten	1.02	R
400	22201050	Pork steak or cutlet, battered, fried, NS as to fat eaten	0.76	R
401	22201100	Pork steak or cutlet, broiled or baked, NS as to fat eaten	0.67	R
402	22201110	Pork steak or cutlet, broiled or baked, lean and fat eaten	0.32	R

403	22201120	Pork steak or cutlet, broiled or baked, lean only eaten	0.99	R
404	22201200	Pork steak or cutlet, fried, NS as to fat eaten	0.98	R
405	22201210	Pork steak or cutlet, fried, lean and fat eaten	0.50	R
406	22201220	Pork steak or cutlet, fried, lean only eaten	0.35	R
407	22201300	Pork steak or cutlet, breaded or floured, broiled or baked, NS as to fat eaten	0.27	R
408	22201310	Pork steak or cutlet, breaded or floured, broiled or baked, lean and fat eaten	0.75	R
409	22201320	Pork steak or cutlet, breaded or floured, broiled or baked, lean only eaten	0.39	R
410	22201400	Pork steak or cutlet, breaded or floured, fried, NS as to fat eaten	0.68	R
411	22201410	Pork steak or cutlet, breaded or floured, fried, lean and fat eaten	1.15	R
412	22201420	Pork steak or cutlet, breaded or floured, fried, lean only eaten	1.18	R
413	22210300	Pork, tenderloin, cooked, NS as to cooking method	0.96	R
414	22210310	Pork, tenderloin, breaded, fried	0.72	R
415	22210350	Pork, tenderloin, braised	0.39	R
416	22210400	Pork, tenderloin, baked	0.18	R
417	22210450	Pork, tenderloin, battered, fried	1.10	R
418	22301000	Ham, fresh, cooked, NS as to fat eaten	0.56	R
419	22301110	Ham, fresh, cooked, lean and fat eaten	0.31	R
420	22301120	Ham, fresh, cooked, lean only eaten	0.16	R
421	22311200	Ham, smoked or cured, low sodium, cooked, NS as to fat eaten	2.46	R
422	22311210	Ham, smoked or cured, low sodium, cooked, lean and fat eaten	2.46	R
423	22311220	Ham, smoked or cured, low sodium, cooked, lean only eaten	2.46	R
424	22400100	Pork roast, NS as to cut, cooked, NS as to fat eaten	0.37	R
425	22400110	Pork roast, NS as to cut, cooked, lean and fat eaten	0.49	R
426	22400120	Pork roast, NS as to cut, cooked, lean only eaten	0.15	R

427	22401000	Pork roast, loin, cooked, NS as to fat eaten	0.56	R
428	22401010	Pork roast, loin, cooked, lean and fat eaten	0.56	R
429	22401020	Pork roast, loin, cooked, lean only eaten	0.56	R
430	22411000	Pork roast, shoulder, cooked, NS as to fat eaten	0.17	R
431	22411020	Pork roast, shoulder, cooked, lean only eaten	0.19	R
432	22601040	Bacon or side pork, fresh, cooked	4.06	R
433	22621100	Fat back, cooked	0.02	R
434	22701000	Pork, spareribs, cooked, NS as to fat eaten	0.65	R
435	22701010	Pork, spareribs, cooked, lean and fat eaten	0.24	R
436	22701020	Pork, spareribs, cooked, lean only eaten	0.14	R
437	22701030	Pork, spareribs, barbecued, with sauce, NS as to fat eaten	0.85	R
438	22701040	Pork, spareribs, barbecued, with sauce, lean and fat eaten	0.85	R
439	22701050	Pork, spareribs, barbecued, with sauce, lean only eaten	0.82	R
440	22705010	Pork ears, tail, head, snout, miscellaneous parts, cooked	0.21	R
441	22706010	Pork, neck bones, cooked	1.69	R
442	22707010	Pork, pig's feet, cooked	0.08	R
443	22708010	Pork, pig's hocks, cooked	0.39	R
444	23000100	Lamb, NS as to cut, cooked	0.74	R
445	23101000	Lamb chop, NS as to cut, cooked, NS as to fat eaten	0.17	R
446	23101010	Lamb chop, NS as to cut, cooked, lean and fat eaten	0.99	R
447	23101020	Lamb chop, NS as to cut, cooked, lean only eaten	0.54	R
448	23104020	Lamb, loin chop, cooked, lean only eaten	0.43	R
449	23110000	Lamb, ribs, cooked, lean only eaten	0.63	R
450	23110010	Lamb, ribs, cooked, NS as to fat eaten	0.60	R
451	23120100	Lamb, roast, cooked, NS as to fat eaten	0.21	R
452	23120110	Lamb, roast, cooked, lean and fat eaten	0.17	R
453	23120120	Lamb, roast, cooked, lean only eaten	0.58	R

454	23132000	Lamb, ground or patty, cooked	0.21	R
455	23150100	Goat, boiled	0.63	R
456	23150250	Goat, baked	0.63	R
457	23150300	Goat ribs, cooked	0.63	R
458	23200100	Veal, NS as to cut, cooked, NS as to fat eaten	0.22	R
459	23200120	Veal, NS as to cut, cooked, lean only eaten	1.01	R
460	23201030	Veal chop, NS as to cooking method, lean only eaten	1.16	R
461	23203020	Veal chop, fried, lean and fat eaten	1.15	R
462	23203030	Veal chop, fried, lean only eaten	0.70	R
463	23203100	Veal chop, broiled, NS as to fat eaten	0.24	R
464	23203120	Veal chop, broiled, lean only eaten	0.29	R
465	23204010	Veal cutlet or steak, NS as to cooking method, NS as to fat eaten	1.00	R
466	23204030	Veal cutlet or steak, NS as to cooking method, lean only eaten	1.01	R
467	23204220	Veal cutlet or steak, broiled, lean only eaten	0.20	R
468	23205010	Veal cutlet or steak, fried, NS as to fat eaten	1.15	R
469	23205030	Veal cutlet or steak, fried, lean only eaten	0.20	R
470	23210010	Veal, roasted, NS as to fat eaten	0.22	R
471	23210020	Veal, roasted, lean and fat eaten	0.63	R
472	23210030	Veal, roasted, lean only eaten	0.27	R
473	23220010	Veal, ground or patty, cooked	1.03	R
474	23220030	Veal patty, breaded, cooked	0.83	R
475	23310000	Rabbit, NS as to domestic or wild, cooked	0.27	R
476	23311120	Rabbit, NS as to domestic or wild, breaded, fried	0.96	R
477	23311200	Rabbit, wild, cooked	0.53	R
478	23321000	Venison/deer, NFS	0.74	R
479	23321100	Venison/deer, roasted	0.21	R
480	23321200	Venison/deer steak, cooked, NS as to cooking method	1.32	R
481	23321250	Venison/deer steak, breaded or floured, cooked, NS as to cooking method	0.56	R

482	23322100	Deer bologna	2.49	R
483	23322350	Venison/deer ribs, cooked	0.74	R
484	23322400	Venison/deer, stewed	0.46	R
485	23323500	Bear, cooked	0.59	R
486	23324100	Caribou, cooked	0.15	R
487	23326100	Bison, cooked	0.14	R
488	23333100	Squirrel, cooked	0.71	R
489	24100000	Chicken, boneless, NS as to part and cooking method, light or dark meat, NS as to skin eaten	1.03	R
490	24100020	Chicken, boneless, NS as to part and cooking method, light or dark meat, skin not eaten	1.04	R
491	24101000	Chicken, boneless, NS as to part, broiled, light or dark meat, NS as to skin eaten	0.21	R
492	24101010	Chicken, boneless, NS as to part, broiled, light or dark meat, skin eaten	1.03	R
493	24101020	Chicken, boneless, NS as to part, broiled, light or dark meat, skin not eaten	0.22	R
494	24102000	Chicken, boneless, NS as to part, roasted, light or dark meat, NS as to skin eaten	1.03	R
495	24102010	Chicken, boneless, NS as to part, roasted, light or dark meat, skin eaten	0.25	R
496	24102020	Chicken, boneless, NS as to part, roasted, light or dark meat, skin not eaten	0.22	R
497	24103000	Chicken, boneless, NS as to part, stewed, light or dark meat, NS as to skin eaten	0.99	R
498	24103010	Chicken, boneless, NS as to part, stewed, light or dark meat, skin eaten	0.99	R
499	24103020	Chicken, boneless, NS as to part, stewed, light or dark meat, skin not eaten	0.71	R
500	24104000	Chicken, boneless, NS as to part, fried, no coating, light or dark meat, NS as to skin eaten	1.07	R
501	24104010	Chicken, boneless, NS as to part, fried, no coating, light or dark meat, skin eaten	1.07	R

502	24104020	Chicken, boneless, NS as to part, fried, no coating, light or dark meat, skin not eaten	0.23	R
503	24105000	Chicken, boneless, NS as to part, floured, baked or fried, light or dark meat, prepared with skin, NS as	1.03	R
504	24105010	Chicken, boneless, NS as to part, floured, baked or fried, light or dark meat, prepared with skin, skin/c	1.03	R
505	24105020	Chicken, boneless, NS as to part, floured, baked or fried, light or dark meat, prepared with skin, skin/c	1.05	R
506	24106000	Chicken, boneless, NS as to part, breaded, baked or fried, light or dark meat, prepared with skin, NS as	0.81	R
507	24106040	Chicken, boneless, NS as to part, breaded, baked or fried, light or dark meat, prepared skinless, NS as t	1.08	R
508	24106050	Chicken, boneless, NS as to part, breaded, baked or fried, light or dark meat, prepared skinless, coating	0.91	R
509	24107000	Chicken, boneless, NS as to part, battered, fried, light or dark meat, prepared with skin, NS as to skin/	0.74	R
510	24107010	Chicken, boneless, NS as to part, battered, fried, light or dark meat, prepared with skin, skin/coating e	0.74	R
511	24107020	Chicken, boneless, NS as to part, battered, fried, light or dark meat, prepared with skin, skin/coating n	0.23	R
512	24110000	Chicken, with bone, NS as to part and cooking method, light or dark meat, NS as to skin eaten	1.03	R
513	24111000	Chicken, with bone, NS as to part, broiled, light or dark meat, NS as to skin eaten	1.03	R
514	24111010	Chicken, with bone, NS as to part, broiled, light or dark meat, skin eaten	0.21	R
515	24111020	Chicken, with bone, NS as to part, broiled, light or dark meat, skin not eaten	1.04	R

516	24112000	Chicken, with bone, NS as to part, roasted, light or dark meat, NS as to skin eaten	1.03	R
517	24112010	Chicken, with bone, NS as to part, roasted, light or dark meat, skin eaten	1.03	R
518	24112020	Chicken, with bone, NS as to part, roasted, light or dark meat, skin not eaten	0.43	R
519	24113000	Chicken, with bone, NS as to part, stewed, light or dark meat, NS as to skin eaten	0.99	R
520	24113020	Chicken, with bone, NS as to part, stewed, light or dark meat, skin not eaten	0.35	R
521	24115000	Chicken, with bone, NS as to part, floured, baked or fried, light or dark meat, prepared with skin, NS as	1.03	R
522	24115020	Chicken, with bone, NS as to part, floured, baked or fried, light or dark meat, prepared with skin, skin/	1.05	R
523	24116010	Chicken, with bone, NS as to part, breaded, baked or fried, light or dark meat, prepared with skin, skin/	0.95	R
524	24117000	Chicken, with bone, NS as to part, battered, fried, light or dark meat, prepared with skin, NS as to skin	0.74	R
525	24117010	Chicken, with bone, NS as to part, battered, fried, light or dark meat, prepared with skin, skin/coating	0.74	R
526	24120100	Chicken, breast, with or without bone, NS as to cooking method, NS as to skin eaten	0.87	R
527	24120110	Chicken, breast, with or without bone, NS as to cooking method, skin eaten	1.00	R
528	24120120	Chicken, breast, with or without bone, NS as to cooking method, skin not eaten	0.66	R
529	24121100	Chicken, breast, with or without bone, broiled, NS as to skin eaten	0.18	R
530	24121110	Chicken, breast, with or without bone, broiled, skin eaten	1.00	R
531	24121120	Chicken, breast, with or without bone, broiled, skin not eaten	0.38	R
532	24122100	Chicken, breast, with or without bone, roasted, NS as to skin eaten	0.18	R

533	24122110	Chicken, breast, with or without bone, roasted, skin eaten	0.27	R
534	24122120	Chicken, breast, with or without bone, roasted, skin not eaten	0.21	R
535	24123100	Chicken, breast, with or without bone, stewed, NS as to skin eaten	0.67	R
536	24123110	Chicken, breast, with or without bone, stewed, skin eaten	0.32	R
537	24123120	Chicken, breast, with or without bone, stewed, skin not eaten	0.80	R
538	24124100	Chicken, breast, with or without bone, fried, no coating, NS as to skin eaten	0.20	R
539	24124110	Chicken, breast, with or without bone, fried, no coating, skin eaten	0.49	R
540	24124120	Chicken, breast, with or without bone, fried, no coating, skin not eaten	0.20	R
541	24125100	Chicken, breast, with or without bone, floured, baked or fried, prepared with skin, NS as to skin/coating	0.19	R
542	24125110	Chicken, breast, with or without bone, floured, baked or fried, prepared with skin, skin/coating eaten	1.01	R
543	24125120	Chicken, breast, with or without bone, floured, baked or fried, prepared with skin, skin/coating not eate	1.02	R
544	24125140	Chicken, breast, with or without bone, floured, baked or fried, prepared skinless, NS as to coating eaten	0.23	R
545	24126100	Chicken, breast, with or without bone, breaded, baked or fried, prepared with skin, NS as to skin/coating	0.93	R
546	24126110	Chicken, breast, with or without bone, breaded, baked or fried, prepared with skin, skin/coating eaten	0.93	R
547	24126120	Chicken, breast, with or without bone, breaded, baked or fried, prepared with skin, skin/coating not eate	0.63	R
548	24126150	Chicken, breast, with or without bone, breaded, baked or fried, prepared skinless, coating eaten	1.12	R
549	24126160	Chicken breast, with or without bone, breaded, baked or fried, prepared skinless, coating not eaten	0.52	R

550	24127100	Chicken, breast, with or without bone, battered, fried, prepared with skin, NS as to skin/coating eaten	0.70	R
551	24127110	Chicken, breast, with or without bone, battered, fried, prepared with skin, skin/coating eaten	0.70	R
552	24127120	Chicken, breast, with or without bone, battered, fried, prepared with skin, skin/coating not eaten	1.02	R
553	24127140	Chicken, breast, with or without bone, battered, fried, prepared skinless, NS as to coating eaten	0.76	R
554	24127150	Chicken, breast, with or without bone, battered, fried, prepared skinless, coating eaten	0.76	R
555	24127160	Chicken breast, with or without bone, battered, fried, prepared skinless, coating not eaten	1.02	R
556	24130200	Chicken, leg (drumstick and thigh), with or without bone, NS as to cooking method, NS as to skin eaten	1.04	R
557	24130210	Chicken, leg (drumstick and thigh), with or without bone, NS as to cooking method, skin eaten	1.04	R
558	24130220	Chicken, leg (drumstick and thigh), with or without bone, NS as to cooking method, skin not eaten	0.46	R
559	24131200	Chicken, leg (drumstick and thigh), with or without bone, broiled, NS as to skin eaten	1.04	R
560	24131210	Chicken, leg (drumstick and thigh), with or without bone, broiled, skin eaten	0.22	R
561	24131220	Chicken, leg (drumstick and thigh), with or without bone, broiled, skin not eaten	0.23	R
562	24132200	Chicken, leg (drumstick and thigh), with or without bone, roasted, NS as to skin eaten	0.44	R
563	24132210	Chicken, leg (drumstick and thigh), with or without bone, roasted, skin eaten	0.22	R
564	24132220	Chicken, leg (drumstick and thigh), with or without bone, roasted, skin not eaten	0.34	R

565	24133200	Chicken, leg (drumstick and thigh), with or without bone, stewed, NS as to skin eaten	0.37	R
566	24133210	Chicken, leg (drumstick and thigh), with or without bone, stewed, skin eaten	0.34	R
567	24133220	Chicken, leg (drumstick and thigh), with or without bone, stewed, skin not eaten	0.53	R
568	24134200	Chicken, leg (drumstick and thigh), with or without bone, fried, no coating, NS as to skin eaten	0.23	R
569	24134210	Chicken, leg (drumstick and thigh), with or without bone, fried, no coating, skin eaten	1.07	R
570	24134220	Chicken, leg (drumstick and thigh), with or without bone, fried, no coating, skin not eaten	0.24	R
571	24135200	Chicken, leg (drumstick and thigh), with or without bone, floured, baked or fried, prepared with skin, NS	0.30	R
572	24135210	Chicken, leg (drumstick and thigh), with or without bone, floured, baked or fried, prepared with skin, sk	1.04	R
573	24135220	Chicken, leg (drumstick and thigh), with or without bone, floured, baked or fried, prepared with skin, sk	0.48	R
574	24136200	Chicken, leg (drumstick and thigh), with or without bone, breaded, baked or fried, prepared with skin, NS	0.96	R
575	24136210	Chicken, leg (drumstick and thigh), with or without bone, breaded, baked or fried, prepared with skin, sk	0.96	R
576	24136220	Chicken, leg (drumstick and thigh), with or without bone, breaded, baked or fried, prepared with skin, sk	0.56	R
577	24137200	Chicken, leg (drumstick and thigh), with or without bone, battered, fried, prepared with skin, NS as to s	0.71	R
578	24137210	Chicken, leg (drumstick and thigh), with or without bone, battered, fried, prepared with skin, skin/coati	0.71	R
579	24137220	Chicken, leg (drumstick and thigh), with or without bone, battered, fried, prepared with skin, skin/coati	1.06	R

580	24140200	Chicken, drumstick, with or without bone, NS as to cooking method, NS as to skin eaten	0.66	R
581	24140210	Chicken, drumstick, with or without bone, NS as to cooking method, skin eaten	0.23	R
582	24140220	Chicken, drumstick, with or without bone, NS as to cooking method, skin not eaten	1.06	R
583	24141200	Chicken, drumstick, with or without bone, broiled, NS as to skin eaten	0.26	R
584	24141210	Chicken, drumstick, with or without bone, broiled, skin eaten	0.23	R
585	24141220	Chicken, drumstick, with or without bone, broiled, skin not eaten	0.24	R
586	24142200	Chicken, drumstick, with or without bone, roasted, NS as to skin eaten	0.23	R
587	24142210	Chicken, drumstick, with or without bone, roasted, skin eaten	1.04	R
588	24142220	Chicken, drumstick, with or without bone, roasted, skin not eaten	0.24	R
589	24143200	Chicken, drumstick, with or without bone, stewed, NS as to skin eaten	0.30	R
590	24143210	Chicken, drumstick, with or without bone, stewed, skin eaten	0.19	R
591	24143220	Chicken, drumstick, with or without bone, stewed, skin not eaten	0.55	R
592	24144200	Chicken, drumstick, with or without bone, fried, no coating, NS as to skin eaten	0.92	R
593	24144210	Chicken, drumstick, with or without bone, fried, no coating, skin eaten	0.23	R
594	24144220	Chicken, drumstick, with or without bone, fried, no coating, skin not eaten	0.25	R
595	24145200	Chicken, drumstick, with or without bone, floured, baked or fried, prepared with skin, NS as to skin/coat	0.26	R
596	24145210	Chicken, drumstick, with or without bone, floured, baked or fried, prepared with skin, skin/coating eaten	1.05	R
597	24145220	Chicken, drumstick, with or without bone, floured, baked or fried, prepared with skin, skin/coating not e	0.61	R

598	24145250	Chicken, drumstick, with or without bone, floured, baked or fried, prepared skinless, coating eaten	0.25	R
599	24146200	Chicken, drumstick, with or without bone, breaded, baked or fried, prepared with skin, NS as to skin/coat	0.82	R
600	24146210	Chicken, drumstick, with or without bone, breaded, baked or fried, prepared with skin, skin/coating eaten	0.82	R
601	24146220	Chicken, drumstick, with or without bone, breaded, baked or fried, prepared with skin, skin/coating not e	1.06	R
602	24146250	Chicken, drumstick, with or without bone, breaded, baked or fried, prepared skinless, coating eaten	1.19	R
603	24146260	Chicken, drumstick, with or without bone, breaded, baked or fried, prepared skinless, coating not eaten	1.19	R
604	24147200	Chicken, drumstick, with or without bone, battered, fried, prepared with skin, NS as to skin/coating eate	0.69	R
605	24147210	Chicken, drumstick, with or without bone, battered, fried, prepared with skin, skin/coating eaten	0.69	R
606	24147220	Chicken, drumstick, with or without bone, battered, fried, prepared with skin, skin/coating not eaten	1.06	R
607	24150200	Chicken, thigh, with or without bone, NS as to cooking method, NS as to skin eaten	1.03	R
608	24150210	Chicken, thigh, with or without bone, NS as to cooking method, skin eaten	1.03	R
609	24150220	Chicken, thigh, with or without bone, NS as to cooking method, skin not eaten	1.04	R
610	24151200	Chicken, thigh, with or without bone, broiled, NS as to skin eaten	0.21	R
611	24151210	Chicken, thigh, with or without bone, broiled, skin eaten	0.24	R
612	24151220	Chicken, thigh, with or without bone, broiled, skin not eaten	0.22	R
613	24152200	Chicken, thigh, with or without bone, roasted, NS as to skin eaten	0.21	R

