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

DRAFT for
Public Review and Comment

March 2005

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Table of contents

Table of contents 2

Table of Figures 7

Table of Tables 9

Acknowledgements 11

Executive Summary 12

1. Scope and Mandate 18
- 1.1. Scope 18
- 1.2. Public Health and Regulatory Context 19
  - 1.2.1. Public Health Background 19
  - 1.2.2. Policy Context 20

2. Hazard Identification for *Clostridium Perfringens* 21
- 2.1. Effects, and incidence 21
- 2.2. Epidemiology of outbreaks 21
- 2.3. Clonal characteristics of *C. perfringens* from outbreaks 21
- 2.4. Outbreaks of *C. perfringens* foodborne illness 22
- 2.5. Clinical presentation 26

3. Exposure assessment 28
- 3.1. Outline of the approach 28
- 3.2. Principle steps in the assessment 30
- 3.3. General approach to deriving variability and uncertainty distributions 36
- 3.4. Selection and identification of servings, treatment in this assessment, and evaluation of w, f_m, and f_{sj} 38
- 3.5. Vegetative cell concentration in heat-treated meat — C_m for RTE foods 41
  - 3.5.1. Selection of studies 41
  - 3.5.2. Preliminary analysis of distribution of concentrations 44
  - 3.5.3. Selected study data — RTE foods 45
  - 3.5.4. Evaluation of certain types of false negatives or positives 48
  - 3.5.5. Analysis of selected study data for vegetative cell concentrations in RTE foods 49
- 3.6. Spore concentrations in the meat fraction — c_m 51
  - 3.6.1. Spore concentration c_m for RTE foods 52
  - 3.6.2. Spore concentration c_m for partially cooked foods 52

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

3.7. Vegetative cell concentrations in raw meat — C_m for partially cooked commodities 52
3.7.1. Selected study data — raw meat 52
3.7.2. Analysis of selected study data for partially cooked foods 55

3.8. Concentrations of *C. perfringens* vegetative cells (C_{sj}) and spores (c_{sj}) in spices 57
3.8.1. Study selection for *C. perfringens* in spices 57
3.8.2. Analysis of studies for "as measured" *C. perfringens* concentrations in spices 59
3.8.3. Vegetative cell and spore concentrations in spices 63

3.9. The fraction of spores that germinate 64
3.9.1. The effect of common food additives on germination 64
3.9.2. The effect of physiologic properties of the food matrix on germination. 64
3.9.3. The effect of heat treatment temperature and duration, and strain, on germination 65
3.9.4. Spore germination fractions after heat treatment — η and g_p 67
3.9.5. Spore germination in favorable conditions without heat treatment 68

3.10. The fraction ($f_{vma,}$, f_{smA}, f_{vsA}, and f_{ssA}) of *C. perfringens* cells that are type A, CPE-positive 69
3.10.1. Selection of studies measuring prevalence of type A strains, prevalence of CPE-positive strains, or both 69
3.10.2. Analysis of selected studies for the fraction of *C. perfringens* in raw meat and spices that are type A, CPE-positive 72

3.11. The growth of *C. perfringens* and *C. botulinum* 75
3.11.1. Modeling growth of *C. perfringens* and *C. botulinum* as a function of temperature and time 75
3.11.2. Method of evaluation of growth rates of *C. perfringens* and *C. botulinum* 77
3.11.3. Results for growth rates of *C. perfringens* and *C. botulinum* 80
3.11.4. Comparison with published growth rates. 82
3.11.5. Modifications of growth rate by environmental factors 86
3.11.5.1. Presence of oxygen. 86
3.11.5.2. Salt and Nitrite effect on growth rate 86
3.11.5.3. The effect of salt and nitrite on the length of delay time 87
3.11.5.4. The effect of pH 89
3.11.5.5. Water activity 89
3.11.5.6. The maximum vegetative cell density 91

3.12. Growth during chilling, stabilization and secondary cooking steps — the factor G_c .. 91

3.13. Storage and transport phases of the distribution system for RTE foods 92
3.13.1. Spontaneous germination of spores during storage and transport — the fraction g_s 92
3.13.2. Survival or growth of *C. perfringens* during storage and transport — the factor G_s 92
3.13.2.1. Selection of studies on the lethal effect of low temperatures 93
3.13.2.2. Analysis of selected studies for lethality at low temperatures 96

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

3.13.2.3. Further assumptions for modeling cold lethality 99
3.13.2.4. Growth during storage 100
3.13.3. Duration and temperature of post-manufacturing storage 100

3.14. Re-heating and hot holding of RTE foods 106
3.14.1. Evaluation of experimental data on death of *C. perfringens* vegetative cells during heating 107
3.14.2. Re-heating times and temperatures. 111
3.14.3. Spore germination during re-heating — the factor g_p 114
3.14.4. Hot-holding temperature and time 115
3.14.5. Growth of *C. perfringens* vegetative cells during hot-holding 118

3.15. Numbers of servings 118
3.15.1. Total number of servings of RTE and partially cooked foods 118
3.15.2. Fraction of servings that are hot-held 119