614	24152210	Chicken, thigh, with or without bone, roasted, skin eaten	0.38	R
615	24152220	Chicken, thigh, with or without bone, roasted, skin not eaten	0.44	R
616	24153200	Chicken, thigh, with or without bone, stewed, NS as to skin eaten	0.36	R
617	24153210	Chicken, thigh, with or without bone, stewed, skin eaten	0.20	R
618	24153220	Chicken, thigh, with or without bone, stewed, skin not eaten	0.62	R
619	24154200	Chicken, thigh, with or without bone, fried, no coating, NS as to skin eaten	1.08	R
620	24154210	Chicken, thigh, with or without bone, fried, no coating, skin eaten	0.23	R
621	24154220	Chicken, thigh, with or without bone, fried, no coating, skin not eaten	0.43	R
622	24155200	Chicken, thigh, with or without bone, floured, baked or fried, prepared with skin, NS as to skin/coating	1.04	R
623	24155210	Chicken, thigh, with or without bone, floured, baked or fried, prepared with skin, skin/coating eaten	0.45	R
624	24155220	Chicken, thigh, with or without bone, floured, baked or fried, prepared with skin, skin/coating not eaten	0.39	R
625	24156200	Chicken, thigh, with or without bone, breaded, baked or fried, prepared with skin, NS as to skin/coating	0.96	R
626	24156210	Chicken, thigh, with or without bone, breaded, baked or fried, prepared with skin, skin/coating eaten	0.96	R
627	24156220	Chicken, thigh, with or without bone, breaded, baked or fried, prepared with skin, skin/coating not eaten	1.06	R
628	24156250	Chicken, thigh, with or without bone, breaded, baked or fried, prepared skinless, coating eaten	1.15	R
629	24156260	Chicken, thigh, with or without bone, breaded, baked or fried, prepared skinless, coating not eaten	1.15	R
630	24157200	Chicken, thigh, with or without bone, battered, fried, prepared with skin, NS as to skin/coating eaten	0.73	R

631	24157210	Chicken, thigh, with or without bone, battered, fried, prepared with skin, skin/coating eaten	0.73	R
632	24157220	Chicken, thigh, with or without bone, battered, fried, prepared with skin, skin/coating not eaten	1.06	R
633	24157250	Chicken, thigh, with or without bone, battered, fried, prepared skinless, coating eaten	0.80	R
634	24157260	Chicken, thigh, with or without bone, battered, fried, prepared skinless, coating not eaten	0.24	R
635	24158210	Chicken, thigh, with or without bone, smoked, skin eaten	1.63	R
636	24158220	Chicken, thigh, with or without bone, smoked, skin not eaten	1.43	R
637	24160100	Chicken, wing, with or without bone, NS as to cooking method, NS as to skin eaten	0.53	R
638	24160110	Chicken, wing, with or without bone, NS as to cooking method, skin eaten	1.03	R
639	24160120	Chicken, wing, with or without bone, NS as to cooking method, skin not eaten	1.05	R
640	24161100	Chicken, wing, with or without bone, broiled, NS as to skin eaten	0.21	R
641	24161110	Chicken, wing, with or without bone, broiled, skin eaten	1.03	R
642	24161120	Chicken, wing, with or without bone, broiled, skin not eaten	0.39	R
643	24162100	Chicken, wing, with or without bone, roasted, NS as to skin eaten	0.21	R
644	24162110	Chicken, wing, with or without bone, roasted, skin eaten	0.92	R
645	24162120	Chicken, wing, with or without bone, roasted, skin not eaten	0.23	R
646	24163100	Chicken, wing, with or without bone, stewed, NS as to skin eaten	0.34	R
647	24163110	Chicken, wing, with or without bone, stewed, skin eaten	0.17	R
648	24163120	Chicken, wing, with or without bone, stewed, skin not eaten	0.29	R

649	24164100	Chicken, wing, with or without bone, fried, no coating, NS as to skin eaten	0.43	R
650	24164110	Chicken, wing, with or without bone, fried, no coating, skin eaten	0.20	R
651	24164120	Chicken, wing, with or without bone, fried, no coating, skin not eaten	0.46	R
652	24165100	Chicken, wing, with or without bone, floured, baked or fried, prepared with skin, NS as to skin/coating e	0.20	R
653	24165110	Chicken, wing, with or without bone, floured, baked or fried, prepared with skin, skin/coating eaten	0.20	R
654	24165120	Chicken, wing, with or without bone, floured, baked or fried, prepared with skin, skin/coating not eaten	1.05	R
655	24166100	Chicken, wing, with or without bone, breaded, baked or fried, prepared with skin, NS as to skin/coating e	0.94	R
656	24166110	Chicken, wing, with or without bone, breaded, baked or fried, prepared with skin, skin/coating eaten	0.94	R
657	24166120	Chicken, wing, with or without bone, breaded, baked or fried, prepared with skin, skin/coating not eaten	0.38	R
658	24167100	Chicken, wing, with or without bone, battered, fried, prepared with skin, NS as to skin/coating eaten	0.81	R
659	24167110	Chicken, wing, with or without bone, battered, fried, prepared with skin, skin/coating eaten	0.81	R
660	24167120	Chicken, wing, with or without bone, battered, fried, prepared with skin, skin/coating not eaten	0.23	R
661	24170210	Chicken, back, with or without bone, NS as to cooking method, skin eaten	1.04	R
662	24171210	Chicken, back, with or without bone, broiled, skin eaten	1.04	R
663	24172210	Chicken, back, with or without bone, roasted, skin eaten	1.04	R
664	24172220	Chicken, back, with or without bone, roasted, skin not eaten	1.06	R
665	24173210	Chicken, back, with or without bone, stewed, skin eaten	0.98	R

666	24173220	Chicken, back, with or without bone, stewed, skin not eaten	0.99	R
667	24174200	Chicken, back, with or without bone, fried, no coating, NS as to skin eaten	1.07	R
668	24174210	Chicken, back, with or without bone, fried, no coating, skin eaten	0.25	R
669	24174220	Chicken, back, with or without bone, fried, no coating, skin not eaten	1.08	R
670	24175200	Chicken, back, with or without bone, floured, baked or fried, prepared with skin, NS as to skin/coating e	1.05	R
671	24175210	Chicken, back, with or without bone, floured, baked or fried, prepared with skin, skin/coating eaten	0.27	R
672	24175220	Chicken, back, with or without bone, floured, baked or fried, prepared with skin, skin/coating not eaten	1.01	R
673	24176210	Chicken, back, with or without bone, breaded, baked or fried, prepared with skin, skin/coating eaten	0.98	R
674	24177210	Chicken, back, with or without bone, battered, fried, prepared with skin, skin/coating eaten	0.80	R
675	24180200	Chicken, neck or ribs, with or without bone, NS as to cooking method, NS as to skin eaten	0.95	R
676	24185220	Chicken, neck or ribs, with or without bone, floured, baked or fried, prepared with skin, skin/coating no	0.25	R
677	24198440	Chicken skin	0.98	R
678	24198500	Chicken feet	0.17	R
679	24198640	Chicken, chicken roll, roasted, NS as to light or dark meat	1.48	R
680	24198710	Chicken patty with cheese, breaded, cooked	1.76	R
681	24198720	Chicken, ground	0.23	R
682	24201000	Turkey, NFS	0.69	R
683	24201010	Turkey, light meat, cooked, NS as to skin eaten	0.57	R
684	24201020	Turkey, light meat, cooked, skin not eaten	0.57	R
685	24201030	Turkey, light meat, cooked, skin eaten	0.16	R

686	24201060	Turkey, light meat, breaded, baked or fried, skin not eaten	0.16	R
687	24201110	Turkey, light meat, roasted, NS as to skin eaten	0.57	R
688	24201120	Turkey, light meat, roasted, skin not eaten	0.16	R
689	24201130	Turkey, light meat, roasted, skin eaten	0.16	R
690	24201210	Turkey, dark meat, roasted, NS as to skin eaten	0.32	R
691	24201220	Turkey, dark meat, roasted, skin not eaten	0.32	R
692	24201230	Turkey, dark meat, roasted, skin eaten	0.19	R
693	24201310	Turkey, light and dark meat, roasted, NS as to skin eaten	0.58	R
694	24201320	Turkey, light and dark meat, roasted, skin not eaten	0.21	R
695	24201330	Turkey, light and dark meat, roasted, skin eaten	0.35	R
696	24201350	Turkey, light or dark meat, battered, fried, NS as to skin eaten	2.03	R
697	24201400	Turkey, light or dark meat, stewed, NS as to skin eaten	0.70	R
698	24201410	Turkey, light or dark meat, stewed, skin not eaten	1.18	R
699	24201500	Turkey, light or dark meat, smoked, cooked, NS as to skin eaten	2.53	R
700	24201520	Turkey, light or dark meat, smoked, cooked, skin not eaten	2.53	R
701	24202000	Turkey, drumstick, cooked, NS as to skin eaten	0.20	R
702	24202010	Turkey, drumstick, cooked, skin not eaten	0.80	R
703	24202020	Turkey, drumstick, cooked, skin eaten	0.20	R
704	24202050	Turkey, drumstick, roasted, NS as to skin eaten	1.01	R
705	24202060	Turkey, drumstick, roasted, skin not eaten	0.20	R
706	24202070	Turkey, drumstick, roasted, skin eaten	1.01	R
707	24202120	Turkey, drumstick, smoked, cooked, skin eaten	2.53	R
708	24202450	Turkey, thigh, cooked, NS as to skin eaten	0.33	R

709	24202460	Turkey, thigh, cooked, skin eaten	1.01	R
710	24202500	Turkey, thigh, cooked, skin not eaten	1.02	R
711	24202600	Turkey, neck, cooked	0.96	R
712	24203000	Turkey, wing, cooked, NS as to skin eaten	0.15	R
713	24203010	Turkey, wing, cooked, skin not eaten	0.70	R
714	24203020	Turkey, wing, cooked, skin eaten	0.16	R
715	24203120	Turkey, wing, smoked, cooked, skin eaten	2.53	R
716	24204000	Turkey, rolled roast, light or dark meat, cooked	1.73	R
717	24205000	Turkey, tail, cooked	0.16	R
718	24205100	Turkey, back, cooked	1.00	R
719	24207000	Turkey, ground	0.51	R
720	24300110	Duck, cooked, skin eaten	0.56	R
721	24300120	Duck, cooked, skin not eaten	0.58	R
722	24301000	Duck, roasted, NS as to skin eaten	0.56	R
723	24301010	Duck, roasted, skin eaten	0.56	R
724	24301020	Duck, roasted, skin not eaten	0.25	R
725	24400000	Cornish game hen, cooked, NS as to skin eaten	0.57	R
726	24400010	Cornish game hen, cooked, skin eaten	0.57	R
727	24400020	Cornish game hen, cooked, skin not eaten	0.16	R
728	24401000	Cornish game hen, roasted, NS as to skin eaten	0.57	R
729	24401010	Cornish game hen, roasted, skin eaten	0.45	R
730	24401020	Cornish game hen, roasted, skin not eaten	0.32	R
731	24402100	Dove, cooked, NS as to cooking method	0.56	R
732	24402110	Dove, fried	0.14	R
733	24403100	Quail, cooked	0.54	R
734	24404100	Pheasant, cooked	0.52	R
735	25110000	Liver, NS as to type, cooked	0.58	R
736	25110100	Beef liver, cooked, NS as to cooking method	0.27	R
737	25110120	Beef liver, braised	1.35	R
738	25110140	Beef liver, fried or broiled, no coating	0.27	R
739	25110150	Beef liver, breaded, fried	1.15	R
740	25110170	Beef liver, battered, fried	0.88	R

741	25110200	Calves liver, cooked, NS as to cooking method	1.07	R
742	25110240	Calves liver, fried or broiled, no coating	0.34	R
743	25110250	Calves liver, breaded, fried	1.25	R
744	25110300	Pork liver, cooked, NS as to cooking method	0.94	R
745	25110320	Pork liver, braised	0.94	R
746	25110340	Pork liver, breaded, fried	1.61	R
747	25110400	Chicken liver, cooked, NS as to cooking method	1.61	R
748	25110410	Chicken liver, battered, fried	0.20	R
749	25110420	Chicken liver, braised	0.95	R
750	25110440	Chicken liver, fried or sauteed, no coating	0.29	R
751	25110450	Chicken liver, breaded, fried	1.10	R
752	25112200	Liver paste or pate, chicken	0.98	R
753	25120000	Heart, cooked, NS as to cooking method	0.98	R
754	25120150	Heart, fried	0.61	R
755	25130000	Kidney, cooked, NS as to cooking method	1.16	R
756	25130150	Kidney, breaded, fried	2.48	R
757	25150000	Brains, cooked	1.20	R
758	25160000	Tongue, cooked, NS as to cooking method	0.97	R
759	25160100	Tongue, braised	0.15	R
760	25160110	Tongue, smoked, cured, or pickled, cooked	2.64	R
761	25160130	Tongue pot roast, Puerto Rican style (Lengua al caldero)	3.15	R
762	25170110	Tripe, cooked	0.99	R
763	25170210	Chitterlings, cooked	0.92	R
764	25170310	Hog maws (stomach), cooked	0.09	R
765	25170420	Gizzard, cooked	0.41	R
766	25220110	Beef sausage, brown and serve, links, cooked	2.63	R
767	25220140	Beef sausage, fresh, bulk, patty or link, cooked	2.41	R
768	25220210	Blood sausage	1.73	R
769	25220350	Bratwurst, cooked	1.42	R

770	25221410	Pork sausage, fresh, bulk, patty or link, cooked	3.28	R
771	25221450	Pork sausage rice links, brown and serve, cooked	1.65	R
772	25221460	Pork and beef sausage	2.05	R
773	25221470	Pork and beef sausage, brown and serve, cooked	2.05	R
774	25221510	Salami, soft, cooked	2.71	R
775	25221610	Scrapple, cooked	1.94	R
776	25221860	Turkey sausage, reduced fat, brown and serve, cooked	1.57	R
777	25221870	Turkey and pork sausage, fresh, bulk, patty or link, cooked	2.23	R
778	25221890	Turkey, pork, and beef sausage, lowfat, smoked	2.02	R
779	25230810	Veal loaf	3.38	R
780	27111050	Spaghetti sauce with beef or meat other than lamb or mutton, homemade-style	0.87	R
781	27111200	Beef burgundy	0.12	R
782	27114000	Beef with (mushroom) soup (mixture)	0.85	R
783	27115100	Steak teriyaki with sauce (mixture)	1.44	R
784	27116350	Stewed, seasoned, ground beef, Mexican style (Picadillo de carne de rez)	0.59	R
785	27116400	Steak tartare (raw ground beef and egg)	0.30	R
786	27118110	Meatballs, Puerto Rican style (Albondigas)	2.07	R
787	27120090	Ham or pork with (mushroom) soup (mixture)	0.81	R
788	27120110	Sausage with tomato-based sauce (mixture)	2.06	R
789	27121000	Pork with chili and tomatoes (mixture) (Puerco con chile)	0.64	R
790	27121010	Stewed pork, Puerto Rican style	1.61	R
791	27130010	Lamb or mutton with gravy (mixture)	0.72	R
792	27130040	Spaghetti sauce with lamb or mutton, homemade-style	1.20	R
793	27130100	Lamb curry	0.53	R
794	27133010	Stewed goat, Puerto Rican style (Cabrito en fricase, chilindron de chivo)	2.09	R
795	27135040	Veal with butter sauce (mixture)	1.46	R
796	27135050	Veal Marsala	0.58	R

797	27136050	Venison/deer with tomato-based sauce (mixture)	0.54	R
798	27136080	Venison/deer with gravy (mixture)	0.71	R
799	27136100	Chili con carne with venison/deer and beans	1.40	R
800	27141030	Spaghetti sauce with poultry, home-made style	1.21	R
801	27141050	Stewed chicken with tomato-based sauce, Mexican style (mixture) (Pollo guisado con tomate)	0.35	R
802	27142100	Chicken or turkey fricassee	0.53	R
803	27144000	Chicken or turkey with (mushroom) soup (mixture)	0.81	R
804	27146400	Chicken kiev	0.89	R
805	27148010	Stuffed chicken, drumstick or breast, Puerto Rican style (Muslo de pollo o pechuga rellena)	2.06	R
806	27150190	Lobster sauce (broth-based)	2.20	R
807	27162050	Spaghetti sauce with combination of meats, homemade-style	1.23	R
808	27211170	Beef and potatoes with (mushroom) soup (mixture)	0.57	R
809	27211550	Stewed, seasoned, ground beef with potatoes, Mexican style (Picadillo de carne de rez con papas)	0.43	R
810	27212400	Beef and noodles with (mushroom) soup (mixture)	0.66	R
811	27213000	Beef and rice, no sauce (mixture)	0.76	R
812	27213120	Porcupine balls with tomato-based sauce (mixture)	1.16	R
813	27213420	Porcupine balls with (mushroom) soup (mixture)	1.27	R
814	27214100	Meat loaf made with beef	0.31	R
815	27214110	Meat loaf made with beef, with tomato-based sauce	1.00	R
816	27218310	Stewed corned beef, Puerto Rican style ("Corned beef" guisado)	1.74	R
817	27220050	Ham or pork with stuffing (mixture)	2.07	R
818	27220150	Sausage and rice with (mushroom) soup (mixture)	1.56	R
819	27220190	Sausage and noodles with cream or white sauce (mixture)	1.16	R

820	27235000	Meat loaf made with venison / deer	0.64	R
821	27236000	Venison/deer and noodles with cream or white sauce (mixture)	0.76	R
822	27242250	Chicken or turkey and noodles with (mushroom) soup (mixture)	0.62	R
823	27243400	Chicken or turkey and rice with (mushroom) soup (mixture)	1.16	R
824	27246500	Meat loaf made with chicken or turkey	0.39	R
825	27250270	Clams Casino	0.80	R
826	27260010	Meat loaf, NS as to type of meat	0.63	R
827	27260090	Meat loaf made with beef, veal and pork	0.29	R
828	27260510	Liver dumpling	1.95	R
829	27311610	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), (mushroom) soup (m	0.63	R
830	27311620	Beef, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), (mushroom) soup (mixt	0.65	R
831	27313310	Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), (mushroom) soup (mi	0.79	R
832	27313320	Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), (mushroom) soup (mixtu	0.78	R
833	27315270	Stuffed grape leaves with beef and rice	0.18	R
834	27315310	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), (mushroom) soup (mixtu	0.76	R
835	27315320	Beef, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), (mushroom) soup (mixture)	0.58	R
836	27319010	Stuffed green pepper, Puerto Rican style (Pimiento relleno)	1.78	R
837	27330060	Lamb or mutton, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based	1.17	R
838	27330170	Stuffed grape leaves with lamb and rice	0.17	R
839	27331150	Veal fricassee, Puerto Rican style (ternera en fricase)	1.67	R
840	27335100	Rabbit stew with potatoes and vegetables	0.71	R

841	27336100	Venison/deer stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy), to	0.65	R
842	27336200	Venison/deer, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mix	0.51	R
843	27336310	Venison/deer, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based s	0.60	R
844	27345410	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), (mushroom	0.38	R
845	27345420	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), (mushroom) s	0.71	R
846	27350020	Paella with seafood	1.34	R
847	27362000	Stewed tripe, Puerto Rican style, with potatoes (Mondongo)	1.37	R
848	27363000	Gumbo with rice (New Orleans type with shellfish, pork, and/or poultry, tomatoes, okra, rice)	0.96	R
849	27363100	Jambalaya with meat and rice	0.35	R
850	27410250	Beef shish kabob with vegetables, excluding potatoes	0.72	R
851	27411120	Swiss steak	0.64	R
852	27411150	Beef rolls, stuffed with vegetables or meat mixture, tomato-based sauce	0.92	R
853	27414100	Beef with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), (mushroom) sou	1.22	R
854	27414200	Beef with vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), (mushroom) soup (	0.81	R
855	27416150	Pepper steak	0.66	R
856	27416200	Beef, ground, with egg and onion (mixture)	0.53	R
857	27418110	Seasoned shredded soup meat (Ropa vieja, sopa de carne ripiada)	0.60	R
858	27418310	Corned beef with tomato sauce and onion, Puerto Rican style (mixture)	1.99	R
859	27418410	Beef steak with onions, Puerto Rican style (mixture) (Biftec encebollado)	2.54	R

860	27420010	Cabbage with ham hocks (mixture)	1.02	R
861	27420400	Pork and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), tomato-based sa	0.69	R
862	27420460	Sausage and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), tomato-based sa	1.96	R
863	27430500	Veal goulash with vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), tomato-ba	0.36	R
864	27430510	Veal goulash with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), tomato	0.64	R
865	27450410	Shrimp and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sau	0.72	R
866	27450420	Shrimp and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), soy-based sauce	0.76	R
867	27450600	Shellfish mixture and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), so	0.29	R
868	27460750	Liver, beef or calves, and onions	0.74	R
869	27463000	Stewed gizzards, Puerto Rican style (Mollejitas guisadas)	1.11	R
870	27464000	Gumbo, no rice (New Orleans type with shellfish, pork, and/or poultry, tomatoes, okra)	1.11	R
871	27510210	Cheeseburger, plain, on bun	1.39	R
872	27510220	Cheeseburger, with mayonnaise or salad dressing, on bun	1.34	R
873	27510230	Cheeseburger, with mayonnaise or salad dressing and tomatoes, on bun	1.17	R
874	27510240	Cheeseburger, 1/4 lb meat, plain, on bun	1.43	R
875	27510250	Cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing, on bun	1.47	R
876	27510260	Cheeseburger, 1/4 lb meat, with mushrooms in sauce, on bun	1.45	R
877	27510270	Double cheeseburger (2 patties), plain, on bun	1.43	R
878	27510280	Double cheeseburger (2 patties), with mayonnaise or salad dressing, on bun	1.45	R

879	27510300	Double cheeseburger (2 patties), with mayonnaise or salad dressing, on double-decker bun	1.42	R
880	27510310	Cheeseburger with tomato and/or catsup, on bun	1.41	R
881	27510311	Cheeseburger, 1 oz meat, plain, on miniature bun	1.21	R
882	27510320	Cheeseburger, 1/4 lb meat, with tomato and/or catsup, on bun	1.63	R
883	27510330	Double cheeseburger (2 patties), with tomato and/or catsup, on bun	1.61	R
884	27510340	Double cheeseburger (2 patties), with mayonnaise or salad dressing and tomatoes, on bun	1.32	R
885	27510350	Cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun	1.31	R
886	27510360	Cheeseburger with mayonnaise or salad dressing, tomato and bacon, on bun	1.39	R
887	27510370	Double cheeseburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing, on bun	1.19	R
888	27510380	Triple cheeseburger (3 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes, on bun	1.11	R
889	27510390	Double bacon cheeseburger (2 patties, 1/4 lb meat each), on bun	1.43	R
890	27510400	Bacon cheeseburger, 1/4 lb meat, with tomato and/or catsup, on bun	1.83	R
891	27510420	Taco burger, on bun	1.44	R
892	27510430	Double bacon cheeseburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes,	1.21	R
893	27510440	Bacon cheeseburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun	1.24	R
894	27510480	Cheeseburger (hamburger with cheese sauce), 1/4 lb meat, with grilled onions, on rye bun	1.01	R
895	27510500	Hamburger, plain, on bun	1.14	R
896	27510510	Hamburger, with tomato and/or catsup, on bun	1.24	R

897	27510520	Hamburger, with mayonnaise or salad dressing and tomatoes, on bun	0.82	R
898	27510530	Hamburger, 1/4 lb meat, plain, on bun	1.10	R
899	27510540	Double hamburger (2 patties), with tomato and/or catsup, on bun	1.34	R
900	27510550	Double hamburger (2 patties), with mayonnaise or salad dressing and tomatoes, on double-decker bun	1.09	R
901	27510560	Hamburger, 1/4 lb meat, with mayonnaise or salad dressing and tomatoes, on bun	1.07	R
902	27510590	Hamburger, with mayonnaise or salad dressing, on bun	1.13	R
903	27510600	Hamburger, 1 oz meat, plain, on miniature bun	1.73	R
904	27510610	Hamburger, 1 oz meat, with tomato and/or catsup, on miniature bun	1.18	R
905	27510620	Hamburger, 1/4 lb meat, with tomato and/or catsup, on bun	1.34	R
906	27510630	Hamburger, 1/4 lb meat, with mayonnaise or salad dressing, on bun	1.20	R
907	27510640	Hamburger, 1/4 lb meat (beef modified in fat content), with tomato and/or catsup, on bun	0.95	R
908	27510670	Double hamburger (2 patties), with mayonnaise or salad dressing and tomatoes, on bun	1.06	R
909	27510680	Double hamburger (2 patties, 1/4 lb meat each), with tomato and/or catsup, on bun	0.98	R
910	27510690	Double hamburger (2 patties, 1/4 lb meat each), with mayonnaise or salad dressing and tomatoes and/or cat	0.90	R
911	27515000	Steak submarine sandwich, on roll, with lettuce and tomato	0.81	R
912	27515010	Steak sandwich, plain, on roll	0.96	R
913	27515020	Steak and cheese submarine sandwich, on roll, with lettuce and tomato	1.13	R
914	27515030	Steak and cheese sandwich, plain, on roll	1.21	R
915	27515040	Steak and cheese submarine sandwich, plain, on roll	1.62	R
916	27515080	Steak sandwich, plain, on biscuit	1.67	R

917	27515150	Steak patty (breaded, fried) sandwich, with mayonnaise or salad dressing, lettuce, and tomato, on bun	1.51	R
918	27516010	Gyro sandwich (pita bread, beef, lamb, onion, condiments), with tomato and spread	0.66	R
919	27520120	Bacon and cheese sandwich, with spread	2.33	R
920	27520140	Bacon and egg sandwich	1.12	R
921	27520150	Bacon, lettuce, and tomato sandwich with spread	1.30	R
922	27520170	Bacon on biscuit	2.82	R
923	28310160	Beef broth, with tomato, home recipe	0.44	R
924	28310170	Beef broth, without tomato, home recipe	0.50	R
925	28330110	Scotch broth (lamb, vegetables, and barley)	1.07	R
926	28340120	Chicken broth, without tomato, home recipe	0.36	R
927	28340130	Chicken broth, with tomato, home recipe	0.40	R
928	28340590	Chicken corn soup, home recipe	0.47	R
929	28340660	Chicken or turkey vegetable soup, home recipe	0.55	R
930	28500050	Gravy, giblet	1.33	R
931	28500150	Gravy, redeye	0.10	R
932	28510010	Gravy or sauce, poultry-based from Puerto Rican-style chicken fricasse	1.27	R
933	32105030	Egg omelet or scrambled egg, with ham or bacon	1.56	R
934	32105060	Egg omelet or scrambled egg, with peppers, onion, and ham	0.73	R
935	32105080	Egg omelet or scrambled egg, with cheese and ham or bacon	1.43	R
936	32105085	Egg omelet or scrambled egg, with cheese, ham or bacon, and tomatoes	1.27	R
937	32105110	Egg omelet or scrambled egg, with beef	0.83	R
938	32105120	Egg omelet or scrambled egg, with sausage and mushrooms	1.28	R
939	32105121	Egg omelet or scrambled egg, with sausage and cheese	0.98	R
940	32105122	Egg omelet or scrambled egg, with sausage	1.44	R

941	32105160	Egg omelet or scrambled egg, with chorizo	1.34	R
942	32105170	Egg omelet or scrambled egg with chicken	0.69	R
943	32105190	Egg casserole with bread, cheese, milk and meat	1.11	R
944	32202070	Egg, cheese, and bacon on biscuit	2.57	R
945	32202080	Egg, cheese, and bacon on English muffin	1.71	R
946	32202090	Egg and bacon on biscuit	1.69	R
947	32202130	Egg and steak on biscuit	2.19	R
948	41101000	Beans, dry, cooked, NS as to type and as to fat added in cooking	0.79	R
949	41101010	Beans, dry, cooked, NS as to type, fat added in cooking	0.52	R
950	41101100	White beans, dry, cooked, NS as to fat added in cooking	0.79	R
951	41101110	White beans, dry, cooked, fat added in cooking	0.04	R
952	41102000	Black, brown, or Bayo beans, dry, cooked, NS as to fat added in cooking	0.89	R
953	41102010	Black, brown, or Bayo beans, dry, cooked, fat added in cooking	0.05	R
954	41102210	Fava beans, cooked, fat added in cooking	0.61	R
955	41103000	Lima beans, dry, cooked, NS as to fat added in cooking	0.29	R
956	41103010	Lima beans, dry, cooked, fat added in cooking	0.03	R
957	41103050	Pink beans, dry, cooked, NS as to fat added in cooking	0.78	R
958	41103070	Pink beans, dry, cooked, fat added in cooking	0.01	R
959	41104000	Pinto, calico, or red Mexican beans, dry, cooked, NS as to fat added in cooking	0.79	R
960	41104010	Pinto, calico, or red Mexican beans, dry, cooked, fat added in cooking	0.04	R
961	41106000	Red kidney beans, dry, cooked, NS as to fat added in cooking	0.78	R
962	41106010	Red kidney beans, dry, cooked, fat added in cooking	0.11	R
963	41205100	Black bean sauce	2.45	R

964	41207030	Beans, dry, cooked with ground beef	1.09	R
965	41208100	Beans, dry, cooked with pork	1.04	R
966	41210100	Stewed dry red beans, Puerto Rican style (Habichuelas coloradas guisadas)	0.49	R
967	41210110	Stewed dry lima beans, Puerto Rican style	0.64	R
968	41210150	Stewed pink beans with viandas, ham, Puerto Rican style	0.21	R
969	41301000	Cowpeas, dry, cooked, NS as to fat added in cooking	0.88	R
970	41301010	Cowpeas, dry, cooked, fat added in cooking	0.23	R
971	41302000	Chickpeas, dry, cooked, NS as to fat added in cooking	0.91	R
972	41302010	Chickpeas, dry, cooked, fat added in cooking	0.20	R
973	41304130	Cowpeas, dry, cooked with pork	1.85	R
974	41310100	Stewed pigeon peas, Puerto Rican style (Gandules guisados, Gandur, Gandules)	0.23	R
975	41310200	Chickpeas stewed with pig's feet, Puerto Rican style (Garbanzos guisados con patitos de cerdo)	0.32	R
976	41601180	Bean and ham soup, home recipe	0.53	R
977	58101800	Ground beef with tomato sauce and taco seasonings on a cornbread crust	1.38	R
978	58101820	Mexican casserole made with ground beef, beans, tomato sauce, cheese, taco seasonings, and corn chips	0.54	R
979	58101830	Mexican casserole made with ground beef, tomato sauce, cheese, taco seasonings, and corn chips	0.64	R
980	58105110	Pupusa, meat-filled	0.22	R
981	58107000	Ground beef with tomato sauce on a pizza crust	2.16	R
982	58109010	Italian pie with meat	1.92	R
983	58116110	Meat turnover, Puerto Rican style (Pastelillo de carne; Empanadilla)	1.39	R
984	58120110	Crepes, filled with meat, fish, or poultry, with sauce	1.11	R
985	58127350	Croissant sandwich with bacon, egg, and cheese	1.62	R
986	58128110	Chicken cornbread	0.89	R

987	58128250	Dressing with meat and vegetables	1.74	R
988	58155110	Rice with chicken, Puerto Rican style (Arroz con Pollo)	1.76	R
989	58155310	Paella, Valenciana style, with meat (Paella Valenciana)	1.87	R
990	58155320	Seafood paella, Puerto Rican style (Paella a la marinera)	0.71	R
991	58155410	Soupy rice with chicken, Puerto Rican style (Asopao de pollo)	0.74	R
992	58155510	Soupy rice mixture with chicken and potatoes, Puerto Rican style	0.69	R
993	58155810	Stewed rice, Puerto Rican style (arroz quisado)	1.79	R
994	58160140	Rice with beans and pork	0.18	R
995	58160150	Red beans and rice	0.45	R
996	58163450	Spanish rice with ground beef	1.05	R
997	58402100	Beef noodle soup, home recipe	0.26	R
998	58403040	Chicken noodle soup, home recipe	0.08	R
999	58404030	Chicken or turkey rice soup, home recipe	0.36	R
1000	58406020	Turkey noodle soup, home recipe	0.36	R
1001	58409000	Noodle soup, with fish ball, shrimp, and dark green leafy vegetable	0.87	R
1002	58421010	Sopa Seca de Fideo, Mexican style, made with dry noodles	1.14	R
1003	71411000	White potato skins, with adhering flesh, fried, with cheese and bacon	0.89	R
1004	71508060	White potato, stuffed, baked, peel eaten, stuffed with bacon and cheese	0.96	R
1005	71508070	White potato, stuffed, baked, peel not eaten, stuffed with bacon and cheese	1.12	R
1006	74415110	Tomato and sofrito stewing sauce, Puerto Rican style	1.11	R
1007	75414020	Mushrooms, stuffed	1.70	R
1008	75649110	Vegetable soup, home recipe	0.66	R
1009	75649150	Vegetable noodle soup, home recipe	0.67	R
1010	75651000	Minestrone soup, home recipe	0.61	R
1011	75652010	Vegetable beef soup, home recipe	0.27	R
1012	75652040	Vegetable beef soup with noodles or pasta, home recipe	0.25	R

1013	75652050	Vegetable beef soup with rice, home recipe	0.25	R	
1014	77250110	Stuffed tannier fritters, Puerto Rican style (Alcapurrias)	3.08	R	
1015	77316010	Stuffed cabbage, with meat, Puerto Rican style (Repollo relleno con carne)	2.25	R	
1016	77316510	Stuffed cabbage, with meat and rice, Syrian dish, Puerto Rican style (Repollo relleno con carne y con arr	0.33	R	
1017	81201000	Bacon grease or meat drippings	1.38	R	
1018	91361050	Duck sauce	0.00	R	
1019	25210150	Frankfurter or hot dog, cheese-filled	2.78	1	a
1020	25210210	Frankfurter or hot dog, beef	2.64	1	a
1021	25210220	Frankfurter or hot dog, beef and pork	2.87	1	a
1022	25210250	Frankfurter or hot dog, meat and poultry, fat free	2.67	1	a
1023	25210610	Frankfurter or hot dog, beef, lowfat	2.67	1	a
1024	25210700	Frankfurter or hot dog, meat & poultry, lowfat	2.37	1	a
1025	27120250	Frankfurters or hot dogs with tomato-based sauce (mixture)	2.26	1	a
1026	27560330	Frankfurter or hot dog, with cheese, plain, on bun	2.43	1	a
1027	27560340	Frankfurter or hot dog, with catsup and/or mustard, on bun	2.33	1	a
1028	27560350	Pig in a blanket (frankfurter or hot dog wrapped in dough)	2.44	1	a
1029	27560370	Frankfurter or hot dog with chili and cheese, on bun	2.21	1	a
1030	27560400	Chicken frankfurter or hot dog, plain, on bun	2.60	1	a
1031	14620320	Pizza topping from meat pizza	2.82	1	b
1032	21416120	Corned beef, cooked, lean only eaten	2.55	1	b
1033	22300150	Ham, breaded or floured, fried, NS as to fat eaten	2.81	1	b
1034	22300160	Ham, breaded or floured, fried, lean and fat eaten	2.81	1	b
1035	22321110	Ham, smoked or cured, ground patty	2.70	1	b
1036	22431000	Pork roll, cured, fried	2.64	1	b
1037	22602010	Pork bacon, smoked or cured, lower sodium	2.62	1	b

1038	25220010	Cold cut, NFS	2.68	1	b
1039	25220100	Beef sausage, NFS	2.59	1	b
1040	25220130	Beef sausage, smoked	2.59	1	b
1041	25220390	Bologna, beef, lowfat	2.87	1	b
1042	25220400	Bologna, pork and beef	2.57	1	b
1043	25220410	Bologna, NFS	2.59	1	b
1044	25220430	Bologna, beef	2.49	1	b
1045	25220440	Bologna, turkey	2.23	1	b
1046	25220450	Bologna ring, smoked	2.59	1	b
1047	25220480	Bologna, chicken, beef, and pork	2.47	1	b
1048	25220500	Bologna, beef and pork, lowfat	2.82	1	b
1049	25220650	Chicken and beef sausage, smoked	2.59	1	b
1050	25221110	Knockwurst	2.57	1	b
1051	25221310	Polish sausage	2.74	1	b
1052	25221350	Italian sausage	2.34	1	b
1053	25221480	Mettwurst	2.73	1	b
1054	25221500	Salami, NFS	2.71	1	b
1055	25221660	Smoked link sausage, pork and beef	2.41	1	b
1056	25221710	Souse	2.62	1	b
1057	25221850	Turkey sausage, smoked	2.23	1	b
1058	25221880	Turkey, pork, and beef sausage, reduced fat, smoked	2.43	1	b
1059	25230220	Ham, sliced, low salt, prepackaged or deli, luncheon meat	2.46	1	b
1060	25230560	Liverwurst	2.90	1	b
1061	25230790	Turkey ham, sliced, extra lean, prepackaged or deli, luncheon meat	2.64	1	b
1062	25230800	Turkey ham	2.53	1	b
1063	25230820	Turkey pastrami	2.66	1	b
1064	25230840	Turkey salami	2.55	1	b
1065	25240220	Ham salad spread	2.32	1	b
1066	27120100	Ham or pork with tomato-based sauce (mixture)	2.50	1	b
1067	27220010	Meat loaf made with ham (not luncheon meat)	2.32	1	b
1068	27220080	Ham croquette	2.27	1	b
1069	27420020	Ham or pork salad	2.26	1	b
1070	27520300	Ham sandwich, with spread	2.34	1	b

1071	27520320	Ham and cheese sandwich, with lettuce and spread	2.38	1	b
1072	27520350	Ham and cheese sandwich, with spread, grilled	2.53	1	b
1073	27520360	Ham and cheese sandwich, on bun, with lettuce and spread	2.31	1	b
1074	27520370	Hot ham and cheese sandwich, on bun	2.23	1	b
1075	27560650	Sausage on biscuit	2.20	1	b
1076	27560670	Sausage and cheese on English muffin	2.29	1	b
1077	32202020	Egg, cheese, and ham on biscuit	2.68	1	b
1078	32202050	Egg, cheese, and sausage on biscuit	2.38	1	b
1079	58156310	Rice with Spanish sausage, Puerto Rican style	2.54	1	b
1080	74410110	Sofrito, Puerto Rican seasoning	2.29	1	b
1081	25220470	Bologna, beef, lower sodium	1.73	2	
1082	25230310	*Chicken or turkey loaf, prepackaged or deli, luncheon meat	1.41	2	
1083	25230710	Sandwich loaf, luncheon meat	3.52	2	
1084	25231110	Beef, sliced, prepackaged or deli, luncheon meat	3.66	2	
1085	25240110	Chicken salad spread	0.96	2	
1086	27416250	Beef salad	0.41	2	
1087	27446200	Chicken or turkey salad	0.40	2	
1088	27446220	Chicken or turkey salad with egg	0.63	2	
1089	27446300	Chicken or turkey garden salad (chicken and/or turkey, tomato and/or carrots, other vegetables), no dress	0.09	2	
1090	27446310	Chicken or turkey garden salad (chicken and/or turkey, other vegetables excluding tomato and carrots), no	0.10	2	
1091	27446350	Oriental chicken or turkey garden salad (chicken and/or turkey, lettuce, fruit, nuts), no dressing	0.19	2	
1092	27460490	Julienne salad (meat, cheese, eggs, vegetables), no dressing	0.46	2	
1093	27460510	Antipasto with ham, fish, cheese, vegetables	1.60	2	
1094	27513010	Roast beef sandwich	0.99	2	
1095	27513040	Roast beef submarine sandwich, on roll, with lettuce, tomato and spread	0.61	2	
1096	27513050	Roast beef sandwich with cheese	1.32	2	

1097	27520130	Bacon, chicken, and tomato club sandwich, with lettuce and spread	0.88	2	
1098	27520160	Bacon, chicken, and tomato club sandwich, on multigrain roll with lettuce and spread	1.10	2	
1099	27520340	Ham salad sandwich	1.94	2	
1100	27520390	Ham and cheese submarine sandwich, on multigrain roll, with lettuce, tomato and spread	1.84	2	
1101	27520540	Ham and tomato club sandwich, with lettuce and spread	2.10	2	
1102	27540120	Chicken salad or chicken spread sandwich	0.89	2	
1103	27540310	Turkey sandwich, with spread	0.87	2	
1104	27540320	Turkey salad or turkey spread sandwich	0.89	2	
1105	27540350	Turkey submarine sandwich, on roll, with cheese, lettuce, tomato and spread	2.21	2	
1106	27560110	Bologna sandwich, with spread	1.83	2	
1107	27560120	Bologna and cheese sandwich, with spread	2.13	2	
1108	27560910	Submarine, cold cut sandwich, on bun, with lettuce	2.03	2	
1109	58148170	Macaroni salad with chicken	1.00	2	
1110	58148550	Pasta salad with meat (macaroni or noodles, vegetables, meat, dressing)	1.57	2	
1111	74304000	Tomato juice with clam or beef juice	0.90	2	
1112	75145000	Seven-layer salad (lettuce salad made with a combination of onion, celery, green pepper, peas, mayonnaise	0.71	2	
1113	27111000	Beef with tomato-based sauce (mixture)	0.40	3	a
1114	27112100	Beef bourguignonne	0.36	3	a
1115	27116300	Beef with sweet and sour sauce (mixture)	1.36	3	a
1116	27120030	Ham or pork with barbecue sauce (mixture)	1.88	3	a
1117	27120060	Sweet and sour pork	0.94	3	a
1118	27145000	Chicken or turkey teriyaki (chicken or turkey with soy-based sauce)	3.34	3	a
1119	27146000	Chicken or turkey with barbecue sauce (mixture)	0.54	3	a
1120	27162010	Meat with tomato-based sauce (mixture)	0.67	3	a

1121	27211110	Mexican style beef stew with potatoes, tomato-based sauce (mixture) (Carne guisada con papas)	1.04	3	a
1122	27212100	Beef and noodles with tomato-based sauce (mixture)	0.68	3	a
1123	27213100	Beef and rice with tomato-based sauce (mixture)	0.88	3	a
1124	27220110	Pork and rice with tomato-based sauce (mixture)	1.20	3	a
1125	27220120	Sausage and rice with tomato-based sauce (mixture)	1.55	3	a
1126	27242400	Chicken or turkey and noodles, tomato-based sauce (mixture)	0.92	3	a
1127	27243500	Chicken or turkey and rice with tomato-based sauce (mixture)	0.39	3	a
1128	27260100	Meat loaf made with beef and pork, with tomato-based sauce	1.16	3	a
1129	27313210	Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce	0.29	3	a
1130	27313220	Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based sauce (mi	0.79	3	a
1131	27315210	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sauce (mi	0.71	3	a
1132	27315220	Beef, rice, and vegetables (excluding carrots, broccoli, and/or dark-green leafy), tomato-based sauce (mi	0.70	3	a
1133	27320070	Ham or pork, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based	1.80	3	a
1134	27320080	Sausage, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based sauce	1.28	3	a
1135	27320090	Sausage, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-based sau	1.25	3	a
1136	27320110	Pork, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based sauce (m	1.31	3	a

1137	27343510	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato	0.62	3	a
1138	27343520	Chicken or turkey, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-ba	0.77	3	a
1139	27345520	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), tomato-based	0.55	3	a
1140	27411100	Beef with vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), tomato-based s	0.24	3	a
1141	27411200	Beef with vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), tomato-based sauc	1.01	3	a
1142	27420410	Pork and vegetables (excluding carrots, broccoli, and dark- green leafy (no potatoes)), tomato-based sauc	0.20	3	a
1143	28110620	Beef short ribs, boneless, with barbecue sauce, potatoes, vegetable (frozen meal)	0.49	3	a
1144	28113050	Salisbury steak with vegetables in tomato-based sauce, noodles (diet frozen meal)	1.11	3	a
1145	28140740	Chicken patty, or nuggets, boneless, breaded, with pasta and tomato sauce, fruit, dessert (frozen meal)	0.98	3	a
1146	28141200	Chicken teriyaki with rice, vegetable (frozen meal)	1.77	3	a
1147	28160310	Meat loaf in tomato sauce with potatoes, vegetable (frozen meal)	0.91	3	a
1148	28500010	Gravy, meat or poultry, with wine	1.02	3	a
1149	58126150	Turnover, meat- and cheese-filled, tomato-based sauce	1.88	3	a
1150	58131110	Ravioli, NS as to filling, with tomato sauce	1.02	3	a
1151	58134610	Tortellini, meat-filled, with tomato sauce	1.65	3	a
1152	58134710	Tortellini, spinach-filled, with tomato sauce	1.59	3	a
1153	58301010	Lasagna with cheese, tomato sauce, vegetable, dessert (frozen meal)	0.76	3	a
1154	58302060	Spaghetti or noodles with beef in tomato-based sauce, lowfat, reduced sodium (diet frozen meal)	0.45	3	a