Appendices for Chapter 3 120

Appendix 3.1 Fitting gamma concentration distributions to observed counts 120

Appendix 3.2 Growth models for *C. perfringens* 124
A3.2.1 Some background mathematics 124
A3.2.2 Application 127
A3.2.2.1 Model 1 127
A3.2.2.2 Model 2 128
A3.2.2.3 Model 3 128
A3.2.3 Connection with usual growth curve fitting techniques 129
A3.2.4 Variation of parameter values with temperature 130
A3.2.5 Extension to varying temperature 132

4. Limitations of the Exposure Model 134

4.1. Representativeness assumptions 134

4.2. Other assumptions consistent with but not proved by available data 135

4.3. Limitations introduced by the methods used in modeling 136

4.4. Other limitations 136

5. Hazard Characterization 137

5.1. Data for Dose-response relationship 137

5.2. Data Summary 138
5.2.1. Data included in dose-response modeling 138
5.2.2. Data not included in dose-response modeling 142

5.3. Dose-response modeling 144
5.3.1. Dose-response model employed 144
5.3.2. Evaluation of within-isolate dose-response 145
5.3.3. Evaluation of between-isolate variability of dose-response 146

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

5.4. Uncertainties in dose-response modeling ... 151

6. Risk Characterization ... 152

6.1. Variation of the risk of diarrhea with growth during stabilization ... 152
6.1.1. Primary results ... 152
6.1.2. The principal cause of illnesses ... 154

6.2. Uncertainty estimates ... 155
6.2.1. Uncertainty not incorporated in the model ... 155
6.2.2. Uncertainty incorporated in the model ... 155

6.3. Sources of illness-causing *C. perfringens* ... 158
6.3.1. Meat or spice as source of the *C. perfringens* ... 158
6.3.2. The source of *C. perfringens* by food category ... 159
6.3.3. Illness due entirely to *C. perfringens* growth during stabilization ... 159
6.3.4. Source by storage temperature ... 161

6.4. Response to Risk Management Questions ... 162
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? ... 162
6.4.2. What would the effect of altering stabilization be on *C. botulinum*? ... 163

6.5. Analysis of 'what-if' scenarios: ... 164
6.5.1. The effect of competing psychrotrophic spoilage organisms ... 164
6.5.2. The effect of consumer detection of high *C. perfringens* concentrations. ... 165

6.6. Sensitivity analysis ... 166
6.6.1. The maximum fraction of spores that may ever germinate in two heating steps. ... 168
6.6.2. The fraction of spores that germinate during production of RTE ... 168
6.6.3. The fraction of spores that germinate without any heat step ... 169
6.6.4. The fraction of spores that could be heat-activated that are heat activated by a second heating ... 169
6.6.5. The fraction of spores that germinate during storage and transport ... 169
6.6.6. The storage time between manufacturer and retailer ... 169
6.6.7. The fraction of category 1 foods that are eaten cold ... 170
6.6.8. The fraction of RTE and partially cooked foods that are heated in an oven ... 170
6.6.9. Heating time in a microwave ... 171
6.6.10. Heating time in an oven ... 171
6.6.11. The fraction of Category 1 and 4 foods that are hot-held ... 171
6.6.12. The hot-holding time ... 171
6.6.13. The maximum vegetative cell density ... 171
6.6.14. The fraction of CSFII (USDA, 2000) servings that are RTE and partially cooked ... 172

7. Research Needs ... 173

8. References ... 176

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Appendix A Food Categories to be Modeled in the FSIS *C. perfringens* Risk Assessment ... 188

A.1 Introduction ... 188

A.2 Selection of foods ... 188

A.3 Exclusion Criteria ... 190
A.3.1 Shelf Stability ... 191
A.3.2 Dried Foods ... 192
A.3.3 Retorted Products ... 192
A.3.4 Non-retorted Shelf Stable Jarred Commodities ... 192
A.3.5 Salt ... 194
A.3.6 Salt in the Presence of Nitrites ... 195
A.3.7 Raw Commodities ... 196
A.3.8 Factors Not Employed as Exclusion Criteria ... 197

A.4 Food Categories ... 198
A.4.1 Category 1: Foods Containing Nitrites and between 2.2% and 3% Salt ... 198
A.4.2 Category 2: Foods Unlikely to be Reheated for Consumption ... 198
A.4.3 Category 3: Foods Likely to be Reheated for Immediate Consumption ... 198
A.4.4 Category 4: Foods Served Hot but not Necessarily Prepared for Immediate Consumption ... 199

A.5 Summary ... 199

Appendix B Food code listing ... 201

Appendix C Foods commonly hot-held ... 275

Appendix D Meat content of servings ... 277

Appendix E Using the program ... 291

E.1 Setup and running the program ... 291

E.2 Structure of the control file ... 292

E.3 Output file, and structure of the output ... 294
E.3.1 Command Box (screen) output. ... 294
E.3.2 Output file, Uncertainty_loops=1 ... 295
E.3.3 Output file, Uncertainty_loops>1 ... 296
E.3.4 Both output files ... 296

E.4 Modifying input values — Sensitivity parameters ... 296
E.4.1 Init_Germ_fracs.dat ... 297
E.4.2 Sensitivity.dat ... 298