1155	58304020	Spaghetti and meatballs with tomato sauce, sliced apples, bread (frozen meal)	1.72	3	a
1156	58304300	Cannelloni, cheese-filled, with tomato sauce (diet frozen meal)	1.20	3	a
1157	24198700	Chicken patty, fillet, or tenders, breaded, cooked	1.35	3	b
1158	27246300	Chicken or turkey cake, patty, or croquette	0.65	3	b
1159	41501000	Mexican dinner with fried beans, frozen	1.01	3	c
1160	58100340	Burrito with eggs, sausage, cheese and vegetables	1.37	3	c
1161	58100400	Enchilada with beef, no beans	0.41	3	c
1162	58100510	Enchilada with beef and beans	0.66	3	c
1163	58100520	Enchilada with beef, beans, and cheese	0.74	3	c
1164	58100530	Enchilada with beef and cheese, no beans	0.62	3	c
1165	58100560	Enchilada with ham and cheese, no beans	1.14	3	c
1166	58100600	Enchilada with chicken, tomato-based sauce	0.40	3	c
1167	58100610	Enchilada with chicken and beans, tomato-based sauce	0.65	3	c
1168	58100620	Enchilada with chicken, beans, and cheese, tomato- based sauce	0.70	3	c
1169	58100630	Enchilada with chicken and cheese, no beans, tomato- based sauce	0.61	3	c
1170	58101240	Flauta with chicken	0.44	3	c
1171	58103110	Tamale with meat and/or poultry	1.53	3	c
1172	58103310	Tamale casserole with meat	0.82	3	c
1173	58104080	Nachos with beef, beans, cheese, and sour cream	0.83	3	c
1174	58104130	Nachos with beef, beans, and cheese	0.86	3	c
1175	58104140	Nachos with beef and cheese	0.81	3	c
1176	58104180	Nachos with beef, beans, cheese, tomatoes and onions	0.74	3	c
1177	58104250	Nachos with chicken or turkey and cheese	0.72	3	c
1178	58104310	Chalupa with beans, chicken, cheese, lettuce and tomato	0.54	3	c
1179	58104450	Chimichanga with beef and tomato	1.24	3	c
1180	58104490	Chimichanga, NFS	0.58	3	c

1181	58104500	Chimichanga with beef, beans, lettuce and tomato	0.50	3	c
1182	58104510	Chimichanga with beef, cheese, lettuce and tomato	0.75	3	c
1183	58104530	Chimichanga with chicken and cheese	0.84	3	c
1184	58104550	Chimichanga with chicken, sour cream, lettuce and tomato, no cheese	0.33	3	c
1185	58104600	Chimichanga with beef and rice	0.51	3	c
1186	58104730	Quesadilla with meat and cheese	0.90	3	c
1187	58104810	Taquitoes	1.04	3	c
1188	58105000	Fajita with chicken and vegetables	0.43	3	c
1189	58105050	Fajita with beef and vegetables	0.86	3	c
1190	58115110	Tamale casserole, Puerto Rican style (Tamales en cazuela)	0.72	3	c
1191	58306010	Beef enchilada dinner, NFS (frozen meal)	1.01	3	c
1192	58306020	Beef enchilada, chili gravy, rice, refried beans (frozen meal)	1.01	3	c
1193	58306100	Chicken enchilada (diet frozen meal)	1.07	3	c
1194	58306200	Chicken fajitas (diet frozen meal)	0.78	3	c
1195	58306500	Chicken burritos (diet frozen meal)	3.94	3	c
1196	75410530	Chiles rellenos, filled with meat and cheese (stuffed chili peppers)	0.71	3	c
1197	13412000	Milk gravy, quick gravy	0.59	3	d
1198	21410110	Beef, stew meat, cooked, lean and fat eaten	0.98	3	d
1199	24198740	Chicken nuggets	1.35	3	d
1200	24208000	Turkey, nuggets	2.15	3	d
1201	27111100	Beef goulash	0.46	3	d
1202	27113000	Beef with cream or white sauce (mixture)	0.53	3	d
1203	27115000	Beef with soy-based sauce (mixture)	0.90	3	d
1204	27116100	Beef curry	1.54	3	d
1205	27120020	Ham or pork with gravy (mixture)	0.70	3	d
1206	27135020	Veal scallopini	0.92	3	d
1207	27135110	Veal parmigiana	1.11	3	d
1208	27141000	Chicken or turkey cacciatore	0.26	3	d
1209	27146100	Sweet and sour chicken or turkey	1.42	3	d
1210	27146150	Chicken curry	1.18	3	d

1211	27146200	Chicken or turkey with cheese sauce (mixture)	0.78	3	d
1212	27146250	Chicken or turkey cordon bleu	0.74	3	d
1213	27146300	Chicken or turkey parmigiana	1.10	3	d
1214	27146350	Lemon chicken, Chinese style	1.92	3	d
1215	27150160	Shrimp with lobster sauce (mixture)	1.42	3	d
1216	27163010	Meat with gravy, NS as to type of meat (mixture)	0.66	3	d
1217	27211000	Beef and potatoes, no sauce (mixture)	0.14	3	d
1218	27211150	Beef goulash with potatoes	0.48	3	d
1219	27211190	Beef and potatoes with cream or white sauce (mixture)	0.95	3	d
1220	27211300	Beef (roast) hash	1.17	3	d
1221	27211400	Corned beef hash	1.37	3	d
1222	27211500	Beef and potatoes with cheese sauce (mixture)	0.90	3	d
1223	27212000	Beef and noodles, no sauce (mixture)	0.11	3	d
1224	27212050	Beef and macaroni with cheese sauce (mixture)	0.73	3	d
1225	27212150	Beef goulash with noodles	0.44	3	d
1226	27212200	Beef and noodles with gravy (mixture)	0.79	3	d
1227	27212300	Beef and noodles with cream or white sauce (mixture)	0.18	3	d
1228	27213200	Beef and rice with gravy (mixture)	1.01	3	d
1229	27213300	Beef and rice with cream sauce (mixture)	1.15	3	d
1230	27213400	Beef and rice with (mushroom) soup (mixture)	0.80	3	d
1231	27213500	Beef and rice with soy-based sauce (mixture)	0.91	3	d
1232	27214500	Corned beef patty	1.37	3	d
1233	27220020	Ham and noodles with cream or white sauce (mixture)	1.53	3	d
1234	27220210	Ham and noodles, no sauce (mixture)	1.27	3	d
1235	27220310	Ham or pork and rice, no sauce (mixture)	1.56	3	d
1236	27220510	Ham or pork and potatoes with gravy (mixture)	1.03	3	d
1237	27220520	Ham or pork and potatoes with cheese sauce (mixture)	1.29	3	d
1238	27241000	Chicken or turkey hash	0.82	3	d

1239	27242000	Chicken or turkey and noodles, no sauce (mixture)	0.30	3	d
1240	27242310	Chicken or turkey and noodles with cheese sauce (mixture)	0.66	3	d
1241	27243000	Chicken or turkey and rice, no sauce (mixture)	0.92	3	d
1242	27243300	Chicken or turkey and rice with cream sauce (mixture)	0.78	3	d
1243	27243600	Chicken or turkey and rice with soy-based sauce (mixture)	0.75	3	d
1244	27243700	Chicken in cheese sauce with Spanish rice	1.11	3	d
1245	27246200	Chicken or turkey with stuffing (mixture)	0.65	3	d
1246	27260080	Meat loaf made with beef and pork	0.30	3	d
1247	27260110	Hash, NS as to type of meat	1.37	3	d
1248	27311110	Beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	0.13	3	d
1249	27311120	Beef, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce (mixture)	0.33	3	d
1250	27311210	Corned beef, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (m	0.90	3	d
1251	27311410	Beef stew with potatoes and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy	0.56	3	d
1252	27313010	Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	0.66	3	d
1253	27313020	Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce (mixture)	0.66	3	d
1254	27313110	Beef chow mein or chop suey with noodles	1.10	3	d
1255	27313150	Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mi	0.68	3	d
1256	27313160	Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), soy-based sauce (mixtu	0.69	3	d

1257	27313410	Beef, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	0.80	3	d
1258	27313420	Beef, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mixture)	0.91	3	d
1259	27315010	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	0.66	3	d
1260	27315020	Beef, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce (mixture)	0.66	3	d
1261	27315250	Stuffed cabbage rolls with beef and rice	0.74	3	d
1262	27315410	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	0.88	3	d
1263	27315420	Beef, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mixture)	0.73	3	d
1264	27315510	Beef, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixtu	0.65	3	d
1265	27315520	Beef, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), soy-based sauce (mixture)	0.69	3	d
1266	27317100	Beef, dumplings, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	0.98	3	d
1267	27320030	Ham or pork, noodles and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sauce (mi	1.83	3	d
1268	27320040	Pork, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	1.02	3	d
1269	27320120	Sausage, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	1.22	3	d
1270	27320130	Sausage, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mixture)	1.45	3	d
1271	27320140	Pork, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mixture)	1.13	3	d

1272	27320150	Pork, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mixture)	0.59	3	d
1273	27320210	Pork, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce (mixture)	0.12	3	d
1274	27320310	Pork chow mein or chop suey with noodles	0.98	3	d
1275	27320320	Pork, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixtu	0.60	3	d
1276	27320330	Pork, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), soy-based sauce (mixture)	0.58	3	d
1277	27320450	Ham, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce (mixture)	1.69	3	d
1278	27320500	Sweet and sour pork with rice	0.94	3	d
1279	27341010	Chicken or turkey, potatoes, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sa	0.62	3	d
1280	27341020	Chicken or turkey, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce	0.21	3	d
1281	27343010	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sau	0.34	3	d
1282	27343020	Chicken or turkey, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce	0.66	3	d
1283	27343410	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy	0.81	3	d
1284	27343470	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), cream,	0.38	3	d
1285	27343480	Chicken or turkey, noodles, and vegetables (excluding carrots, broccoli, and/or dark-green leafy), cream,	0.81	3	d
1286	27343910	Chicken or turkey chow mein or chop suey with noodles	1.22	3	d

1287	27343950	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese	0.76	3	d
1288	27343960	Chicken or turkey, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sa	0.39	3	d
1289	27343970	Chicken or turkey, noodles, and vegetables (including carrots, broccoli, and/or dark-green leafy), cream	0.29	3	d
1290	27343980	Chicken or turkey, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), cream or	0.29	3	d
1291	27345010	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sauce	0.09	3	d
1292	27345020	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce (mi	0.06	3	d
1293	27345210	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy (mi	0.71	3	d
1294	27345220	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mixtu	0.86	3	d
1295	27345310	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based	0.83	3	d
1296	27345320	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), soy-based sa	1.08	3	d
1297	27345440	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), cheese sa	0.47	3	d
1298	27345450	Chicken or turkey, rice, and vegetables (excluding carrots, broccoli, and dark-green leafy), cheese sauce	0.79	3	d
1299	27345510	Chicken or turkey, rice, and vegetables (including carrots, broccoli, and/or dark-green leafy), tomato-ba	0.45	3	d
1300	27347200	Chicken or turkey, stuffing, and vegetables (including carrots, broccoli, and/or dark-green leafy), no sa	1.02	3	d

1301	27347210	Chicken or turkey,stuffing, and vegetables (excluding carrots, broccoli, and dark green leafy), no sauce	0.66	3	d
1302	27347220	Chicken or turkey, stuffing, and vegetables (including carrots, broccoli, and/or dark-green leafy), gravy	0.61	3	d
1303	27347230	Chicken or turkey, stuffing, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (m	0.87	3	d
1304	27350050	Shrimp chow mein or chop suey with noodles	1.15	3	d
1305	27360050	Meat pie, NFS	1.23	3	d
1306	27360080	Chow mein or chop suey, NS as to type of meat, with noodles	1.11	3	d
1307	27360120	Chow mein or chop suey, various types of meat, with noodles	1.33	3	d
1308	27410210	Beef and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), no sauce (mixtu	0.84	3	d
1309	27410220	Beef and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), no sauce (mixture)	0.81	3	d
1310	27415100	Beef and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-based sauce	0.40	3	d
1311	27415120	Beef, tofu, and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-base	1.46	3	d
1312	27415150	Beef chow mein or chop suey, no noodles	1.07	3	d
1313	27415200	Beef and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), soy-based sauce (m	0.54	3	d
1314	27415220	Beef, tofu, and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), soy-based s	1.43	3	d
1315	27416450	Beef and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), gravy (mixture)	0.14	3	d
1316	27416500	Beef and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), gravy (mixture)	0.86	3	d

1317	27420060	Pork and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), no sauce (mixtu	0.36	3	d
1318	27420080	Greens with ham or pork (mixture)	0.92	3	d
1319	27420100	Pork, tofu, and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), soy-base	1.43	3	d
1320	27420160	Moo Shu (Mu Shi) Pork, without Chinese pancake	1.81	3	d
1321	27420170	Pork and onions with soy-based sauce (mixture)	1.13	3	d
1322	27420270	Ham and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), no sauce (mixture)	1.52	3	d
1323	27420350	Pork and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), no sauce (mixture)	0.58	3	d
1324	27420370	Pork, tofu, and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), soy-based s	1.55	3	d
1325	27420390	Pork chow mein or chop suey, no noodles	1.07	3	d
1326	27420470	Sausage and peppers, no sauce (mixture)	1.68	3	d
1327	27420500	Pork and vegetables (including carrots, broccoli, and/or dark-green leafy), soy-based sauce (mixture)	0.87	3	d
1328	27420510	Pork and vegetables (excluding carrots, broccoli, and dark- green leafy), soy-based sauce (mixture)	0.54	3	d
1329	27440110	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), no	0.66	3	d
1330	27440120	Chicken or turkey and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), no sa	0.29	3	d
1331	27442110	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), gr	0.42	3	d
1332	27442120	Chicken or turkey and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), gravy	0.73	3	d
1333	27443150	Chicken or turkey divan	0.48	3	d

1334	27445110	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), so	1.13	3	d
1335	27445120	Chicken or turkey and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), soy-b	1.23	3	d
1336	27445150	General Tso (General Gau) chicken	1.58	3	d
1337	27445180	Moo Goo Gai Pan	0.36	3	d
1338	27445220	Kung pao chicken	1.42	3	d
1339	27445250	Almond chicken	0.55	3	d
1340	27446100	Chicken or turkey chow mein or chop suey, no noodles	1.11	3	d
1341	27446400	Chicken or turkey and vegetables (including carrots, broccoli, and/or dark-green leafy (no potatoes)), ch	0.62	3	d
1342	27446410	Chicken or turkey and vegetables (excluding carrots, broccoli, and dark-green leafy (no potatoes)), chees	1.12	3	d
1343	27450040	Shrimp chow mein or chop suey, no noodles	0.85	3	d
1344	27460010	Chow mein or chop suey, NS as to type of meat, no noodles	1.09	3	d
1345	27500100	Meat sandwich, NFS	1.83	3	d
1346	27510950	Reuben sandwich (corned beef sandwich with sauerkraut and cheese), with spread	1.89	3	d
1347	27513020	Roast beef sandwich, with gravy	1.16	3	d
1348	27513060	Roast beef sandwich with bacon and cheese sauce	1.41	3	d
1349	27513070	Roast beef submarine sandwich, on roll, au jus	0.81	3	d
1350	27515050	Fajita-style beef sandwich with cheese, on pita bread, with lettuce and tomato	0.95	3	d
1351	27515070	Steak and cheese submarine sandwich, with fried peppers and onions, on roll	0.77	3	d
1352	27520380	Ham and cheese on English muffin	1.96	3	d
1353	27540110	Chicken sandwich, with spread	0.91	3	d
1354	27540130	Chicken barbecue sandwich	0.90	3	d
1355	27540140	Chicken fillet (breaded, fried) sandwich	1.06	3	d
1356	27540150	Chicken fillet (breaded, fried) sandwich with lettuce, tomato and spread	0.87	3	d

1357	27540170	Chicken patty sandwich, miniature, with spread	1.39	3	d
1358	27540180	Chicken patty sandwich or biscuit	2.58	3	d
1359	27540190	Chicken patty sandwich, with lettuce and spread	1.29	3	d
1360	27540200	Fajita-style chicken sandwich with cheese, on pita bread, with lettuce and tomato	0.96	3	d
1361	27540230	Chicken patty sandwich with cheese, on wheat bun, with lettuce, tomato and spread	1.29	3	d
1362	27540240	Chicken fillet, (broiled), sandwich, on whole wheat roll, with lettuce, tomato and spread	1.03	3	d
1363	27540260	Chicken fillet, broiled, sandwich, on oat bran bun, with lettuce, tomato, spread	0.95	3	d
1364	27540270	Chicken fillet, broiled, sandwich, with lettuce, tomato, and non-mayonnaise type spread	0.65	3	d
1365	27540280	Chicken fillet, broiled, sandwich with cheese, on bun, with lettuce, tomato and spread	0.99	3	d
1366	27560300	Corn dog (frankfurter or hot dog with cornbread coating)	2.03	3	d
1367	28101000	Frozen dinner, NFS	0.66	3	d
1368	28110000	Beef dinner, NFS (frozen meal)	0.66	3	d
1369	28110110	Beef with potatoes (frozen meal)	0.91	3	d
1370	28110220	Sirloin, chopped, with gravy, mashed potatoes, vegetable (frozen meal)	1.45	3	d
1371	28110230	Sirloin, chopped, or swiss steak with gravy, vegetable, potatoes, dessert or muffin (frozen meal)	0.62	3	d
1372	28110250	Sirloin tips with gravy, potatoes, vegetable (frozen meal)	0.67	3	d
1373	28110260	Sirloin tips, potato, vegetable, fruit (diet frozen meal)	0.36	3	d
1374	28110290	Sirloin tips and mushrooms in wine sauce with rotini (diet frozen entree)	1.07	3	d
1375	28110310	Salisbury steak with gravy, potatoes, vegetable (frozen meal)	0.71	3	d

1376	28110330	Salisbury steak with gravy, whipped potatoes, vegetable, dessert (frozen meal)	1.51	3	d
1377	28110340	Salisbury steak with gravy, potatoes, vegetable, soup or macaroni and cheese, dessert (frozen meal)	0.99	3	d
1378	28110350	Salisbury steak with gravy, potatoes, vegetable, dessert (frozen meal, large meat portion)	0.88	3	d
1379	28110370	Salisbury steak with gravy, macaroni and cheese, vegetable (frozen meal)	1.10	3	d
1380	28110390	Salisbury steak, potatoes, vegetable, dessert (diet frozen meal)	0.35	3	d
1381	28110500	Beef, sliced, with gravy, barley and wild rice, vegetables (diet frozen meal)	0.27	3	d
1382	28110510	Beef, sliced, with gravy, potatoes, vegetable (frozen meal)	0.66	3	d
1383	28110520	Beef, sliced, with gravy, potatoes, vegetable, dessert (frozen meal)	1.01	3	d
1384	28110540	Beef, sliced, with vegetable in sauce, au gratin potatoes (frozen meal)	0.85	3	d
1385	28110600	Beef with noodles, vegetable (frozen meal)	2.35	3	d
1386	28110640	Meatballs, Swedish, in sauce, with noodles (frozen meal)	0.75	3	d
1387	28110650	Meatballs, Swedish, in sauce, with noodles and vegetable medley (frozen meal)	0.80	3	d
1388	28110660	Meatballs, Swedish, in gravy, with noodles (diet frozen meal)	0.66	3	d
1389	28113040	Beef, oriental style, with vegetable, rice, and fruit dessert (diet frozen meal)	0.50	3	d
1390	28113140	Beef with spaetzle or rice, vegetable (frozen meal)	0.77	3	d
1391	28113150	Beef steak with rice, vegetable (diet frozen meal)	0.95	3	d
1392	28120230	Pork, sliced, with gravy, mashed potatoes, vegetable, dessert (frozen meal)	0.77	3	d
1393	28130000	Veal dinner, NFS (frozen meal)	1.45	3	d
1394	28133340	Veal parmigiana with vegetable, fettuccine alfredo, dessert (frozen meal)	1.00	3	d

1395	28133410	Veal parmigiana with potatoes, vegetable (frozen meal)	1.08	3	d
1396	28140100	Chicken dinner, NFS (frozen meal)	1.22	3	d
1397	28140150	Chicken divan (frozen meal)	0.71	3	d
1398	28140250	Chicken, boneless, with gravy, dressing, rice, vegetable, dessert (frozen meal, large meat portion)	0.84	3	d
1399	28140610	Chicken, fried, with potatoes (frozen meal)	1.40	3	d
1400	28140620	Chicken, fried, with potatoes (frozen meal, large meat portion)	1.59	3	d
1401	28140710	Chicken, fried, with potatoes, vegetable (frozen meal)	1.28	3	d
1402	28140720	Chicken patty, or nuggets, boneless, breaded, potatoes, vegetable (frozen meal)	1.22	3	d
1403	28140730	Chicken patty, breaded, with tomato sauce and cheese, fettuccine alfredo, vegetable (frozen meal)	0.70	3	d
1404	28140810	Chicken, fried, with potatoes, vegetable, dessert (frozen meal)	1.22	3	d
1405	28141010	Chicken, fried, with potatoes, vegetable, dessert (frozen meal, large meat portion)	0.87	3	d
1406	28141060	Chicken patty with vegetable (diet frozen meal)	1.35	3	d
1407	28141210	Chicken, fried in honey sauce, with Oriental style rice and vegetables, in soy-based sauce (frozen meal)	0.59	3	d
1408	28141250	Chicken with rice-vegetable mixture (diet frozen meal)	0.86	3	d
1409	28141300	Chicken with rice and vegetable, reduced fat and sodium (diet frozen meal)	0.41	3	d
1410	28141600	Chicken a la king with rice (frozen meal)	1.03	3	d
1411	28141610	Chicken and vegetables in cream or white sauce (diet frozen meal)	0.65	3	d
1412	28141650	Chicken and vegetables au gratin with rice-vegetable mixture (diet frozen entree)	1.10	3	d
1413	28142000	Chicken in cream sauce, with brown and wild rice, vegetable, and fruit dessert (diet frozen meal)	0.32	3	d

1414	28143010	Chicken and vegetable entree with rice, Oriental (frozen meal)	0.65	3	d
1415	28143020	Chicken and vegetable entree with rice, Oriental (diet frozen meal)	0.63	3	d
1416	28143030	Chicken and vegetable entree, oriental (diet frozen meal)	0.89	3	d
1417	28143040	Chicken chow mein with rice (diet frozen meal)	0.85	3	d
1418	28143050	Chicken chow mein with rice, reduced fat and sodium (diet frozen meal)	0.66	3	d
1419	28143080	Chicken with noodles and cheese sauce (diet frozen meal)	0.27	3	d
1420	28143110	Chicken cacciatore with noodles (diet frozen meal)	0.77	3	d
1421	28143130	Chicken and vegetable entree with noodles (frozen meal)	0.64	3	d
1422	28143150	Chicken and vegetable entree with noodles (diet frozen meal)	0.57	3	d
1423	28143170	Chicken in cream sauce with noodles and vegetable (frozen meal)	1.04	3	d
1424	28143180	Chicken in butter sauce with potatoes and vegetable (diet frozen meal)	0.24	3	d
1425	28143190	Chicken in mushroom sauce, white and wild rice, vegetable (frozen meal)	0.90	3	d
1426	28143200	Chicken in soy-based sauce, rice and vegetables (frozen meal)	0.90	3	d
1427	28143210	Chicken in orange sauce with almond rice (diet frozen meal)	0.76	3	d
1428	28143220	Chicken in barbecue sauce, with rice, vegetable and dessert, reduced fat and sodium (diet frozen meal)	0.39	3	d
1429	28144100	Chicken and vegetable entree with noodles and cream sauce (frozen meal)	0.14	3	d
1430	28145000	Turkey dinner, NFS (frozen meal)	0.52	3	d
1431	28145010	Turkey with dressing, gravy, potato (frozen meal)	1.48	3	d
1432	28145100	Turkey with dressing, gravy, vegetable and fruit (diet frozen meal)	0.37	3	d
1433	28145110	Turkey with vegetable, stuffing (diet frozen meal)	0.48	3	d
1434	28145210	Turkey with gravy, dressing, potatoes, vegetable (frozen meal)	0.99	3	d

1435	28145310	Turkey with gravy, dressing, potatoes, vegetable, dessert (frozen meal)	1.38	3	d
1436	28145610	Turkey with gravy, dressing, potatoes, vegetable, dessert (frozen meal, large meat portion)	0.92	3	d
1437	28145810	Turkey breast with gravy, long-grain and wild rice, vegetable (frozen meal)	0.97	3	d
1438	28154010	Shrimp and vegetables in sauce with noodles (diet frozen meal)	0.90	3	d
1439	28160300	Meat loaf dinner, NFS (frozen meal)	1.42	3	d
1440	28160650	Stuffed green pepper (frozen meal)	0.78	3	d
1441	28160710	Stuffed cabbage, with meat and tomato sauce (diet frozen meal)	0.58	3	d
1442	28500000	Gravy, poultry	1.47	3	d
1443	28500020	Gravy, meat, with fruit	1.07	3	d
1444	28500040	Gravy, beef or meat	1.43	3	d
1445	28522000	Mole poblano (sauce)	0.58	3	d
1446	32101500	Egg, Benedict	1.65	3	d
1447	32105210	Chicken egg foo yung (young)	0.39	3	d
1448	32105220	Pork egg foo yung (young)	0.39	3	d
1449	32202010	Egg, cheese, and ham on English muffin	1.83	3	d
1450	32202030	Egg, cheese, and sausage on English muffin	1.63	3	d
1451	32202060	Egg and sausage on biscuit	1.61	3	d
1452	32202110	Egg and ham on biscuit	1.83	3	d
1453	35001000	Scrambled eggs, sausage, hash brown potatoes (frozen meal)	1.21	3	d
1454	35002000	Scrambled eggs, bacon, home fried potatoes (frozen meal)	1.13	3	d
1455	53306070	Pie, mince, individual size or tart	1.12	3	d
1456	58108010	Calzone, with meat and cheese	1.08	3	d
1457	58110130	Egg roll, with beef and/or pork	1.09	3	d
1458	58110170	Egg roll, with chicken or turkey	0.65	3	d
1459	58111110	Won ton (wonton), fried, meat filled	1.47	3	d
1460	58112110	Dim sum, meat filled (egg roll-type)	0.92	3	d
1461	58112510	Dumpling, steamed, filled with meat, poultry, or seafood	1.08	3	d
1462	58113110	Dumpling, fried, pork	0.92	3	d
1463	58122310	Knish, potato	1.02	3	d
1464	58125110	Quiche with meat, poultry or fish	0.85	3	d

1465	58126000	Bierock (turnover filled with ground beef and cabbage mixture)	0.65	3	d
1466	58126110	Turnover, meat-filled, no gravy	1.11	3	d
1467	58126120	Turnover, meat-filled, with gravy	1.38	3	d
1468	58126130	Turnover, meat- and cheese-filled, no gravy	1.12	3	d
1469	58126140	Turnover, meat- and bean-filled, no gravy	0.99	3	d
1470	58126170	Turnover, meat-and vegetable- filled (no potatoes, no gravy)	0.89	3	d
1471	58126270	Turnover, chicken- or turkey-, and cheese-filled, no gravy	1.13	3	d
1472	58126280	Turnover, chicken- or turkey-, and vegetable-filled	1.19	3	d
1473	58127210	Croissant sandwich, filled with ham and cheese	1.97	3	d
1474	58127220	Croissant sandwich, filled with chicken, broccoli, and cheese sauce	1.24	3	d
1475	58127270	Croissant sandwich with sausage and egg	1.57	3	d
1476	58127310	Croissant sandwich with ham, egg, and cheese	1.91	3	d
1477	58127330	Croissant sandwich with sausage, egg, and cheese	1.65	3	d
1478	58128000	Biscuit with gravy	1.58	3	d
1479	58128120	Cornmeal dressing with chicken or turkey and vegetables	1.47	3	d
1480	58128220	Dressing with chicken or turkey and vegetables	1.42	3	d
1481	58131100	Ravioli, NS as to filling, no sauce	0.59	3	d
1482	58131310	Ravioli, meat-filled, no sauce	0.49	3	d
1483	58131530	Ravioli, cheese-filled, with meat sauce	1.56	3	d
1484	58133130	Manicotti, cheese-filled, with meat sauce	1.11	3	d
1485	58134130	Stuffed shells, cheese-filled, with meat sauce	0.70	3	d
1486	58134650	Tortellini, meat-filled, no sauce	1.10	3	d
1487	58134720	Tortellini, spinach-filled, no sauce	0.52	3	d
1488	58135110	Chow fun noodles with meat and vegetables	0.76	3	d
1489	58136110	Lo mein, NFS	0.50	3	d

1490	58145130	Macaroni or noodles with cheese and beef	0.77	3	d
1491	58145150	Macaroni or noodles with cheese and pork or ham	1.52	3	d
1492	58146130	Pasta with carbonara sauce	0.66	3	d
1493	58150310	Rice, fried, NFS	1.05	3	d
1494	58160130	Rice with beans and chicken	0.66	3	d
1495	58162110	Stuffed pepper, with rice and meat	0.76	3	d
1496	58163110	Rice with gravy	1.19	3	d
1497	58163130	Dirty rice	0.59	3	d
1498	58163510	Rice dressing	1.34	3	d
1499	58163610	Rice-vegetable medley	1.48	3	d
1500	58301050	Lasagna with cheese and meat sauce (diet frozen meal)	0.63	3	d
1501	58301080	Lasagna with cheese and meat sauce, reduced fat and sodium (diet frozen meal)	0.46	3	d
1502	58302050	Beef and noodles with meat sauce and cheese (diet frozen meal)	0.97	3	d
1503	58304010	Spaghetti and meatballs dinner, NFS (frozen meal)	0.75	3	d
1504	58304030	Spaghetti and meatballs with vegetable, dessert (frozen meal)	0.73	3	d
1505	58304050	Spaghetti with meat and mushroom sauce (diet frozen meal)	1.05	3	d
1506	58304060	Spaghetti with meat sauce (diet frozen meal)	0.89	3	d
1507	58304220	Rigatoni with meat sauce and cheese (diet frozen meal)	0.77	3	d
1508	58305100	Macaroni or noodles, spinach, with chicken and cheese sauce (diet frozen meal)	0.59	3	d
1509	58306800	Noodles and chicken with gravy, vegetable, and dessert (frozen meal)	0.80	3	d
1510	58310310	Pancakes and sausage (frozen meal)	1.79	3	d
1511	71305110	White potato, scalloped, with ham	1.23	3	d
1512	71507100	White potato, stuffed, baked, peel not eaten, stuffed with chicken, broccoli and cheese sauce	0.53	3	d
1513	71508050	White potato, stuffed, baked, peel eaten, stuffed with meat in cream sauce	0.68	3	d

1514	71508120	White potato, stuffed with ham, broccoli and cheese sauce, baked, peel eaten	0.55	3	d
1515	77316600	Eggplant and meat casserole	0.37	3	d
1516	14620330	Pizza topping from meat and vegetable pizza	2.03	4	a
1517	27111500	Beef sloppy joe (no bun)	1.30	4	a
1518	27113100	Beef stroganoff	0.67	4	a
1519	27116200	Beef with barbecue sauce (mixture)	0.61	4	a
1520	27121410	Chili con carne with beans, made with pork	1.20	4	a
1521	27146050	Chicken wing with hot pepper sauce	0.52	4	a
1522	27160010	Meat with barbecue sauce, NS as to type of meat (mixture)	0.60	4	a
1523	27211100	Beef stew with potatoes, tomato-based sauce (mixture)	0.38	4	a
1524	27212350	Beef stroganoff with noodles	0.81	4	a
1525	27510110	Beef barbecue or Sloppy Joe, on bun	1.62	4	a
1526	27510130	Beef barbecue submarine sandwich, on bun	0.94	4	a
1527	27510700	Meatball and spaghetti sauce submarine sandwich, on roll	0.79	4	a
1528	27520500	Pork, barbecue sauce, onions and dill pickles on white roll	1.20	4	a
1529	27520510	Pork barbecue or Sloppy Joe, on bun	1.29	4	a
1530	58100120	Burrito with beef, beans, and cheese	1.04	4	a
1531	58106520	Pizza with meat, thin crust	1.95	4	a
1532	58106530	Pizza with meat, thick crust	1.73	4	a
1533	58106710	Pizza with meat and vegetables, NS as to type of crust	1.64	4	a
1534	58106720	Pizza with meat and vegetables, thin crust	1.64	4	a
1535	58106730	Pizza with meat and vegetables, thick crust	1.53	4	a
1536	58106740	Pizza with meat and fruit, NS as to type of crust	1.56	4	a
1537	58106760	Pizza with meat and fruit, thick crust	1.47	4	a
1538	58106780	Pizza with meat and vegetables, lowfat, thin crust	1.33	4	a
1539	58108050	Pizza rolls	1.93	4	a
1540	58130010	Lasagna with meat and/or poultry	0.81	4	a
1541	58130020	Lasagna with meat and spinach	0.77	4	a

1542	58130150	Lasagna, with chicken or turkey, and spinach	0.45	4	a
1543	58130610	Lasagna with meat, whole wheat noodles	0.81	4	a
1544	58132910	Spaghetti with tomato sauce and chicken or turkey	0.53	4	a
1545	58162090	Stuffed pepper, with meat	0.47	4	a
1546	27212120	Chili con carne with beans and macaroni	1.04	4	c
1547	27416300	Beef taco filling: beef, cheese, tomato, taco sauce	1.18	4	c
1548	58100100	Burrito with beef, no beans	1.22	4	c
1549	58100110	Burrito with beef and beans	0.91	4	c
1550	58100130	Burrito with beef and cheese, no beans	1.37	4	c
1551	58100140	Burrito with beef, beans, cheese, and sour cream	0.88	4	c
1552	58100150	Burrito with beef and potato, no beans	0.65	4	c
1553	58100180	Burrito with pork and beans	0.86	4	c
1554	58100200	Burrito with chicken, no beans	1.00	4	c
1555	58100210	Burrito with chicken and beans	0.86	4	c
1556	58100220	Burrito with chicken, beans, and cheese	1.05	4	c
1557	58100230	Burrito with chicken and cheese	1.23	4	c
1558	58100240	Burrito with chicken, NFS	1.11	4	c
1559	58101300	Taco or tostada with beef, cheese and lettuce	1.50	4	c
1560	58101310	Taco or tostada with beef, lettuce, tomato and salsa	0.67	4	c
1561	58101320	Taco or tostada with beef, cheese, lettuce, tomato and salsa	1.11	4	c
1562	58101350	Soft taco with beef, cheese, lettuce, tomato and sour cream	1.24	4	c
1563	58101400	Soft taco with beef, cheese, and lettuce	1.64	4	c
1564	58101450	Soft taco with chicken, cheese, and lettuce	1.10	4	c
1565	58101510	Taco or tostada with chicken or turkey, lettuce, tomato and salsa	1.01	4	c
1566	58101520	Taco or tostada with chicken, cheese, lettuce, tomato and salsa	1.06	4	c
1567	58101730	Taco or tostada with beans, cheese, meat, lettuce, tomato and salsa	1.20	4	c
1568	58101910	Taco or tostada salad with beef and cheese, corn chips	0.74	4	c

1569	58101930	Taco or tostada salad with beef and cheese, fried flour tortilla	1.01	4	c
1570	58106750	Pizza with meat and fruit, thin crust	1.55	4	c
1571	21407110	Beef, pot roast, braised or boiled, lean and fat eaten	0.16	4	d
1572	21417100	Beef brisket, cooked, NS as to fat eaten	0.16	4	d
1573	21417110	Beef brisket, cooked, lean and fat eaten	0.32	4	d
1574	21417120	Beef brisket, cooked, lean only eaten	0.18	4	d
1575	25210510	Frankfurter or hot dog, low salt	0.80	4	d
1576	25220360	Bratwurst, with cheese	1.64	4	d
1577	25221840	Turkey breakfast sausage, bulk	1.75	4	d
1578	25231150	Corned beef, pressed	2.56	4	d
1579	27111400	Chili con carne, NS as to beans	1.31	4	d
1580	27111410	Chili con carne with beans	1.31	4	d
1581	27111420	Chili con carne without beans	1.72	4	d
1582	27111430	Chili con carne, NS as to beans, with cheese	0.97	4	d
1583	27111440	Chili con carne with beans and cheese	1.11	4	d
1584	27112000	Beef with gravy (mixture)	0.27	4	d
1585	27112010	Salisbury steak with gravy (mixture)	1.01	4	d
1586	27113300	Swedish meatballs with cream or white sauce (mixture)	1.29	4	d
1587	27120120	Sausage gravy	0.75	4	d
1588	27120210	Frankfurter or hot dog, with chili, no bun	1.96	4	d
1589	27141500	Chili con carne with chicken or turkey and beans	1.31	4	d
1590	27142000	Chicken with gravy (mixture)	0.86	4	d
1591	27142200	Turkey with gravy (mixture)	0.84	4	d
1592	27143000	Chicken or turkey with cream sauce (mixture)	0.32	4	d
1593	27160100	Meatballs, NS as to type of meat, with sauce (mixture)	1.64	4	d
1594	27241010	Chicken or turkey and potatoes with gravy (mixture)	1.17	4	d
1595	27242200	Chicken or turkey and noodles with gravy (mixture)	1.15	4	d
1596	27242300	Chicken or turkey and noodles with cream or white sauce (mixture)	0.63	4	d
1597	27242350	Chicken or turkey tetrazzini	0.73	4	d

1598	27246100	Chicken or turkey with dumplings (mixture)	0.96	4	d
1599	27260050	Meatballs, with breading, NS as to type of meat, with gravy	1.07	4	d
1600	27311220	Corned beef, potatoes, and vegetables (excluding carrots, broccoli, and dark-green leafy), no sauce (mixt	0.77	4	d
1601	27311510	Shepherd's pie with beef	0.69	4	d
1602	27317010	Beef pot pie	1.04	4	d
1603	27320020	Ham pot pie	1.55	4	d
1604	27343420	Chicken or turkey, noodles, and vegetables (excluding carrots, broccoli, and dark-green leafy), gravy (mi	1.06	4	d
1605	27347100	Chicken or turkey pot pie	0.66	4	d
1606	27347240	Chicken or turkey, dumplings, and vegetables (including carrots, broccoli, and/or dark green leafy), grav	0.43	4	d
1607	27347250	Chicken or turkey, dumplings, and vegetables (excluding carrots, broccoli, and dark green leafy), gravy (	0.75	4	d
1608	27420040	Frankfurters or hot dogs and sauerkraut (mixture)	1.99	4	d
1609	27443110	Chicken or turkey a la king with vegetables (including carrots, broccoli, and/or dark-green leafy (no pot	0.69	4	d
1610	27443120	Chicken or turkey a la king with vegetables (excluding carrots, broccoli, and dark-green leafy (no potato	1.07	4	d
1611	27520520	Pork sandwich	1.18	4	d
1612	27540330	Turkey sandwich, with gravy	1.14	4	d
1613	27560320	Frankfurter or hot dog, plain, on bun	2.11	4	d
1614	27560360	Frankfurter or hot dog, with chili, on bun	1.88	4	d
1615	41201010	Baked beans, NFS	0.24	4	d
1616	41201040	Baked beans, with pork and sweet sauce	0.85	4	d
1617	41204020	Boston baked beans	0.24	4	d
1618	41205030	Refried beans with meat	1.11	4	d
1619	41206030	Beans and franks	1.09	4	d
1620	41208030	Pork and beans	1.12	4	d
1621	58106510	Pizza with meat, NS as to type of crust	1.95	4	d
1622	58121510	Dumpling, meat-filled	1.33	4	d

1623	58145160	Macaroni or noodles with cheese and frankfurters or hot dogs	1.31	4	d
1624	58145190	Macaroni or noodles with cheese and chicken or turkey	0.64	4	d
1625	71602010	Potato salad, German style	0.64	4	d

Appendix C Foods commonly hot-held

The following list of foods was supplied by US Foodservice, and used to assist selection of foods in CSFII that should be placed in Category 4.

Precooked Bacon
Beef Barbeque
Chicken Barbeque
Pork Barbeque
Turkey Barbeque
Beef Brisket
Beef Burgandy with Mushrooms and Onions
Cream Chipped Beef
Cooked Breaded Beef Fingers
Cooked Breaded Beef Patty Nuggets
Cooked Beef Patty
Beef Pot Roast
Beef Pot Roast with Vegetables
Cooked Beef Prime Rib
Roast Beef
Cooked Shredded Roast Beeg
Cooked Beef Steakexe
Stew Beef
Cooked Beef Steak breaded
Salisbury Beef Steak
Cooked Sirloin Strip Steak
Beef Taco Fillings
Beef Tips
Beef Steak Biscuit
Ham Biscuit
Sausage and Cheese Biscuit
Sausage Biscuit
Beef Burrito
Beef and Bean Burrito
Stuffed Cabbage Rolls
Beef and Bean Chili
Hot Dog Chili
4 Piece Cooked Breaded Chicken
8 Piece Cooked Breaded Chicken
Chicken and Dumplings
Chicken Fettucci with Vegetables
ChIcken Fricasses
Chicken with Mushrooms and Sausage
Chicken Parmigiana
Chicken Primavera
Roasted Chicken with Glaze Sauce
Shredded Chicken with Vegetables and Sauce
Sweet and Sour Chicken
Cooked Buffalo Wings
Cooked Teriyaki Chicken Wings
Corned Beef
Honey Chicken Drummies
Chicken Egg Rolls
Turkey Vegetable Egg Rolls
Beef and Beef Enchalada
Chicken Enchalada
Beef Fajitas
Chicken Fajitas
Chicken Pot Pie Filing
Frank in a Blanket
Sausage Gravy
Sliced Chicken Gyron
Beef Gyro Cone
Beef and Lamb Gyro Cone
Cooked Turkey Meatballs
Sausage Lasagna
Chicken Gyro Cone
Loaf Lamb Gryo
Honey Baked Bavarian Ham
Cooked Ham in Natural Juice
Black Forest Ham
Canned Ham
Black Pepper Ham, Water Added
Maple and Brown Sugar Cooked Ham
Cured Ham
Half Ham
Honey Roasted Ham
Tavern Honey Ham
Precooked Ham Patty
Smoked Pit Ham
Prosciuto Ham
Smoked Ham
Spiral Ham
Ham Steaks
Breakfast Turkey Ham
Smoked Turkey Ham
Virginia Ham
Beef and Cheddar Cheese Hot Pockets
Ham and Cheese Hot Pockets
Jalapeno and Cheese Hot Pockets
Pepperoni Hot Pockets
Pizza Stick Hot Pockets
Classic Lasagna
Meat Lasagna

Meat and Cheese Lasagna
Macaroni and Beef
Meatballs with Sauce
Beef Meatballs
Cooked Italian Meatballs
Swedish Meatballs
Meatloaf
Pastrami Brisket
Cooked Pastrami
Flat Pastrami
Pastrami
Stuffed Peppers
Pepperoni Pizza Pockets
Ham and Cheese Pockets
Roast Beef
Eye of Round Roast Beef
Top Round Roast Beef
Prime Rib Roast Beef
Pork Rib Barbeque Sandwich
Flame Broiled Cheeseburger Sandwich
Ham and Turkey Club Sandwich
Turkey, Bologna and Cheese Sandwich
Beef Stroganoff
Spicy Beef Taco
Pulled Turkey with Gravy
Turkey Tetrazzini
Turkey and Dumplings
Turkey Breast

Appendix D Meat content of servings

The meat content (that could be a source of spores or vegetative cells) of each selected serving in the CSFII was estimated by using the ingredient database component of the CSFII (USDA, 2000). Each serving in the CSFII has an associated food code[104] (see Appendix B) and mass for that serving, and each food code has an associated ingredient list in the CSFII recipe database. The recipe database includes the masses of each ingredient in a recipe, allowing the calculation of the mass fraction of each ingredient associated with each food code, hence the mass of that ingredient in each serving. We classified ingredients as to whether they contain meat products capable of being a source of spores or vegetative cells. Because there is no information within the CSFII database concerning the fraction of meat in the listed ingredients, we assumed that each ingredient that we classified as containing meat is 100% meat. This potentially overestimates the meat content of many ingredients. The ingredients that are associated with the food codes included in the risk assessment (Appendix B) are listed below, sorted by meat classification and then by CSFII ingredient code.