E.5 Specification of distributions. ... 300

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Table of Figures

Figure ES - 1 The rate of illnesses per million servings, with 90% confidence intervals for the uncertainties explicitly included in the risk assessment, as a function of growth during stabilization. 15
Figure ES - 2 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. 16

Figure 2-1 Temporal distribution (year) of *C. perfringens* outbreaks (1990-1999). 23
Figure 2-2 Temporal distribution (month) of *C. perfringens* outbreaks (1990-1999)............ 23
Figure 2-3 Geographical distribution (state) of *C. perfringens* outbreaks (1990-1999)......... 24
Figure 2-4 Geographical distribution (state) of *C. perfringens* cases (1990-1999)............... 24
Figure 2-5 Distribution of food item for *C. perfringens* outbreaks (1990-1999). 25
Figure 2-6 Location of *C. perfringens* outbreaks (1990-1999). 25
Figure 2-7 The proportion of USDA regulated foods associated with *C. perfringens* outbreaks (1990-1999). 26
Figure 2-8 Simplified schematic of the bacterial sporulation process. Adapted from Boyd and Hoerl (1991). 27
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)................ 32
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. 45
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. 50
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. 82
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)........ 84
Figure 3-6 Rates of decline of *C. perfringens* concentrations in refrigerated storage (± standard errors). 98
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... 101
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. 102
Figure 3-9 Cumulative frequency distribution for average home storage time (American Meat Institute, 2001)... 103

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Figure 3-10 Empirical distribution for retail storage temperatures of frozen entrées (based on Audits International/FDA, 1999). 104
Figure 3-11 Empirical distribution for home freezer temperatures (based on Audits International/FDA, 1999)........ 105
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. 106
Figure 3-13 D-values where the cells were subjected to substantial heat shock. 110
Figure 3-14 D-values obtained under conditions with less heat-shock. 111
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). 112
Figure 3-16 Cumulative distributions of cooking temperatures for poultry, ground beef, and beef/pork/lamb categories (Audits International/FDA, 1999). 113
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. 113
Figure 3-18 Distribution of all hot-holding temperatures found in the FDA survey (FDA, 2000) on a normal scale. 116
Figure 3-19 Observed distribution of hot-holding temperatures for foods of Categories 1 and 4 (based on FDA, 2000). 117
Figure 5-1 Dose-response relationship for *C. perfringens* (total cells). 142
Figure 5-2 Distribution of maximum likelihood estimates for potency (k). 148
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. 150
Figure 6-1 Variation in risk of diarrhea with growth during stabilization (MLE and median). 153
Figure 6-2 Uncertainty estimates for rate of diarrhea for fixed growth during stabilization. 156
Figure 6-3 Uncertainty distributions at fixed growth during stabilization. 157
Figure 6-4 Rate of illnesses due entirely to growth of *C. perfringens* during stabilization. 161
Figure 6-5 Fraction of illnesses caused by storage at abnormally high temperatures. 162
Figure 6-6 Approximate variation in MLE of illness rate for sensitive parameters. 170

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Table of Tables

Table 3.1 RTE and partially cooked foods that could support the growth of *C. perfringens*. ... 39
Table 3.2 *C. perfringens* in meat products. ... 43
Table 3.3 *C. perfringens* vegetative cells in raw meat blends following heat treatment (Kalinowski *et al.*, 2003) ... 46
Table 3.4 Putative *C. perfringens* vegetative cells in raw meat product mixtures following heat treatment (Taormina *et al*., 2003) ... 47
Table 3.5 Efficiency of *C. perfringens* media (Table 1; Araujo *et al*., 2001). ... 48
Table 3.6 Maximum likelihood estimates for the distribution parameters for *C. perfringens* concentration in cooked RTE foods. ... 50
Table 3.7 Standard deviation/correlation coefficient matrix for transformed parameters for *C. perfringens* concentration in cooked RTE foods. ... 51
Table 3.8 Prevalence and levels of *C. perfringens* in raw meats. ... 53
Table 3.9 Maximum likelihood estimates for parameter values for gamma distributions for concentrations in partially cooked food. ... 56
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. ... 57
Table 3.11 Levels and prevalence of *C. perfringens* spores in spices. ... 58
Table 3.12 Occurrence of spices in foods in the selected CSFII servings (RTE and partially cooked). ... 59
Table 3.13 Parameter estimates for *C. perfringens* in mustard, cumin, cinnamon, chili, cayenne pepper and black pepper combined. ... 61
Table 3.14 Parameter estimates for *C. perfringens* in garlic (as a spice) ... 62
Table 3.15 Parameter estimates for *C. perfringens* in oregano ... 62
Table 3.16 Parameter estimates for *C. perfringens* in all other spices ... 62
Table 3.17 Proportion of *C. perfringens* environmental isolates that were type A. ... 70
Table 3.18 Proportion of heat resistant *C. perfringens* among food samples. ... 72
Table 3.19 Summary of selected data analyzed for fraction of *C. perfringens* expected to be type A, CPE+. ... 73
Table 3.20 Probabilities for each entry in Table 3.19. ... 74
Table 3.21 Maximum likelihood estimates for the fractions of cells that are type A, CPE-positive. ... 74
Table 3.22 Standard deviations (main diagonal) and correlation coefficient (off-diagonal) for the uncertainty distribution of *u* and *v*. ... 75
Table 3.23 Maximum likelihood estimates for growth parameters for *C. perfringens* in cooked cured beef and cooked cured chicken. ... 80
Table 3.24 Standard deviations (diagonal) and correlation coefficients (off-diagonal) for the parameter estimates of Table 3.23 ... 81
Table 3.25 Mean lag phase and generation time of *C. perfringens* NCTC 8797 at 43 °C. (Riha and Solberg, 1975). ... 88
Table 3.26 Lag phase of *C. perfringens* NCTC 8798 at 45°C (Labbe and Duncan, 1970). ... 88
Table 3.27 Water activity values of meat items (Chirife and Ferro Fontan, 1982; Alzamora and Chirife, 1983; Taormina *et al*., 2003; Fett, 1973). ... 90