CSFII ingredient code	CSFII ingredient description	Classified as a meat ingredient
4001	FAT,BF TALLOW	yes
4002	FAT,LARD	yes
4542	FAT,CHICKEN	yes
5004	CHICK,WHL,RSTD	yes
5006	CHICK,MEAT&SKIN,RAW	yes
5007	CHICK,MEAT&SKIN,FRIED,BATTER	yes
5008	CHICK,MEAT&SKIN,FRIED,FLR	yes
5009	CHICK,MEAT&SKIN,RSTD	yes
5010	CHICK,MEAT&SKIN,STWD	yes
5011	CHICK,MEAT,RAW	yes
5013	CHICK,MEAT,RSTD	yes
5014	CHICK,MEAT,STWD	yes
5018	CHICK,SKIN,RSTD	yes
5020	CHICK,GIBLETS,RAW	yes
5022	CHICK,GIBLETS,SIMMRD	yes
5031	CHICK,LT MEAT&SKIN,FRIED,FLR	yes
5041	CHICK,LT MEAT,RSTD	yes
5042	CHICK,LT MEAT,STWD	yes
5045	CHICK,DK MEAT,RSTD	yes
5047	CHICK,SEPARABLE FAT,RAW	yes
5058	CHICK,BREAST,MEAT&SKIN,FRIED,BATTER	yes
5060	CHICK,BREAST,MEAT&SKIN,RSTD	yes
5062	CHICK,BREAST MEAT,RAW	yes
5063	CHICK,BREAST MEAT,FRIED	yes

[104] We used only the FOODCODE entry of record type rt30 in CSFII. The MODTYPE code was ignored. The documented recipe modifications should have a negligible effect on estimated meat fractions.

5064	CHICK,BREAST MEAT,RSTD	yes
5065	CHICK,BREAST MEAT,STWD	yes
5078	CHICK,LEG,MEAT&SKIN,RSTD	yes
5103	CHICK,WING,MEAT&SKIN,RSTD	yes
5118	CHICK,ROASTING,LT MEAT,RSTD	yes
5122	CHICK,STEWING,WHL,STWD	yes
5166	TURKEY,MEAT&SKIN,RSTD	yes
5168	TURKEY,MEAT ONLY,RSTD	yes
5174	TURKEY,GIZZARD,SIMMRD	yes
5182	TURKEY,LT MEAT&SKIN,RSTD	yes
5186	TURKEY,LT MEAT,RSTD	yes
5200	TURKEY,FRYER-ROASTERS,MEAT&SKIN,RSTD	yes
5220	TURKEY,BREAST,MEAT,RSTD	yes
5277	CHICK,CND,BONED,W/BROTH	yes
5296	TURKEY ROAST,BNLESS,FRZ,LT&DK MEAT,RSTD	yes
5306	TURKEY,GROUND,CKD	yes
6075	SOUP,BF BROTH/BOUILLON,PDR,DRY	yes
6076	SOUP,BF BROTH,CUBED,DRY	yes
6116	GRAVY,BF,CND	yes
6119	GRAVY,CHICK,CND	yes
6125	GRAVY,TURKEY,CND	yes
6475	SOUP,BF BROTH/BOUILLON,PDR,PREP W/H2O	yes
6480	SOUP,CHICK BROTH,DEHYD,PREP W/H2O	yes
6524	GRAVY,PORK,DEHYD,PREP W/H2O	yes
7007	BOLOGNA,BF	yes
7008	BOLOGNA,BF&PORK	yes
7011	BOLOGNA,TURKEY	yes
7013	BRATWURST	yes
7014	BRAUNSCHWEIGER,PORK	yes
7016	CHEESEFURTER,BF&PORK	yes
7017	CHICK ROLL,LT MEAT	yes
7021	DUTCH BRAND LOAF,BF&PORK	yes
7022	FRANKFURTER,BF	yes
7023	FRANKFURTER,BF&PORK	yes
7024	FRANKFURTER,CHICK	yes
7029	HAM,SLICED,11% FAT,REG	yes
7031	HAM SALAD SPRD	yes
7034	HEADCHEESE,PORK	yes
7037	METTWURST	yes
7038	KNACKWURST,KNOCKWURST,PORK,BF	yes
7043	LUNCH MEAT,BF,THIN SLICED	yes
7050	MORTADELLA,BF,PORK	yes
7052	PASTRAMI,TURKEY	yes
7056	PEPPERED LOAF,PORK,BF	yes
7057	PEPPERONI,PORK,BF	yes
7064	SAUSAGE,PORK,FRESH,CKD	yes

7065	SAUSAGE,PORK&BF,FRESH,CKD	yes
7067	POULTRY SALAD SANDWICH SPRD	yes
7068	SALAMI,CKD,BF	yes
7069	SALAMI,CKD,BF&PORK	yes
7070	SALAMI,CKD,TURKEY	yes
7074	SMOKED LINK SAUSAGE,PORK	yes
7075	SMOKED LINK SAUSAGE,PORK&BF	yes
7076	SMOKED LINK SAUSAGE,PORK &BF,W/FLOUR & NFDM	yes
7079	TURKEY BREAST MEAT	yes
7080	TURKEY HAM,CURED THIGH MEAT	yes
7081	TURKEY ROLL,LT MEAT	yes
7082	TURKEY ROLL,LT & DK MEAT	yes
7089	SAUSAGE,ITALIAN,CKD,PORK	yes
7905	FRANKFURTER,BF,PORK,&TURKEY,FAT FREE	yes
10002	PORK,FRSH,COMP,LN,RAW	yes
10003	PORK,FRSH,COMP,LN&FAT,RAW	yes
10011	PORK,FRSH,LEG,WHL,LN,RSTD	yes
10020	PORK,FRSH,LOIN,WHL,LN&FAT,RAW	yes
10021	PORK,FRSH,LOIN,WHL,LN&FAT,BRSD	yes
10022	PORK,FRSH,LOIN,WHL,LN&FAT,BRLD	yes
10023	PORK,FRSH,LOIN,WHL,LN&FAT,RSTD	yes
10024	PORK,FRSH,LOIN,WHL,LN,RAW	yes
10025	PORK,FRSH,LOIN,WHL,LN,BRSD	yes
10027	PORK,FRSH,LOIN,WHL,LN,RSTD	yes
10036	PORK,FRSH,CNTR LOIN,LN&FAT,RAW	yes
10060	PORK,FRSH,TENDERLOIN,LN,RAW	yes
10078	PORK,FRSH,ARM PICNIC,LN,BRSD	yes
10085	PORK,FRSH,BLADE,BOSTON,LN,BRSD	yes
10086	PORK,FRSH,BLADE,BOSTON,LN,BRLD	yes
10093	PORK,FRSH,COMPOSITE,LN,CKD	yes
10124	PORK,CURED,BACON,BRLD/PAN-FRIED/RSTD	yes
10134	PORK,CURED,HAM,BNLESS,EX LN,RSTD	yes
10136	PORK,CURED,HAM,BNLESS,REG,RSTD	yes
10141	PORK,CNTR SLICE,COUNTRY-STYLE,LN,RAW	yes
10147	Pork roll,cured,fried	yes
10151	PORK,CURED,HAM,WHL,LN&FAT,RSTD	yes
10152	PORK,CURED,HAM,WHL,LN,UNHTD	yes
10153	PORK,CURED,HAM,WHL,LN,RSTD	yes
10165	PORK,CURED,SALT PORK,RAW	yes
10182	PORK,CURED,HAM,BNLESS,UNHTD	yes
10183	PORK,CURED,HAM,BNLESS,RSTD	yes
10184	PORK,CURED,HAM,CND,UNHTD	yes
10185	PORK,CURED,HAM,CND,RSTD	yes
10220	PORK,FRSH,GROUND,CKD	yes
10226	PORK,FRSH,COMP LOIN&SHOULDER,LN&FAT,RAW	yes

10227	PORK,FRSH,COMP LOIN&SHOULDER,LN&FAT,CKD	yes
13020	BF,RETAIL CUTS,FAT,CKD	yes
13022	BF,BRISKET,WHL,LN+FT,1/4",ALL,BRSD	yes
13024	BF,BRISKET,WHL,LN,1/4",ALL,BRSD	yes
13034	BF,ARM POT RST,LN+FT,1/4",ALL,BRSD	yes
13036	BF,ARM POT RST,LN+FT,1/4",CHOIC,BRSD	yes
13038	BF,ARM POT RST,LN+FT,1/4",SEL,BRSD	yes
13043	BF,ARM POT RST,LN,CHOIC,RAW	yes
13044	BF,ARM POT RST,LN,1/4",CHOIC,BRSD	yes
13046	BF,ARM POT RST,LN,1/4",SEL,BRSD	yes
13050	BF,BLADE RST,LN+FT,1/4",ALL,BRSD	yes
13058	BF,BLADE RST,LN,1/4",ALL,BRSD	yes
13061	BF,BLADE RST,LN,SEL,RAW	yes
13062	BF,BLADE RST,LN,1/4",SEL,BRSD	yes
13065	BF,FLANK,LN+FT,CHOIC,0",RAW	yes
13068	BF,FLANK,LN,ALL,RAW	yes
13088	BF,RIB,WHL,LN,1/4",CHOIC,RSTD	yes
13143	BF,RIB,SML END,LN,1/4",SEL,RSTD	yes
13150	BF,SHORTRIBS,LN,CHOIC,BRSD	yes
13151	BF,RND,FULL,LN+FT,1/4",CHOIC,RAW	yes
13152	BF,RND,FULL,LN+FT,1/4",CHOIC,BRLD	yes
13155	BF,RND,FULL,LN,CHOIC,RAW	yes
13156	BF,RND,FULL,LN,1/4",CHOIC,BRLD	yes
13160	BF,BTTM RND,LN+FT,1/4",ALL,BRSD	yes
13162	BF,BTTM RND,LN+FT,1/4",CHOIC,BRSD	yes
13168	BF,BTTM RND,LN,1/4",ALL,BRSD	yes
13194	BF,TIP RND,LN+FT,1/4",CHOIC,RSTD	yes
13202	BF,TIP RND,LN,1/4",CHOIC,RSTD	yes
13204	BF,TIP RND,LN,1/4",SEL,RSTD	yes
13281	BF,TOP SIRLOIN,LN+FT,1/4",CHOIC,PAN-FRIED	yes
13288	BF,TOP SIRLOIN,LN,CHOIC,RAW	yes
13289	BF,TOP SIRLOIN,LN,1/4",CHOIC,BRLD	yes
13291	BF,TOP SIRLOIN,SEL,LN,RAW	yes
13292	BF,TOP SIRLOIN,SEL,LN,1/4",BRLD	yes
13295	BF,GROUND,EX LN,RAW	yes
13298	BF,GROUND,EX LN,BRLD,MED	yes
13299	BF,GROUND,EX LN,BRLD,WELL DONE	yes
13302	BF,GROUND,LN,RAW	yes
13306	BF,GROUND,LN,BRLD,WELL DONE	yes
13312	BF,GROUND,REG,BRLD,MED	yes
13313	BF,GROUND,REG,BRLD,WELL DONE	yes
13314	BF,GROUND,REG,PAN-FRIED,MED	yes
13347	BF,CURED,CORNED,BRISKET,CKD	yes
13348	BF,CURED,CORNED,BRISKET,CND	yes
13367	BF,BRISKET,WHL,LN+FT,0",ALL,BRSD	yes
13368	BF,BRISKET,WHL,LN,0",ALL,BRSD	yes

13373	BF,ARM POT RST,LN+FT,0",ALL,BRSD	yes
13376	BF,ARM POT RST,LN,0",ALL,BRSD	yes
13379	BF,BLADE RST,LN+FT,0",ALL,BRSD	yes
13398	BF,BTTM RND,LN+FT,0",ALL,BRSD	yes
13454	BF,TOP SIRLOIN,ALL,LN,0",BRLD	yes
16008	BNS,BKD,CND,W/FRANKS	yes
16010	BNS,BKD,CND,W/PORK&SWT SAU	yes
16011	BNS,BKD,CND,W/PORK&TOMATO SAU	yes
17042	LAMB,US,SHOULDER,WHL,LN,CHOIC,RSTD	yes
17089	VEAL,LN&FAT,CKD	yes
17104	VEAL,LOIN,LN&FAT,RAW	yes
17117	VEAL,SHOULDER,WHL,LN&FAT,BRSD	yes
17134	VEAL,SIRLOIN,LN&FAT,RAW	yes
17136	VEAL,SIRLOIN,LN&FAT,RSTD	yes
21004	BISCUIT W/EGG & HAM	yes
21005	BISCUIT W/EGG & SAUSAGE	yes
21008	BISCUIT W/HAM	yes
21009	BISCUIT,W/SAUSAGE	yes
21020	ENGLISH MUFFIN W/CHS & SAUSAGE	yes
21037	CHICK,BREADED,FRIED,BNLESS	yes
22401	HEALTHY CHOIC SPAGHETTI BOLOGNESE,FRZ ENTREE	yes
22402	HEALTHY CHOIC BF MACARONI,FRZ ENTREE	yes
42004	CHICK,BREAST,MEAT,FRIED W/O ABSORB FAT	yes
42128	TURKEY,HAM,EX LN,PREPACK/DELI	yes
42129	BOLOGNA,BF&PORK,LO FAT	yes
42161	BOLOGNA,BF,LO FAT	yes
42179	FRANKFURTER,BF,LO FAT	yes
42241	SAUSAGE,TURKEY,PORK&BF,RED FAT,SMOKED	yes
42262	CHICK &BF SAUSAGE,SMOKED	yes
42280	FRANKFURTER,MEAT& POULTRY,LOFAT	yes
43325	HAM,SMOKED/CURED,LO NA,CKD,NS FAT	yes
43378	BACON,SMOKED/CURED,RED NA	yes
43384	BOLOGNA,BF,RED NA	yes
43507	FRANKFURTER,LO SALT	yes
73790	BF,CORNED BF HASH,CND,W/POTATO	yes
21540100	GROUND BEEF W/ TEXTURED VEGETABLE PROTEIN, COOKED	yes
24198740	CHICKEN NUGGETS	yes
25220710	CHORIZOS	yes
27111400	CHILI CON CARNE, NS AS TO BEANS	yes
27111410	CHILI CON CARNE W/ BEANS	yes
27112000	BEEF W/ GRAVY (MIXTURE) (INCLUDE COUNTRY STYLE)	yes
27112010	SALISBURY STEAK W/ GRAVY (MIXTURE)	yes
27113100	BEEF STROGANOFF	yes

27116200	BEEF W/ BARBECUE SAUCE (MIXTURE)	yes
27120020	HAM/PORK W/ GRAVY (MIXTURE)	yes
27135110	VEAL PARMIGIANA	yes
27260010	MEATLOAF, NS AS TO TYPE OF MEAT	yes
27443120	CHICKEN A LA KING W/ VEG(NO CAR/DK GRN),WHITE SAUCE	yes
58104500	CHIMICHANGA W/ BEEF, BEANS, LETTUCE AND TOMATO	yes
58104530	CHIMICHANGA W/ CHICKEN & CHEESE	yes
58112510	DUMPLING, STEAMED, FILLED W/ MEAT OR SEAFOOD	yes
1001	BUTTER,W/SALT	no
1009	CHEESE,CHEDDAR,AMERICAN	no
1012	CHEESE,COTTAGE,CRMD	no
1014	CHEESE,COTTAGE,NONFAT,UNCRMD,DRY,LRG OR SML CURD	no
1016	CHEESE,COTTAGE,LOWFAT,1% MILKFAT	no
1025	CHEESE,MONTEREY	no
1026	CHEESE,MOZZARELLA,WHL	no
1027	CHEESE,MOZZARELLA,WHL,LO MOIST	no
1028	CHEESE,MOZZARELLA,PART SKIM	no
1029	CHEESE,MOZZARELLA,PART SKIM,LO MOIST	no
1032	CHEESE,PARMESAN,GRATED	no
1033	CHEESE,PARMESAN,PIECE	no
1035	CHEESE,PROVOLONE	no
1036	CHEESE,RICOTTA,WHL	no
1037	CHEESE,RICOTTA,PART SKIM	no
1038	CHEESE,ROMANO	no
1040	CHEESE,SWISS	no
1042	CHEESE,PAST PROC,AMERICAN	no
1044	CHEESE,PAST PROC,SWISS	no
1046	CHEESE FOOD,PAST PROC,AMERICAN	no
1048	CHEESE SAUCE	no
1049	HALF&HALF,CRM&MILK	no
1050	CREAM,FLUID,LT (COFFEE CRM OR TABLE CRM)	no
1053	CREAM,HVY WHIPPING	no
1056	SOUR CREAM	no
1077	MILK,FLUID,3.25% MILKFAT	no
1085	MILK,NONFAT,FLUID,W/ VIT A (FAT FREE OR SKIM)	no
1088	MILK,BTTRMLK,FLUID,CULTURED,LOWFAT	no
1090	MILK,DRY,WHL	no
1091	MILK,DRY,NONFAT,REG,WO/ VIT A	no
1092	MILK,DRY,NONFAT,INST,W/ VIT A	no
1094	BTTRMLK,DRIED,SWT CRM	no
1113	WHEY,ACID,DRIED	no
1115	WHEY,SWEET,DRIED	no

1123	EGGS,CHICK,WHL,RAW/FRZ	no
1124	EGGS,CHICK,WHITE,RAW/FRZ	no
1125	EGGS,CHICK,YOLK,RAW	no
1128	EGGS,CHICK,WHL,FRIED	no
1129	EGGS,CHICK,WHL,HARD-BLD	no
1131	EGGS,CHICK,WHL,POACHED	no
1132	EGGS,CHICK,WHL,SCRMBLD	no
1154	MILK,DRY,NONFAT,REG,W/ VIT A	no
1168	CHEESE,CHEDDAR,LOFAT	no
2001	ALLSPICE,GROUND	no
2002	ANISE SEED	no
2003	BASIL,GROUND	no
2009	CHILI PDR	no
2010	CINNAMON,GROUND	no
2011	CLOVES,GROUND	no
2014	CUMIN SEED	no
2015	CURRY PDR	no
2020	GARLIC PDR	no
2021	GINGER,GROUND	no
2024	MUSTARD SEED,YEL	no
2025	NUTMEG,GROUND	no
2026	ONION PDR	no
2027	OREGANO,GROUND	no
2028	PAPRIKA	no
2029	PARSLEY,DRIED	no
2030	PEPPER,BLACK	no
2031	PEPPER,RED/CAYENNE	no
2034	POULTRY SEASONING	no
2038	SAGE,GROUND	no
2042	THYME,GROUND	no
2046	MUSTARD,PREP,YEL	no
2047	SALT,TABLE	no
2048	VINEGAR,CIDER	no
2053	VINEGAR,DISTILLED	no
2054	CAPERS,CND,DRND	no
4017	SALAD DRSNG,THOUSAND ISLAND,COMM,REG	no
4018	SALAD DRSNG,MAYO TYPE,REG	no
4025	SALAD DRSNG,MAYO,SOYBN	no
4027	honey mustard sauce	no
4031	SHORTENING,HOUSEHOLD,SOYBN,CTTNSD,HYDR	no
4034	OIL,SOYBN,HYDR	no
4042	OIL,PNUT	no
4044	OIL,SOYBN	no
4053	OIL,OLIVE	no
4058	OIL,SESAME	no
4105	MARGARINE,LIQ,SOYBN(HYDR&REG)&CTTNSD	no

4114	SALAD DRSNG,ITALIAN,COMM,REG	no
4120	SALAD DRSNG,FRENCH,COMM,REG	no
4131	MARGARINE,REG,UNSPEC OILS,WO/SALT	no
4132	MARGARINE,REG,UNSPEC OILS,W/SALT	no
4502	OIL,COTTONSEED	no
4518	OIL,CORN	no
4521	MARGARINE,REG,SUNFLOWER,SOYBN&CTTNSD(HYDR)	no
4531	OIL,SOYBN LECITHIN	no
4543	OIL,SOYBN,HYDR&CTTNSD	no
4610	MARGARINE,REG,STICK,COMP,80%FAT	no
4615	SHORTENING,HOUSEHOLD,COMP	no
4616	SHORTENING,INSTITUTIONAL,COMP	no
6008	SOUP,BF BROTH OR BOUILLON CND,RTS	no
6013	SOUP,CHICK BROTH,COND,COMM	no
6016	SOUP,CRM OF CHICK,COND,COMM	no
6043	SOUP,CRM OF MUSHROOM,COND,COMM	no
6134	SAUCE,SOY	no
6150	SAUCE,BARBECUE	no
6164	SALSA,COMMERCIAL	no
6165	SAUCE,HOME-PREP,WHITE,THIN	no
6166	SAUCE,HOME-PREP,WHITE,MED	no
6303	SAUCE,CHEESE,DEHYD,PREP W/MILK	no
6313	SAUCE,WHITE,DEHYD,PREP W/MILK	no
6413	SOUP,CHICK BROTH,PREP W/H2O,COMM	no
6555	SAUCE,HOLLANDAISE,DEHYD,PREP W/H2O	no
6931	SAUCE,PASTA,SPAGHETTI/MARINARA,RTS	no
8120	CEREAL,OATS,WO/FORT,DRY	no
9005	APPLES,RAW,WO/SKIN,BLD	no
9006	APPLES,RAW,WO/SKIN,MICROWAVE	no
9007	APPLES,CND,SWTND,DRND	no
9009	APPLS,DEHYD,SULFURED	no
9016	APPL JUC,CND,UNSWTND,WO/+VIT C	no
9019	APPLSAUC,CND,UNSWTND,WO/+VIT C	no
9020	APPLSAUC,CND,SWTND,WO/SALT	no
9036	APRICOT NECTAR,CND,WO/+VIT C	no
9037	AVOCADOS,RAW,ALL VAR	no
9063	CHERRIES,SOUR,RED,RAW	no
9066	CHERRIES,SOUR,RED,CND,HVY SYRUP	no
9071	CHERRIES,SWT,CND,H2O PK	no
9072	CHERRIES,SWT,CND,JUC PK	no
9078	CRANBERRIES,RAW	no
9150	LEMONS,RAW,WO/PEEL	no
9152	LEMON JUC,RAW	no
9153	LEMON JUC,CND/BTLD	no
9156	LEMON PEEL,RAW	no

9193	OLIVES,RIPE,CND(SML-EX LRG)	no
9206	ORANGE JUC,RAW	no
9214	ORANGE JUC,FRZ,UNSWTND,UNDIL	no
9215	ORANGE JUC,FRZ,UNSWTND,DIL	no
9216	ORANGE PEEL,RAW	no
9232	PASSION-FRUIT JUC,PURPLE,RAW	no
9237	PEACHES,CND,H2O PK	no
9238	PEACHES,CND,JUC PK	no
9266	PNAPPL,RAW	no
9267	PNAPPL,CND,H2O PK	no
9268	PNAPPL,CND,JUC PK	no
9270	PNAPPL,CND,HVY SYRUP	no
9273	PNAPPL JUC,CND,UNSWTND	no
9279	PLUMS,RAW	no
9298	RAISINS,SEEDLESS	no
9299	RAISINS,SEEDED	no
9354	PNAPPL,CND,JUC PK,DRND	no
11026	BAMBOO SHOOTS,RAW	no
11028	BAMBOO SHOOTS,CND,DRND	no
11032	BNS,LIMA,IMMAT,BLD,DRND	no
11037	BNS,LIMA,IMMAT,FORDHOOK,FRZ	no
11038	BNS,LIMA,IMMAT,FORDHOOK,FRZ,BLD,DRND	no
11043	BNS,MUNG,MATURE,SPROUTED,RAW	no
11044	BNS,MUNG,MATURE,SPROUTED,BLD,DRND	no
11052	BNS,SNAP,GRN,RAW	no
11053	BNS,SNAP,GRN,BLD,DRND	no
11061	BNS,SNAP,GRN,FRZ,BLD,DRND	no
11090	BROCCOLI,RAW	no
11091	BROCCOLI,BLD,DRND	no
11092	BROCCOLI,FRZ,CHOPD	no
11093	BROCCOLI,FRZ,CHOPD,BLD,DRND	no
11095	BROCCOLI,FRZ,SPEARS,BLD,DRND	no
11109	CABBAGE,RAW	no
11110	CABBAGE,BLD,DRND	no
11112	CABBAGE,RED,RAW	no
11116	CABBAGE,PAK-CHOI,RAW	no
11117	CABBAGE,PAK-CHOI,BLD,DRND	no
11119	CABBAGE,PE-TSAI,RAW	no
11124	CARROTS,RAW	no
11125	CARROTS,BLD,DRND	no
11130	CARROTS,FRZ	no
11131	CARROTS,FRZ,BLD,DRND	no
11136	CAULIFLOWER,BLD,DRND	no
11138	CAULIFLOWER,FRZ,BLD,DRND	no
11143	CELERY,RAW	no
11144	CELERY,BLD,DRND	no

11156	CHIVES,RAW	no
11162	COLLARDS,BLD,DRND	no
11165	CORIANDER,RAW	no
11168	CORN,SWT,YEL,BLD,DRND	no
11172	CORN,SWT,YEL,CND,BRINE,DRND	no
11174	CORN,SWT,YEL,CND,CRM,REG PK	no
11178	CORN,SWT,YEL,FRZ,KRNLS	no
11179	CORN,SWT,YEL,FRZ,KRNLS,BLD,DRND	no
11205	CUCUMBER,RAW	no
11209	EGGPLANT,RAW	no
11215	GARLIC,RAW	no
11216	GINGER ROOT,RAW	no
11234	KALE,BLD,DRND	no
11246	LEEKS,RAW	no
11252	LETTUCE,ICEBERG,RAW	no
11260	MUSHROOMS,RAW	no
11261	MUSHROOMS,BLD,DRND	no
11264	MUSHROOMS,CND,DRND	no
11269	MUSHROOMS,SHIITAKE,CKD	no
11282	ONIONS,RAW	no
11283	ONIONS,BLD,DRND	no
11284	ONIONS,DEHYD FLAKES	no
11288	ONIONS,FRZ,CHOPD,BLD,DRND	no
11291	ONIONS,SPRING OR SCALLIONS (INCL TOPS&BULB),RAW	no
11297	PARSLEY,RAW	no
11300	PEAS,EDIBLE-PODDED,RAW	no
11301	PEAS,EDIBLE-PODDED,BLD,DRND	no
11304	PEAS,GRN,RAW	no
11305	PEAS,GRN,BLD,DRND	no
11308	PEAS,GRN,CND,REG,DRND	no
11312	PEAS,GRN,FRZ	no
11313	PEAS,GRN,FRZ,BLD,DRND	no
11327	PEAS&ONIONS,FRZ,BLD,DRND	no
11329	PEPPERS,HOT CHILI,GRN,CND	no
11333	PEPPERS,SWT,GRN,RAW	no
11334	PEPPERS,SWT,GRN,BLD,DRND	no
11352	POTATOES,RAW,FLESH	no
11363	POTATOES,BKD,FLESH	no
11365	POTATOES,BLD,CKD W/SKIN,FLESH	no
11367	POTATOES,BLD,CKD WO/SKIN,FLESH	no
11371	POTATO,MSHD,HOMEMADE W/MILK&MARGARINE	no
11378	POTATOES,MSHD,DEHYD,FLAKES WO/MILK	no
11379	POTATO,MSHD,FLAKES,PREP W/MILK&BUTTER	no
11391	POTATOES,HASH BROWN,FRZ,PREP	no
11403	POTATO,FRZ,FRENCH-FR,PART-FRIED,OVEN HTD	no

11429	RADISHES,RAW	no
11439	SAUERKRAUT,DRAINED	no
11457	SPINACH,RAW	no
11458	SPINACH,BLD,DRND	no
11468	SQUASH,SMMR,CROOK&STR NECK,BLD,DRND	no
11478	SQUASH,SMMR,ZUCCHINI,BLD,DRND	no
11529	TOMATOES,RED,RIPE,RAW	no
11530	TOMATOES,RED,RIPE,BLD	no
11531	TOMATOES,RED,CND,WHL,REG PK	no
11540	TOMATO JUC,CND,W/SALT	no
11546	TOMATO PASTE,CND	no
11547	TOMATO PUREE,CND	no
11549	TOMATO SAUCE,CND	no
11584	VEG,MXD,FRZ,BLD,DRND	no
11588	WATERCHESTNUTS,CHINESE,RAW	no
11590	WATERCHESTNUTS,CHINESE,CND	no
11642	SQUASH,SMMR,ALL VAR,BLD,DRND	no
11660	TOMATOES,RED,STWD	no
11670	PEPPERS,HOT CHILI,GRN,RAW	no
11674	POTATOES,BKD,FLESH&SKIN	no
11718	BNS,MUNG SPROUT,BLD,DRND,W/SALT	no
11724	BNS,SNAP,YEL,BLD,DRND	no
11820	PEPPERS,HOT CHILI,RED,CND	no
11821	PEPPERS,SWT,RED,RAW	no
11823	PEPPERS,SWT,RED,BLD,DRND	no
11831	POTATOES,BLD W/SKIN,FLESH,W/SALT	no
11833	POTATOES,BLD WO/SKIN,FLESH,W/SALT	no
11887	TOMATO PASTE,CND,W/SALT	no
11888	TOMATO PUREE,CND,W/SALT	no
11935	CATSUP	no
11937	PICKLES,CUCUMBER,DILL	no
11940	PICKLE,CUCUMBER,SWEET	no
11941	PICKLE,CUCUMBER,SOUR	no
11943	PIMIENTO,CND	no
11945	PICKLE RELISH,SWEET	no
11962	PEPPERS,HOT CHILI,SUN-DRIED	no
11979	PEPPERS,JALAPENO,RAW	no
12014	PUMPKIN&SQUASH SD KRNLS,DRIED	no
12061	ALMONDS,DRIED,UNBLANCHED	no
12062	ALMONDS,DRIED,BLANCHED	no
12063	ALMONDS,DRY RSTD	no
12067	ALMONDS,TSTD	no
12085	CASHEW NUTS,DRY RSTD	no
12201	SESAME SD KERNELS,DRIED	no
14057	WINE,DSSRT,SWEET	no
14175	CHOC FLAV BEV MIX	no

14429	WATER,MUNICIPAL	no
15002	ANCHOVY,EUROPEAN,CND,OIL,DRND	no
15149	SHRIMP,MXD SP,RAW	no
15152	SHRIMP,MXD SP,CND	no
16033	BNS,KIDNEY,RED,MATURE,BLD	no
16034	BNS,KIDNEY,RED,MATURE,CND	no
16049	BNS,WHITE,MATURE,RAW	no
16050	BNS,WHITE,MATURE,BLD	no
16059	CHILI W/BNS,CND	no
16080	BNS,MUNG,MATURE,RAW	no
16103	REFRIED BEANS,CANNED (INCL USDA COMMODITY)	no
16115	SOY FLR,FULL-FAT,RAW	no
16117	SOY FLR,DEFATTED	no
16118	SOY FLR,LO FAT	no
16122	SOY PROT ISOLATE	no
16123	SOY SAUCE,FROM SOY&WHEAT (SHOYU)	no
16124	SOY SAUCE,FROM SOY (TAMARI)	no
16125	SOY SAUCE,FROM HYDROLYZED VEG PROT	no
16127	TOFU,SOFT,PREP W/CA SULFATE&MAGNESIUM CHLORIDE (NIGARI)	no
16390	PNUTS,ALL TYPES,DRY-RSTD	no
18009	BISCUITS,PLN/BTTRMLK,COMM BKD	no
18060	BREAD,RYE	no
18069	BREAD,WHITE,COMM PREP(INCL SOFT BREAD CRUMBS)	no
18070	BREAD,WHITE,COMM PREP,TSTD	no
18075	BREAD,WHL-WHEAT,COMM PREP	no
18079	BREAD CRUMBS,DRY,GRATED,PLN	no
18081	BREAD STUFFING,DRY MIX	no
18173	GRAHAM CRACKERS,PLN/HONEY/CINN	no
18229	CRACKERS,STD SNACK-TYPE,REG	no
18239	CROISSANTS,BUTTER	no
18243	CROUTONS,SEASONED	no
18259	ENG MUFFINS,PLN,TSTD,ENR(INCL SOURDOUGH)	no
18335	PIE CRUST,STD-TYPE,FRZ,RTB,BKD	no
18350	ROLLS,HAMBURGER/HOTDOG,PLN	no
18360	TACO SHELLS,BKD	no
18363	TORTILLAS,RTB/RTF,CORN	no
18364	TORTILLAS,RTB/RTF,FLOUR	no
18369	BAKING PDR,DOUBLE-ACTING,NaAlSO4	no
18370	BAKING PDR,DOUBLE-ACTING,PHOSPHATE	no
18372	BAKING SODA	no
18374	YEAST,BAKER'S,COMPRESSED	no
18375	YEAST,BAKER'S,ACTIVE DRY	no
19003	CORN CHIPS,PLAIN	no

19056	TORTILLA CHIPS,PLAIN	no
19078	CANDIES,BAKING CHOC,UNSWTND	no
19177	GELATINS,DRY,UNSWTND	no
19296	HONEY,STR/EXTRACTED	no
19304	MOLASSES	no
19334	SUGARS,BROWN	no
19335	SUGARS,GRANULATED	no
19350	SYRUP,CORN,LT	no
19719	JAMS&PRESERVES,APRICOT	no
20005	BARLEY,PEARLED,RAW	no
20016	CORN FLR,WHL,YEL	no
20017	CORN FLR,MASA,ENR	no
20022	CORNMEAL,DEGERMED,ENR,YEL	no
20027	CORNSTARCH	no
20037	RICE,BROWN,LONG,CKD	no
20044	RICE,WHITE,LONG,REG,RAW,ENR	no
20045	RICE,WHITE,LONG,REG,CKD,ENR	no
20047	RICE,WHITE,LONG,PARBLD,CKD,ENR	no
20048	RICE,WHITE,LONG,PRECKD/INST,ENR,DRY	no
20061	RICE FLR,WHITE	no
20081	WHEAT FLR,WHITE,ALLPURP,ENR,BLEACH	no
20088	WILD RICE,RAW	no
20099	MACARONI,DRY,ENR	no
20100	MACARONI,CKD,ENR	no
20108	MACARONI,WHL-WHEAT,CKD	no
20110	NOODLES,EGG,CKD,ENR	no
20112	NOODLES,EGG,SPINACH,CKD,ENR	no
20113	NOODLES,CHINESE,CHOW MEIN	no
20121	SPAGHETTI,ENR,CKD,WO/SALT	no
20345	RICE,WHITE,LONG,ENR,CKD,W/SALT	no
20400	MACARONI,CKD,UNENR	no
20410	NOODLES,EGG,UNENR,CKD,WO/SALT	no
20445	RICE,WHITE,LONG,UNENR,CKD,WO/SALT	no
20481	WHEAT FLR,WHITE,ALLPURP,UNENR	no
21018	FAST FD,EGG, SCRMBLD	no
21138	POTATO,FRENCH FRIED,IN VEG OIL	no
42011	BREADING FOR BAKED/FRIED CHICK	no
42061	WINE,NON-ALCOHOLIC	no
42213	TABLE WINE,ALL,BKD/SIMMRD 1-59MIN	no
42214	TABLE WINE,ALL,BKD/SIMMRD 2HR-2HR29MIN	no
42215	TABLE WINE,ALL,BKD/SIMMRD 1HR-1HR29MIN	no
42216	TABLE WINE,ALL,STIRRED INTO HOT LIQ	no
42218	WINE,DSSRT,DRY,STIRRED INTO HOT LIQ	no
42219	WINE,DSSRT,DRY,BKD/SIMMRD 1-29 MIN	no
42221	WINE,DSSRT,DRY,BKD/SIMMRD 46-60MIN	no
43212	BACON BITS,MEATLESS	no

43216	FRUCTOSE SWEETENER	no
43374	SAUCE,WORCESTERSHIRE	no
44005	OIL,CORN,PEANUT&OLIVE	no
44051	RICE MIX,W/OR W/O VERMICELLI&OTHER PASTA,DRY VEG,ENR	no
78862	CORNMEAL,YEL,CKD,DEGERMED,ENR,WO/SALT	no
84060	OLIVES,PICKLED,CND/BTLD,GRN	no
85390	PEPPERS,HOT,CHILI,GRN,CND,CHILI SAUCE	no
85420	PEPPERS,HOT,CHILI,RED,CND,CHILI SAUCE	no
92320	SUGARS,DEXTROSE,ANHYDROUS	no
92330	SUGARS,DEXTROSE,CRYSTAL	no
92871	TACO SAUCE	no
92872	SAUCE,TOMATO CHILI,BTLD,WO/SALT	no
11100000	MILK, NFS	no
11112000	MILK, COW'S, FLUID, NOT WHOLE, NS AS TO % FAT	no
14410200	CHEESE, PROCESSED, AMERICAN/CHEDDAR TYPE	no
41205010	REFRIED BEANS	no
41205100	fermented black beans	no
51109100	BREAD, PITA	no
51150000	ROLL, WHITE, SOFT	no
51157000	ROLL, HOAGIE, SUBMARINE,	no
51182010	BREAD, STUFFING (INCLUDE HOMEMADE; STUFFING, NFS)	no
51186010	MUFFIN, ENGLISH (INCLUDE SOUR DOUGH)	no
51300110	BREAD, WHOLE WHEAT, OTHER THAN 100%/NS AS TO 100%	no
51502100	ROLL, OAT BRAN	no
51620000	ROLL, MULTIGRAIN	no
52202060	CORNBREAD, HOMEMADE	no
52215100	TORTILLA, CORN	no
52215200	TORTILLA, FLOUR (WHEAT)	no
53204010	COOKIE, BROWNIE, W/O ICING	no
53410100	COBBLER, APPLE (INCLUDE FRUIT COBBLER)	no
56117100	CHOW FUN RICE NOODLES, COOKED, NO FAT ADDED	no
56205210	RICE, WILD, 100%, COOKED, NO FAT ADDED	no
58121410	DUMPLING, PLAIN	no
58132110	SPAGHETTI W/ TOMATO SAUCE, MEATLESS	no
58145110	MACARONI OR NOODLES W/ CHEESE	no
63409010	GUACAMOLE, NFS	no
72201230	BROCCOLI, COOKED, NS AS TO FORM, W/ CHEESE SAUCE	no
74404010	SPAGHETTI SAUCE	no
75121400	PEPPER, POBLANO, RAW	no
75510030	OLIVES, GREEN, STUFFED	no
82101000	VEGETABLE OIL, NFS (INCLUDE OIL, NFS)	no

Appendix E Using the program

This section describes how to set up and run the program, what and where the output is, and how to change the sensitivity inputs, in that order.

E.1 Setup and running the program

This program is a "console" application that runs in a Command Box under Windows® (it has been tested only in Windows® XP®). It consists of a single program file, C_perfringens.exe, and the following ASCII text data files:

Basic_growth.dat
Category_12_temps.dat
Category_34_a_temps.dat
Category_34_temps.dat
Cold_storage.dat
Cooking.dat
dose_response.dat
D_values_high.dat
D_values_low.dat
Food_samples.dat
garlic.dat
Growth_corrections.dat
Home_empirical.dat
Home_intra_var.dat
hot_holding.dat
misc_spice.dat
mustard.dat
oregano.dat
Raw_meat.dat
RTE_meat.dat
Type_A_Plus.dat
Category_1a_Cold_Eat.dat

These data files must all have the given names and be placed in the same ("data") sub-directory (which may be the same sub-directory containing the program file, or not, at user preference). In addition, in that same data sub-directory there must be two further files with arbitrary names that specify variability distributions for parameters that could not be adequately evaluated from available data, and that are treated in sensitivity analyses. In the following explanation, these files will have the names:

Sensitivity.dat
Init_Germ_fracs.dat

Finally, a control file (an example called "control.dat" is provided) may be placed in any convenient sub-directory and given any name.

The program is invoked with a command line like:

```
>C_perfringens.exe  ["][directory\]controlfile["]
```

where [ ] indicates an optionally allowed value, "`directory`" specifies a directory path (relative to the current directory, or absolute), and "`controlfile`" is the file name of a control file that provides information equivalent to the "control.dat" example file. If the control file name or directory path includes spaces, surround the whole string with quotes.

Example setup: You have a default directory called Root\. (on my machine, Root\ is "C:Documents and Settings\Edmund\My Documents\PROJECT\B-1640 C Perfringens\", so it is much easier to use relative references).
Create a directory Root\progs and place C_perfringens.exe in it.
Create a directory Root\progdata and place all the data files in it, including the control.dat file.
To run the program, open a Command Box (in Windows XP by selecting Start, then Run, and specifying cmd as the command to run), change directory to Root\progs, and enter

```
>C_perfringens   ..\progdata\control.dat
```

or

```
>C_perfringens  Root\progdata\control.dat
```

[The program can be run from within Windows — it will create its own command box, and a shortcut or PIF file can be set up that automatically provides the name of the control file as a parameter; but it is easier to do it all in a Command Box].

E.2 Structure of the control file

The basic Monte Carlo parameters for the program run are set according to what is specified in the control file (and various further modifications are possible by modifying the sensitivity parameters, see below). The control file format is:

```
#  Any number of comment and/or blank lines, indicated by # as
#  the first character of the line.  Comment lines and blank
#  lines may be interspersed anywhere.
!  ! can also be used as a comment delimiter,
{  as can { (curly left brace}

Data_directory      ..\progdata\
Output_file         output.txt
Sensitivity_file    sensitivity.dat
Init_germ_file      Init_Germ_fracs.dat
Variability_loops   10000000
Uncertainty_loops   1
```

There are six keyword-value pairs. The first entry shown on each of the six non-comment lines are the keywords. The second entries are their values, which may be changed to change the running of the program. Each keyword must occur on a separate line as the first value on that line, they can occur in any order, and are not case-sensitive. Values for each keyword must be on the same line separated by an arbitrary number of spaces from the keyword. If you repeat the same keyword within the file, the last occurrence overrides earlier ones.

Data_directory tells the program where to find the data files and where to put the output. It is the directory path (absolute or relative to the program) where the data files may be found. Note the terminating \, which must be present on the directory path. The example shown corresponds to the example setup described above.

Output_file is the name of the file where output will be placed by the program. It will be created in the Data_directory directory if it does not already exist, and overwritten if it already does exist. Its value must be a valid file name.

Sensitivity_file is the name of the file that corresponds to Sensitivity.dat in the description that follows. For sensitivity analyses, where multiple runs are performed using different values for parameters in the Sensitivity_file, it is convenient to have multiple files of the same format as Sensitivity.dat, each one with a single parameter value changed. Different control files can then be used for each sensitivity run, with each control file specifying a different Sensitivity_file.

Init_germ_file is the name of the file that corresponds to Init_Germ_fracs.dat in the description that follows. For sensitivity analyses, where multiple runs are performed using different values for parameters in the Init_germ_file, it is convenient to have multiple files of the same format as Init_Germ_fracs.dat, each one with a single parameter value changed. Different control files can then be used for each sensitivity run, with each control file specifying a different Init_germ_file.

Variability_loops is the number of variability loops (*i.e*. servings) to run for each value of growth during stabilization and each uncertainty loop. This must be a positive number, less than or equal to 2147483647 (*i.e.* roughly 2 billion). Note: do NOT include commas. A real number in this range (with decimal point or exponential notation) will also work — it will be truncated to the next lowest integer.

Uncertainty_loops is the number of uncertainty loops to run. This must be a positive number, less than or equal to 2147483647 (*i.e.* roughly 2 billion). Note: do NOT include commas. A real number (with decimal point or exponential notation) will also work — it will be truncated to the next lowest integer.

Warning: the total number of servings calculated is (number of growth distributions) × Variability_loops × Uncertainty_loops (see the Growth sensitivity parameter, below, for the number of growth distributions). The program runs at about 400,000 servings per second on a 2.6 GHz Pentium 4 with plenty of memory (512 MByte or more). The factor (distributions of growth steps) occurs because each Monte Carlo run is repeated for each value of growth during stabilization specified in the sensitivity parameter file (see below). To obtain an appropriate number of illnesses requires at least 10 million servings in each variability loop.