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Table 3.28 Measurements on survival of *C. perfringens* vegetative cells under freezing and refrigeration conditions. ... 95
Table 3.29 Summary of rates of decline ($\log_{10}$ reduction/day) of *C. perfringens* concentrations in refrigerated storage. ... 96
Table 3.30 Parameters for the variability and uncertainty distributions for the decline rate of *C. perfringens* cells in refrigerated storage. ... 99
Table 3.31 Summary of available data on D-values (in minutes) for *C. perfringens.* ... 108
Table 3.32 Maximum likelihood estimates for the parameters α, β, and θ. ... 109
Table 3.33 Standard deviations (main diagonal) and correlation coefficients (off diagonal) for the parameters α, β, and θ. ... 110
Table 3.34 Parameters of distributions for hot-holding times. All in °C (except correlations). ... 118
Table 5.1 Evidence for toxin production and consequent inclusion of human clinical data in dose-response modeling. ... 140
Table 5.2 Data used to model the *C. perfringens* dose-response relationship. ... 141
Table 5.3 Evidence for exclusion of clinical data obtained from use of various *C. perfringens* strains. ... 143
Table 5.4 Potency estimates for each of the fifteen strains of *C. perfringens* ... 146
Table 5.5 Parameters characterizing the lognormal distribution of potencies. ... 149
Table 5.6 Percentage points of the strain-averaged dose-response curve shown in Figure 5-3 ... 150
Table 6.1 Estimates for annual numbers and rate of illnesses. ... 152
Table 6.2 Source fractions by meat, spice or germinating spores ... 158
Table 6.3 Fraction of illnesses by each food category, for growth of 0.5 through 3.5 $\log_{10}$ during stabilization. ... 159
Table 6.4 Numbers of illnesses per year (*i.e.* in 55.7 billion servings) due entirely due to growth during stabilization. ... 160
Table 6.5 Estimated annual number of illnesses without and with detection of spoilage by consumers, and the serving discard rate. ... 166
Table 6.6 Summary of numerical estimates of sensitivity. ... 167

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Acknowledgements

This Risk Assessment is the result of a collaborative effort. The Risk Assessment Division of USDA performed the initial selection of foods from the CSFII and their assignation to various relevant categories, performed literature searches, read the relevant literature, made most initial literature selections, and wrote drafts of Chapters 1, 2, 5, 7, Appendix A, and significant parts of other chapters. Edmund Crouch edited these drafts and wrote the rest of the final text, with substantial input from the Risk Assessment Division, assisted at Cambridge Environmental Inc by Mike Ames, who performed the final selections of foods from the CSFII, and Shai Sahay who largely organized the references. Edmund Crouch was responsible for interpretation of the literature for the risk assessment, performed all the analyses, wrote the computer programs, and interpreted the results. Funding for the entire effort was provided by FSIS.

We should like to acknowledge the assistance of many others who were consulted during this project, including B.S. Eblen, D. Eblen, L.V. Cook, P. Levine, D. Schaffner, M. Tamplin, P. Taormina and the Field Laboratories located in Athens, GA, St. Louis, MO, and Alameda, CA. For providing additional information on their published experimental results, we thank L. Huang, V.K. Juneja, R. Kalinowski and H. Marks. However, the responsibility for any errors, omissions, and misinterpretations lies entirely with us.

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

Anne Courtney-Radcliff, Ph.D.[1]
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.
Carl Schroeder, Ph.D.

and providing comments and managing the project were:

Margaret Coleman, M.S.[1]
Uday Dessai, Ph.D., M.P.H.

Finally, we should like to thank the five peer reviewers for their thoughtful contributions and rapid response to a complex document.

[1] Currently not employeed by FSIS.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Executive Summary

We performed a quantitative risk assessment for the occurrence of diarrheal illness due to the bacterium *Clostridium perfringens* (*C. perfringens*) in meat-containing[2] ready-to-eat (RTE) and partially cooked foods. The primary purposes were to evaluate (i) the effect on the annual frequency of diarrheal illnesses of changing the allowed maximal growth of *C. perfringens* during manufacturing stabilization (cooling after the cooking step), and (ii) the uncertainty in the size of any such effect. A secondary purpose was to examine whether steps taken to limit the germination and outgrowth of *C. perfringens* occurring in raw ingredients of RTE and partially cooked foods would also be adequate to protect against germination and outgrowth of similarly occurring *Clostridium botulinum* bacteria.

C. perfringens is a bacterium that grows well on meat, poultry, and their products in the absence of oxygen, and favors relatively high temperatures. It is ubiquitous in the environment, and all sources of raw meat and poultry are likely to be contaminated with it occasionally to some degree. In addition, as spicy foods have been implicated in some *C. perfringens* outbreaks, and spices have been identified in some surveys as having high *C. perfringens* contamination, spices are treated here as an additional source of *C. perfringens* contamination. *C. perfringens* may be present in two forms, vegetative cells that are growing or ready to grow in favorable conditions, but that are vulnerable to decontamination processes such as heating; and spores that form from vegetative cells as the result of adverse environmental conditions and that are then resistant to decontamination processes. Eating foods contaminated with very large numbers of *C. perfringens* vegetative cells of certain strains (those known as Type A, that produce the *C. perfringens* enterotoxin, CPE) may lead to diarrheal illness. The illness is generally self-limiting, lasting one or two days. There have been no known food poisoning cases from the ingestion of spores at the concentrations customarily seen; rather, it is necessary to consume the vegetative cells themselves for illness to occur. This contrasts with such other toxin producing bacteria as *Staphylococcus aureus* where eating food contaminated with toxins produced by the bacteria is sufficient. With *C. perfringens*, diarrheal illness is due to toxin production by the vegetative cells as they sporulate inside the gut.

Vegetative cells of *C. perfringens* are killed by applied heat during the production of RTE foods, although they may survive the incomplete cooking used to prepare partially cooked foods. Spores, on the other hand, are not killed by any of the processes applied to RTE foods. Rather they are activated to germinate, becoming vegetative cells as a result of the cooking procedures used, while being relatively unaffected by procedures used for partially cooked foods. Meat- and poultry-containing partially cooked foods may thus be contaminated by vegetative cells originating in the raw meat or spices used in their production. Meat-containing RTE foods may be contaminated by vegetative cells that start out as spores in the raw meat or spices, are stimulated to germinate during cooking (that kills all the vegetative cells originally present), and that develop as vegetative cells and grow during the post-cooking cooling period(s), known as the "stabilization" step(s) of manufacture. In both cases there is also a small possibility for spores to germinate spontaneously during storage at almost any temperature.

---

[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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Subsequent to manufacture, RTE and partially cooked foods are stored before, during, and after transport from the manufacturing facility to the place of retail. Ideally this storage is at temperatures sufficiently low that no growth of vegetative cells can occur (and refrigeration and freezing temperatures can cause the slow death of vegetative cells). Similarly, storage occurs after retail purchase until the final consumer prepares and eats the food. Preparation may take the form of re-heating/cooking the food, and may include keeping some foods hot for a period before it is served and eaten, although RTE foods may also be eaten cold. At any time during storage and preparation, if the temperature exceeds about 12 °C (53 ° F) or dips below about 53 °C (130 °F) then vegetative cells of *C. perfringens* may be able to grow.

This risk assessment tracks *C. perfringens* spores and vegetative cells all the way from the raw meat or spices to the time when the RTE or partially cooked food is consumed. This is done using 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 considered capable of supporting growth of *C. perfringens* (omitting, for example, shelf-stable foods and foods high in salt and nitrite). In the simulation, a very large number of representative meat-containing RTE and partially cooked food servings are examined one-by-one. For each food serving, the original amount of contamination by spores and vegetative cells of *C. perfringens* is obtained, the resultant amount after manufacture (including the 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 eaten in the serving, the likelihood of those cells to cause illness, and whether that particular serving actually causes illness, is calculated for each serving. Repeating this for a large number of servings that represent the meat-containing RTE and partially cooked food servings eaten in the U.S. in a given year takes into account all the differences among servings (for each serving is unique), and provides an estimate of the number of illnesses that occur each year.

In addition to obtaining a single estimate of the number of illnesses per year, the Monte Carlo simulation performed here also takes account of the known uncertainties in each part of the calculation. The effect of these known uncertainties is thus also calculated to obtain an uncertainty estimate (that is, how sure we are of the result) on the number of illnesses each year. This uncertainty estimate is an underestimate of our true ignorance, since it does not incorporate unknown uncertainties, and it is conditional on how well the calculations and input data reflect what really happens.

To obtain results in the Monte Carlo simulation that represents the number of diarrheal illnesses in the U.S. requires inputs to the simulation that represent what happens in the U.S. for each of those inputs. The amounts and types of RTE and partially cooked food eaten in the U.S. were estimated from the Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 2000). Food servings were selected to correspond potentially to RTE or partially cooked foods, although what fraction of the servings examined in the CSFII actually were RTE and partially cooked is

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

not known. Various factors about each serving, such as the food type, weight, meat content, spice content, and salt concentration, were used in the simulation.