E.3 Output file, and structure of the output

While running, the program produces output to the Command Box (screen) to indicate what is going on, and saves output in the output file (all output is saved immediately, so a power failure will only stop the program, not lose what has been done so far). The saved output file differs for the case Uncertainty_loops=1 versus Uncertainty_loops>1, but what appears on the screen is the same.

E.3.1 Command Box (screen) output.

Example (with Uncertainty_loops=1, Variability_loops=10000000)

```
Creation took   1.08 secs
.................  0.50  1  397563  11  0  1   29.22
.................  1.00  1  407090  18  0  1   57.50
.................  1.50  1  411722  24  0  0   85.59
.................  2.00  1  415962  34  0  1  113.80
.................  2.50  1  414950  27  0  2  141.89
.................  3.00  1  417296  32  0  0  170.67
.................  3.50  1  416444  28  0  1  198.69
Done.
```

Creation refers to setting up the required structures in memory, and reading all the data files. This line appears on screen after these initial procedures have been completed. Each of the dots then appears slowly as the program runs, and indicates continued progress. Each dot corresponds to 500,000 servings (so they appear about 1.6 seconds apart on the machine described, giving feedback that the program is running). After each uncertainty loop for a given growth, and on the same line as the last dot for that uncertainty loop, a summary of seven numbers is given. In order these are:

1. Growth during stabilization, (this is the summary growth value specified in the sensitivity parameter file, see below),
2. Uncertainty loop number,
3. number of servings with non-zero vegetative cells at the time of being eaten (out of the total servings = variability_loops for this uncertainty loop),
4. number of illnesses occurring for this uncertainty loop,
5. number of cases for this uncertainty loop where contamination was detected (and the food thrown out); this occurs only in "what if" situations (see below),
6. number of illnesses in hot-held food in this uncertainty loop,
7. difference in time counter value from the beginning of the program to the time this line was output, in seconds (note: the time counter increments for a maximum of one day, then starts over, so runs over 1 day can give negative numbers here; you would have to add the number of days to get the correct time).

If Uncertainty_loops had been set to more than 1, the above output would have continued with the further uncertainty loops showing an uncertainly loop number larger than 1, before final termination is indicated with the word Done.

E.3.2 Output file, Uncertainty_loops=1

This is an ASCII text file containing tab-delimited values on each line, with each line separated by a carriage-return, line-feed pair. For each uncertainty loop and value of growth during stabilization, first a line is output with the following five numbers:

Growth	Growth during stabilization, (this is the summary growth number specified in the sensitivity parameter file, see below),
Non-zero	Number of servings in this uncertainty loop that had non-zero veg. cells at the time of being eaten
# cutoff	Number of servings with detected contamination (and the serving discarded); this occurs only in "what if" situations (see below),
# ill	Number of illnesses
# hot_hold	Number of illnesses for hot-held servings

Subsequently a line of header information is output, then a line of information for each illness occurring in that uncertainty loop. The header line is a set of key values (separated by tabs). On each output line (one line for each illness occurring), the following information is recorded about the serving causing that illness (the key on the left of this list corresponds to the header value output for that entry on the line):

Randkey	Random key. A integer (currently in the range 0 to 2^{64}–1, so out of the range of most spreadsheet cells to record exactly). This can be used to reproduce this particular entry (to do so would require modification and re-compilation of the program).
Category	Food category
No_spice	Food serving had no spice in it (True/false)
Veg/meat	Serving initially contained vegetative cells derived from meat (True/false). [Initially, here and below, means after any production heat steps and before stabilization].
Spore/meat	Serving initially contained spores derived from meat (True/false)
Veg/spice	Serving initially contained vegetative cells derived from spices (True/false)
Spore/spice	Serving initially contained spores derived from spices (True/false)
Init veg	Initial number of vegetative cells in serving, before growth during stabilization in production
Init spores	Initial number of spores in serving, before stabilization (same as after stabilization)
Veg growth	Number of veg. cells in the serving after stabilization
retail temp	Temperature of retail storage (°C)
veg retail	Number of veg. cells in the serving after retail storage
sp retail	Number of spores in the serving after retail storage
home temp	Temperature of home storage (°C)
veg home	Number of veg. cells in the serving after home storage
sp home	Number of spores in the serving after home storage
hold_hot	True if hot-held, false otherwise
oven	True if heated in an oven, false otherwise

cold eat	True if eaten cold, false otherwise
veg eat	Number of veg. cells in serving at time of eating
sp eat	Number of spores in serving at time of eating

E.3.3 Output file, Uncertainty_loops>1

This is an ASCII text file containing tab-delimited values on each line, with each line separated by a carriage-return, line-feed pair. For each uncertainty loop and value of growth during stabilization, first a line is output with the following five numbers:

Growth	Growth during stabilization, (this is the summary growth number specified in the sensitivity parameter file, see below),
Non-zero	Number of servings in this uncertainty loop that had non-zero veg. cells at the time of being eaten
# cutoff	Number of servings with detected contamination (and the food thrown out); this occurs only in "what if" situations (see below),
# ill	Number of illnesses
# hot_hold	Number of illnesses for hot-held servings

E.3.4 Both output files

The output files may be readily imported into spreadsheets. For Excel, accepting the default values (using Data/Get External Data/Import Text File) works well, except that to retain the complete Randkey value that field should be explicitly imported as text, since it contains more digits than are retained by numbers imported into typical spreadsheets. Failure to explicitly import this field as text is unimportant if it is not necessary to retain the capability of exactly reproducing each line in the output file. Importing into other applications should be just as straightforward; specify a tab-delimited file and, if desired (and possible), set the field type of the Randkey field to be text.

E.4 Modifying input values — Sensitivity parameters

The sensitivity parameters described in the description of the exposure assessment are encoded in the two ASCII text files (as described in Section E.2, these files can have any name, and the names are provided in the control file; for convenience, they are given specific names here corresponding to the example control file):

Init_Germ_fracs.dat
Sensitivity.dat

(Two files were used because, for technical reasons, it increased the speed of the program). Both these files have the same structure, of the form:

```
#  Comment lines begin with #.  Comment and blank lines are ignored.
#  Comment and blank lines may be interspersed throughout the file.
#  The essential part of the file occurs in keyword-value lines.
```

```
#  There are as many such keyword-value lines as necessary.
#  Keywords can occur in any order.

keyword   values   # comments after the first # are ignored
keyword   values
keyword   values
```

The keyword must occur first on the line (*exception* — see the description of vector parameters for the growth keyword below). The rest of the line consists of values associated with that keyword, terminating at the end of the line (*exception* — for keywords specifying vectors, the next few lines contain values associated with that keyword) or at a comment delimiter (anything after the first comment delimiter, any one of #, !, or {, is ignored). Values are separated, and separated from the keyword, by an arbitrary number of spaces.

E.4.1 Init_Germ_fracs.dat

This contains three keywords, all of which must be present:

```
Max_germ_frac
First_heat_frac
No_heat_frac
```

`Max_germ_frac` is a constant, and the default value (in the current control.dat file) is 0.75. It is the maximum fraction of spores that may ever germinate in two heat steps. A single value between 0 and 1 should be entered here in any numerical format. It is up to the user to ensure that it lies in the range 0 to 1.

`First_heat_frac` and `No_heat_frac` are variability distributions. The default entries are:

```
First_heat_frac  triangular  0.05 0.50 0.75 0.50
No_heat_frac  triangular 0.01 0.05 0.10 0.05
```

(*see below for how to specify distributions*).

`First_heat_frac` is the fraction of spores that are activated during production heating (lethality step or steps) of RTE foods. Warning: *it is up to the user to ensure that values returned from any distribution entered for this fraction lie within the range* [0, `Max_germ_frac`].

`No_heat_frac` is the distribution of the fraction of spores that will germinate under mild conditions. Warning: *it is up to the user to ensure that the values returned from any distribution entered for this fraction lie within the range* [0,1].

E.4.2 Sensitivity.dat

This file contains the following keywords, all of which must be present:

```
Growth
Second_heat_frac
Storage_frac
```

```
Pre_retail_time
Category_1_cold
Oven_fraction
Microwave_heat_time
Oven_heat_time
Hot_holding_fraction
Hot_hold_time  triangular
Max_Cell_Density
Max_Allowed_conc  Point 1e15  1/g  ! Units needed
OverGrowthFraction  0.0           ! Constant
OverGrowthTemp    Point  12   K    ! Variability distribution.  Units needed
SpoiledMinConc   1e9   1/g  ! Units needed; constant
SpoiledConc90    1e8   1/g  ! Units needed; constant
```

The keywords, and the values associated with them in the supplied default file, are as follows.

```
Growth  vector 7
Point 0.5
Point 1.0
Point 1.5
Point 2.0
Point 2.5
Point 3.0
Point 3.5
```

The keyword `growth` is associated with a vector (list) of variability distributions, typically set to be seven point distributions of 0.5 through 3.5 by steps of 0.5. During execution of the program, the uncertainty and variability loops are repeated for each entry in this list of growths during stabilization. The keyword `vector` must appear after the keyword `growth`, and be followed by the number of growth variability distributions to be modeled in this run (this can be any number from 1 upwards). Following the `growth` keyword line must be one line for each variability distribution. Each such line describes the variability distribution for $\log_{10}$ growth during stabilization required. The "preferred" value of the distribution (*see below for specification of distributions* — the preferred value for a point distribution is the single point value) is the value that is printed on the screen and in the output file for this growth during stabilization.

```
Second_heat_frac  triangular 0.0 0.5 1.0 0.5
```
The fraction of heat-activatable spores remaining after RTE production that are activated by a second heating step. A variability distribution.

```
Storage_frac  triangular 0.0  0.025 0.05 0.025
```
The fraction of spores that germinate during storage and transport. A variability distribution.

```
Pre_retail_time  uniform 10 30 20 d  ! Note that units are required.
```
Pre-retail storage time for all categories. *Units are required as the last value provided*. Allowable units are abbreviations of standard time units (s, with any standard MKS multiplier prefix,[105] and min, h, d for minute, hour, day, with no prefixes allowed).

```
Category_1_cold  0.2   ! a constant
```

[105] μ is represented by u, but in this application there would be no call to use microseconds

Fraction of Category 1 foods that are eaten cold. A constant.

```
Oven_fraction   0.5    ! a constant
```
Fraction of RTE & partially cooked foods that are heated in an oven, assuming they are heated at all. A constant.

```
Microwave_heat_time  uniform 1  10 5.5 min    ! units needed
```
Variability distribution for times of heating in a microwave oven. *Units are required*. Allowable units are abbreviations of standard time units (s, with any MKS multiplier prefix, and min, h, d for minute, hour, day, with no prefixes allowed).

```
Oven_heat_time       uniform 10 30 20 min     ! units needed
```
Variability distribution for times of heating in a standard oven. *Units are required*. Allowable units are abbreviations of standard time units (s, with any MKS multiplier prefix, and min, h, d for minute, hour, day, with no prefixes allowed).

```
Hot_holding_fraction  0.01   ! a constant
```
Fraction that is hot-held, applied to Categories 1 & 4 servings. A constant.

```
Hot_hold_time  triangular 0.5  2  8  3  h  ! Units needed
```
The variability distribution for hot-holding times. *Units are required*. Allowable units are abbreviations of standard time units (s, with any MKS multiplier prefix, and min, h, d for minute, hour, day, with no prefixes allowed).

```
Max_Cell_Density  lognormal  18.42  1.151  1e8  1/g
```
The variability distribution for maximum cell density in CFU/g. *Units are required*. This default value is 8-$\log_{10}$ with SD 0.5 on $\log_{10}$ scale. Note that "lognormal" requires entries using natural logarithms =2.303*$\log_{10}$. Acceptable units are the inverse of any mass unit (*e.g*. 1/g, 1/kg, 1/lb, etc. for CFU/g, CFU/kg, CFU/lb).

The final set of keywords specify "what if" scenarios.

```
Max_Allowed_conc  Point 1e15  1/g  ! Units needed
```
"What if" the manufacturer could detect *C. perfringens* (all types, not just type A, and CPE-positive or CPE-negative) and throw out servings with more than some concentration of *C. perfringens* in them. *Units are required.* This keyword defines the variability distribution for the concentration that can be detected and eliminated. This "what if" can be ignored by setting a large enough value (*e.g.* 10^{15} CFU/g, as specified here)

```
OverGrowthFraction  0.0            ! Constant
OverGrowthTemp    Point  12   K    ! Variability distribution.  Units needed
```
"What if" at low enough temperatures some other organism would outgrow *C. perfringens*, preventing growth of *C. perfringens*. These two parameters specify the fraction of servings in which *C. perfringens* could grow that are overgrown instead by some other organism (`OverGrowthFraction`) and the temperature below which this might happen (`OverGrowthTemp`). This "what if" is ignored if the `OverGrowthFraction` is set to zero. (It is up to the user to ensure that the fraction is less than unity). For `OverGrowthTemp`, *units are required* (R, Rankine, or F,

Fahrenheit, are acceptable[106] alternatives; but `OverGrowthTemp` specifies a temperature difference from 0 °C or 32 °F, not an absolute temperature).

```
SpoiledMinConc   1e9   1/g  ! Units needed; constant
SpoiledConc90    1e8   1/g  ! Units needed; constant
```

"What if" the consumer can detect spoiled servings and throw them out. These two parameters specify the minimum *C. perfringens* concentration necessary for detection (`SpoiledMinConc`) and the concentration at which the detection probability has increased to 90% (`SpoiledConc90`). For both parameters, *units are required*. The detection probability is assumed to be zero below `SpoiledMinConc`, and to increase as

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

above C_{min}=`SpoiledMinConc`, where C is the concentration of *C. perfringens* in the serving, C_{90}=`SpoiledConc90`, and p is the probability for detecting the serving as spoiled and throwing it out. To ignore this "what if," set `SpoiledConc90` less than or equal to `SpoiledMinConc`.

E.5 Specification of distributions.

The specification of distributions is again by keyword-value pairs, where the keyword is the name of the distribution, and the "value" is a sequence of numbers, followed if necessary by a unit, needed as parameters for the distribution. The following keyword-value sets are available at the moment for distributions. The value of "units" in the following should be left blank in this file except where explicitly needed (the value "nounit" could also be used), as specified in the description of keywords above. Further explanation follows this table.

Point value units
Normal mean sd preferred units
Truncnormalabove mean sd upper preferred units
Truncnormalbelow mean sd lower preferred units
Truncnormalboth mean sd lower upper preferred units
Lognormal median sd preferred units
Lognormal2 mean arithsd preferred units
Trunclognormalabove median sd upper preferred units
Trunclognormalbelow median sd lower preferred units
Trunclognormalboth median sd lower upper preferred units
Uniform lower upper preferred units
Loguniform lower upper preferred units
Triangular lower break upper preferred units
Exponential decayconst preferred units
Gamma parmA parmB preferred units
Chisquared nu parmB preferred units
Beta parmA parmB parmC preferred units
Logistic A B preferred units
Weibull alpha beta preferred units

[106] C for Centigrade or Celcius is not acceptable; in the MKS system C stands for Coulomb, the unit of charge.

Pareto theta alpha preferred units
Geometric probability preferred
Poisson lambda preferred
Pert minimum mode maximum preferred units
Mass_point filename
Piecewise_linear filename

There MUST be values for each of the value entries in the lines defining distributions, except for the "units" value. That is, all values up to and including the "preferred" entry must have a corresponding number in the data file.

"Preferred" always corresponds to a "preferred" value for the output from the distribution. This entry is used in this application to reference the MLE value of uncertainty distributions, so should be set to the MLE value for any distributions specifying uncertainties. For distributions specifying variabilities, this value is generally not used (*exception; it is used to describe the distribution of growth during stabilization on the screen and in the output file*), but some entry should be provided in the input file. Only variability distributions are included in the sensitivity parameter files — the uncertainty distributions for these parameters are not known.

"Units" indicate the units of (one or more of) the values given. Units are specified as a character string specifying MKS or British units (*e.g.* m/s, kg, km-s/Mg-mol). Unit specifiers (like m, kg, mol) are separated by hyphens, and a single / may occur to indicate division. All unit specifiers occurring before any / are multiplied, and all those following any / are applied with inverse power. Any unit specifier may optionally be followed immediately with a single digit to indicate a power of that unit. MKS unit specifiers may be preceded immediately with any of the standard MKS multiplier characters (a, f, p, n, u, m, c, d, h, k, M, G, T, P, E for atto, femto, pico, nano, micro, milli, centi, deci, hecto, kilo, mega, giga, tera, peta, and exa, indicating decimal powers of −18, −15, −12, −9, −6, −3, −1, +2, +3, +6, +9, +12, +15, and +18 respectively; u for micro is non-standard but is the closest available for the Greek μ). All seven dimensions (mass, length, time, current, temperature, amount of substance, and luminous intensity) are handled; the base MKS units are, respectively, the meter [m], kilogram [kg], ampere (A), kelvin (K), mole (mol), and candela (cd). Unit specifiers are case-sensitive (capital letters often mean something other than the lower-case letter).

All inputs requiring dimensional values must be accompanied by a units string to indicate the units of the values supplied. Conversion to standard units is automatic.

Other than for units, keywords are not case-sensitive.

Mass_point and Piecewise_linear are special cases described below.

The other value entries are parameters of the distributions. Most are fairly self-explanatory, and are indicated further in the following summaries.

Point value units

value = single value of the point distribution. Note that this is strictly distinct from a constant — an input that is specified to be a constant can only accept a constant input, not a point distribution. An input specified to be a distribution can accept a point distribution to mimic a constant value. The preferred value of a point distribution is the single value of it.

Normal mean sd preferred units
mean = mean
sd = standard deviation

Truncnormalabove mean sd upper preferred units
This is a normal truncated above at the value "upper"
mean = mean of underlying normal
sd = standard deviation of underlying normal
upper = truncation point

Truncnormalbelow mean sd lower preferred units
This is a normal truncated below at the value "lower"
mean = mean of underlying normal
sd = standard deviation of underlying normal
lower = truncation point

Truncnormalboth mean sd lower upper preferred units
This is a normal truncated both above and below. See above for meanings.

Lognormal median sd preferred units
Median = mean value of the logarithmically transformed values
sd = standard deviation of the logarithmically transformed values

Lognormal2 mean arithsd preferred units
Lognormal distribution, as above, but initialized differently:
mean = arithmetic mean of the distribution
arithsd = arithmetic standard deviation of the distribution

Truncated lognormals. The truncation points are in the arithmetic space, NOT the logarithmically transformed space, even though the median and sd are in the transformed space.
Trunclognormalabove median sd upper preferred units
Trunclognormalbelow median sd lower preferred units
Trunclognormalboth median sd lower upper preferred units

Uniform lower upper preferred units
Lower and upper bounds of a uniform distribution.

Loguniform lower upper preferred units
Lower and upper bounds of a log-uniform distribution.

Triangular lower break upper preferred units

Lower, upper and break point (=mode) of a triangular distribution

Exponential decayconst preferred units
decayconst is the decay constant of the exponential. The units are those of "preferred", that is the units of 1/decayconst.

Gamma parmA parmB preferred units
Gamma distribution, with two parameters. parmA is the shape parameter, parmB the scale parameter. units are those of the scale parameter.

Chisquared nu parmB preferred units
Chisquared, nu=degrees of freedom, parmB =scale parameter. units are those of the scale parameter.

Beta parmA parmB parmC preferred units
Beta distribution. parmC scales the output from [0,1] to [0,parmC], and the units are those of the scale parameter.

Logistic A B preferred units
Logistic distribution. A is the location parameter, and B the scale. units are those of the scale parameter.

Weibull alpha beta preferred units
alpha is the shape parameter, beta the scale. units are those of the scale parameter.

Pareto theta alpha preferred units
theta is the shape, alpha the scale (least possible value). units are those of the scale parameter.

Geometric probability preferred
No scaling possible, and no units. Integer values returned. probability is the probability associated with this geometric distribution.

Poisson lambda preferred
No scaling is possible, and no units. Integer values are returned. lambda is the expected value of the value returned.

Pert minimum mode maximum preferred units
The Pert is a special case of a shifted beta distribution. units are those of the minimum, mode, and maximum.

Mass_point filename
The Mass_point distribution consists of an arbitrary number of values, each associated with a probability (and the sum of the probabilities is unity). This keyword requires that the name of a file defining the distribution be specified as the value associated with the keyword. That file must be in the same directory as the file containing the keyword. The file defining the distribution is laid out as follows:

```
n      units                # first line
p1     v1                   # first pair (second line)
p2     v2                   # second pair
# etc. etc.
...............
# up to:
pn     vn                   # nth pair (line n+1)
preferred                   # preferred value  (line n+2)
```

Any line can have a comment (preceded by #, !, or {) on it, as shown; comment lines can be interspersed throughout; and comment lines are ignored completely. n is the number of (value, probability) pairs, and (p1,v1), (p2, v2) are (probability, value) pairs. "Units" are the units of the values specified, and may be blank if the values are dimensionless. "preferred" is the preferred value for the distribution return value (and in this application should be set to the MLE value). The sum of the probabilities should be unity, within 1 part in 1000 (otherwise an error is generated, terminating the program; in any case the probabilities are re-normalized to sum to unity within machine rounding error). Entries on individual lines within the file are separated by arbitrary numbers of spaces.

Piecewise_linear filename

The Piecewise_linear distribution consists of a cumulative distribution that is piecewise linear between an arbitrary number of values, and continuous everywhere. The density function is uniform between each pair of values. This keyword requires that the name of a file defining the distribution be specified as the value associated with the keyword. That file must be in the same directory as the file containing the keyword. The file defining the distribution is laid out exactly as for the Mass_point distribution, but the entries differ in meaning. n is again the number of points specified, but (p1,v1), (p2, v2) etc. are pairs of (probability, value) pairs defining the cumulative distribution. The probabilities specified are cumulative probabilities, so $0=p1 \le p2 \le p3 \le ... \le pn=1$ necessarily (it is an error if this is not true), and the values must be strictly increasing, so $v1<v2<v3<v4<....<vn$ (again, it is an error if this is not true).