Most of the values used in the Monte Carlo simulation were obtained by a formal synthesis ("meta-analysis") of experimental data presented in the published literature. A large fraction of this document is devoted to a summary of the literature examined, the reasons for such selections of that literature as were made, the meta-analyses performed on the published information, and the results of those meta-analyses as used in the simulation. Some of the inputs evaluated in this way are:

- 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 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
- 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.

Finally, the object of the risk assessment is to evaluate how the number or rate of illnesses is affected by growth during stabilization. Ideally, what is required is an estimate of how changes in regulations on the allowed amount of growth during stabilization would affect actual growth rates in practice, and hence how the number or rate of illnesses changes with changes in regulations. Such estimates are impractical due to lack of information. Insufficient data were

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

located on actual growth rates achieved under current regulations, let alone what would be the industry response to changes in regulation and the growth rates that would occur as a result of such industry response. Instead what is evaluated is the effect of fixed amounts of growth applied uniformly to every serving (although the simulation model has the capability of including a variable amount of growth, should that information become available).

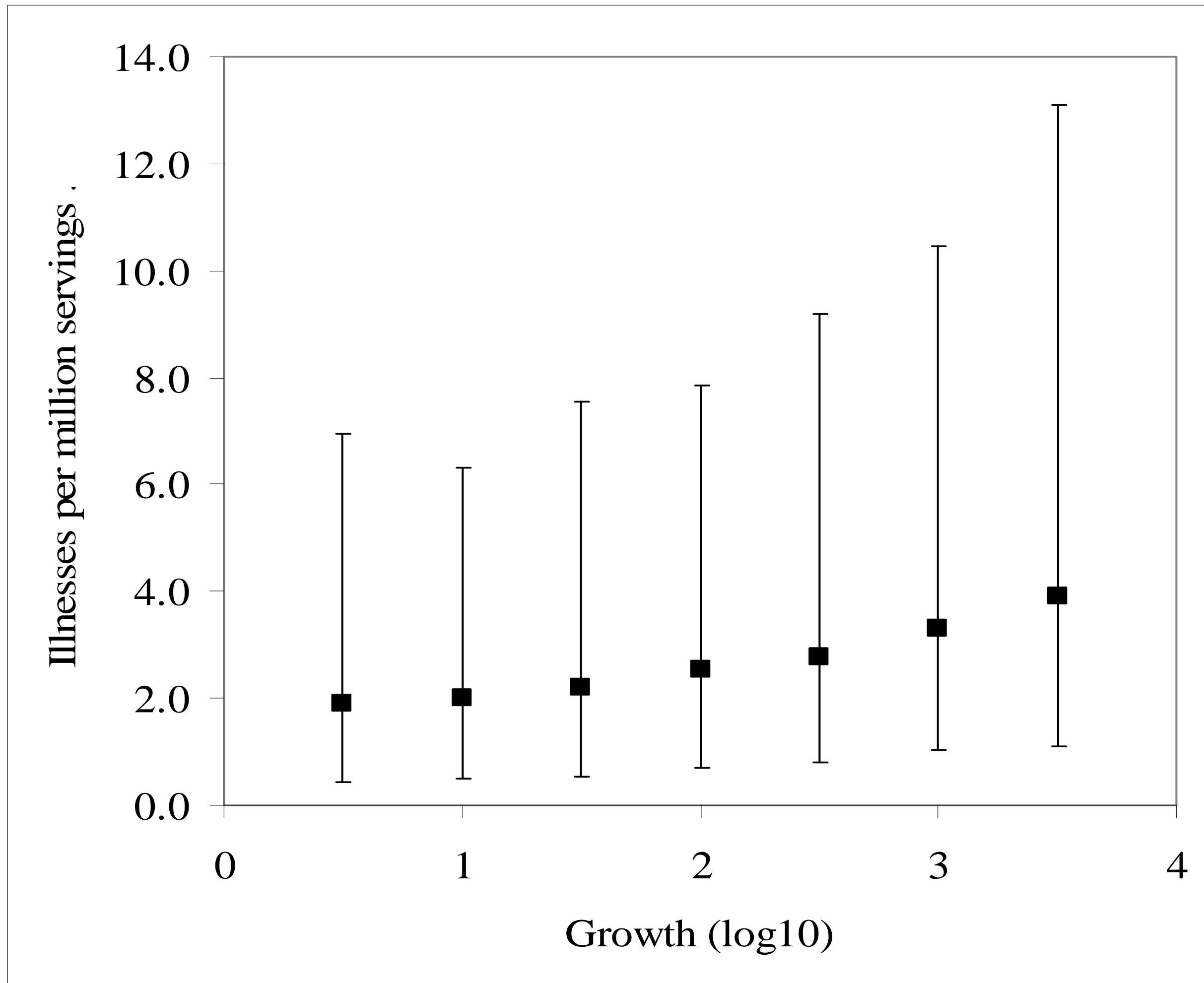


Figure ES - 1 The rate of illnesses per million servings, with 90% confidence intervals for the uncertainties explicitly included in the risk assessment, as a function of growth during stabilization.

The primary results of the risk assessment can be summarized by two graphs (Figure ES - 1 and Figure ES - 2). Figure ES - 1 shows the rate of illness per million servings, and its uncertainty, as estimated using the Monte Carlo simulation. The square boxes show how the rate of illness increases as the growth allowed during stabilization increases, from about 2.0 illnesses per million servings, corresponding to approximately 113,000 illnesses per year in the U.S., at 1 $\log_{10}$ growth (that is, 10×) during stabilization, through 2.5 illnesses per million servings at 2

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

$\log_{10}$ growth (100×) during stabilization, corresponding to approximately 138,000 illnesses per year, to approximately 3.3 illnesses per million servings, corresponding to approximately 183,000 illnesses per year at 3 $\log_{10}$ growth (1000×) during stabilization.[3]

Figure ES - 1 also shows the uncertainty in these estimates; the error bars depict the 90% confidence interval — that is, if all assumptions going into the modeling in the Monte Carlo simulation are correct, there is approximately a 9 in 10 chance that the true rate of illnesses would lie somewhere within the range given by the error bars (which span a range about 3.5× higher and lower than the central estimates shown by the square boxes). However, the illness rate increases smoothly as the allowed growth increases, so whatever the true rate, it would increase in the way shown by the square boxes as the allowed growth during stabilization increased.

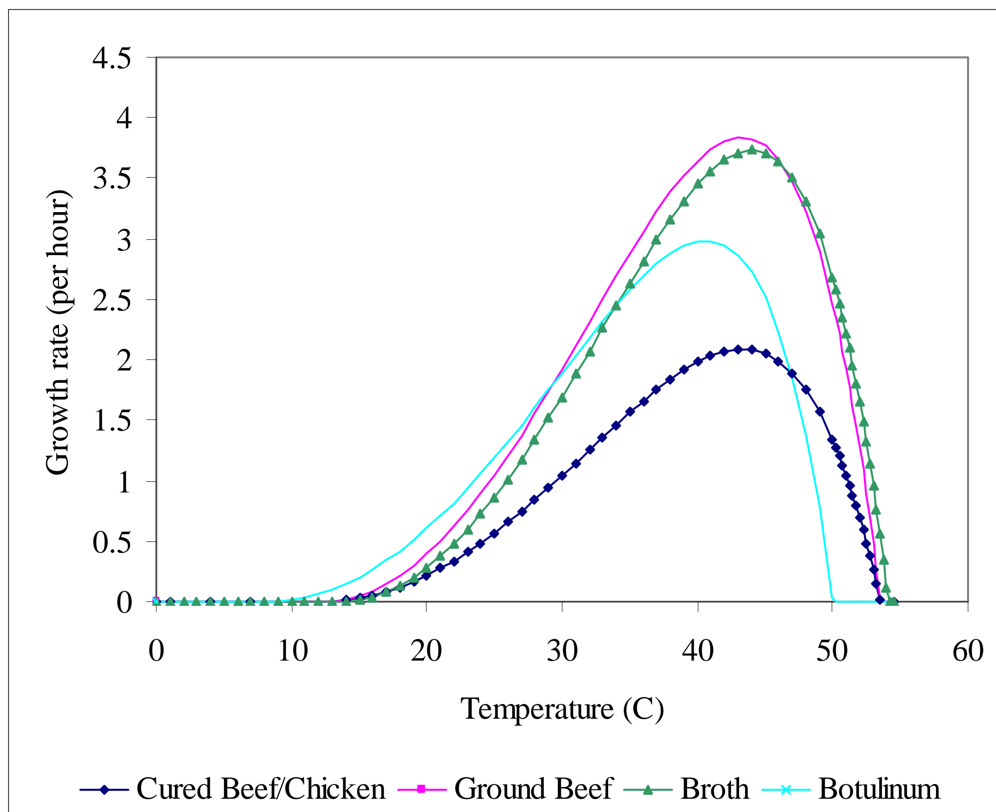


Figure ES - 2 Average growth rates of *C. perfringens* in the three media indicated, and of *C. botulinum* in a laboratory medium, and how these rates are estimated to vary with temperature.

[3] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Figure ES - 2 shows how the average growth rate of *C. perfringens* is estimated to vary with temperature when growing in three different media, and how the estimated average growth rate of *C. botulinum* in a laboratory medium differs. In particular, 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. On the other hand, *C. botulinum* was not observed to grow at 50 ºC, whereas *C. perfringens* is observed to grow rapidly at 50 ºC in broth, and is able to grow at higher temperatures. The variation shown here in the growth rate of *C. perfringens* is incorporated in the risk assessment, but the difference from the growth rate of *C. botulinum* at high or low temperatures shows that any measures taken to reduce or prevent growth of *C. perfringens* will not necessarily have the same effects on growth of *C. botulinum*. Thus prevention of growth of *C. botulinum* requires measures specific to that organism.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

1. Scope and Mandate

1.1. Scope

This risk assessment was initiated in May, 2002 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 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)

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 ready-to-eat (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 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).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 food-borne 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 2.2), only one has been confirmed as having been caused by a Ready-to-Eat (RTE) product, turkey loaf (CDC, 2000; DeWaal *et al*., 2001). 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

- 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 CP cpe-positive 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.

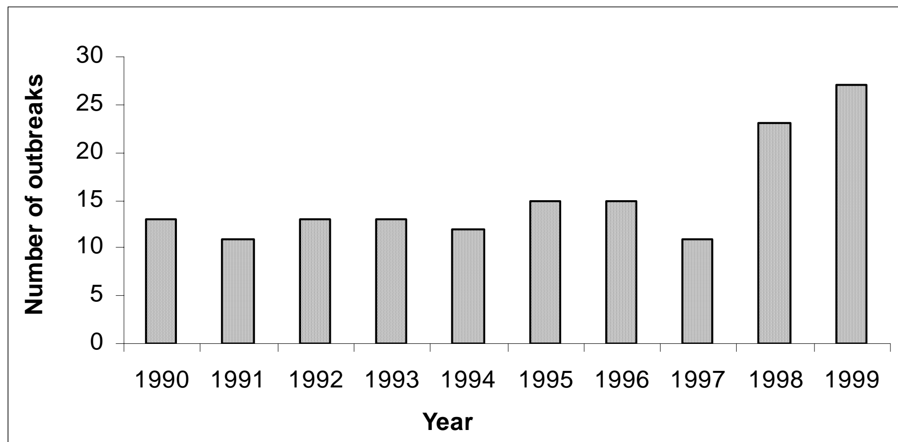
2.4. Outbreaks of *C. perfringens* foodborne illness

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.

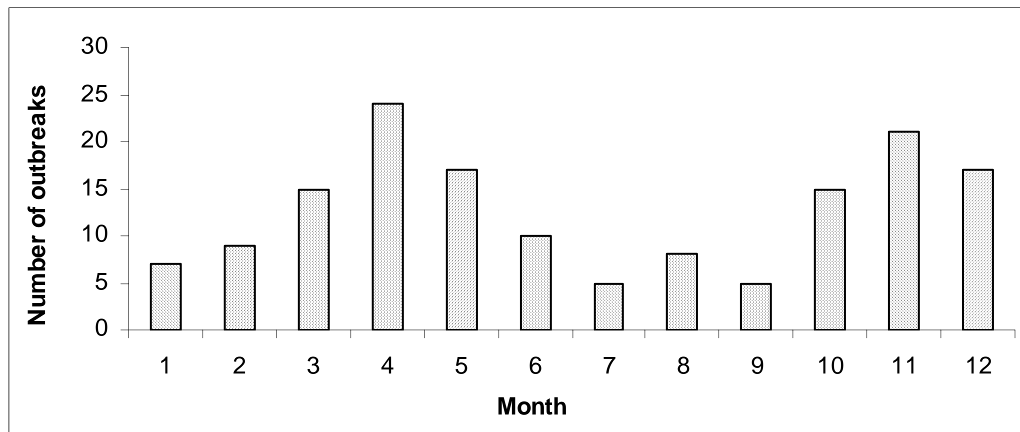
This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).

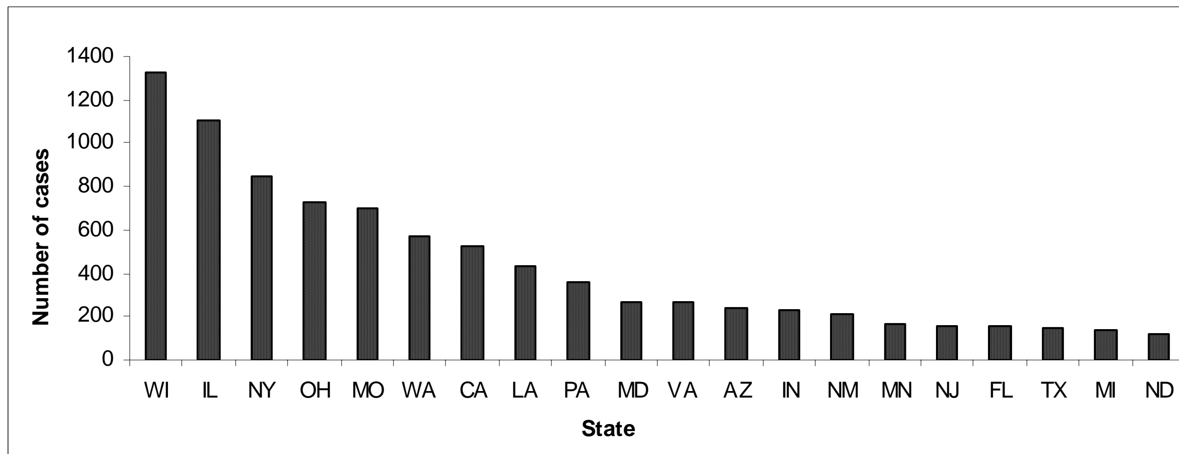


The highest number of reported outbreaks occurred in New York State, followed by Wisconsin, and Illinois (Figure 2-3) while the highest number of individual cases of *C. perfringens* foodborne illness occurred in Wisconsin, followed by Illinois and New York State (Figure 2-4). Note that these differences could be due to the differences in epidemiological investigation programs from state to state.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Figure 2-3 Geographical distribution (state) of *C. perfringens* outbreaks (1990-1999).

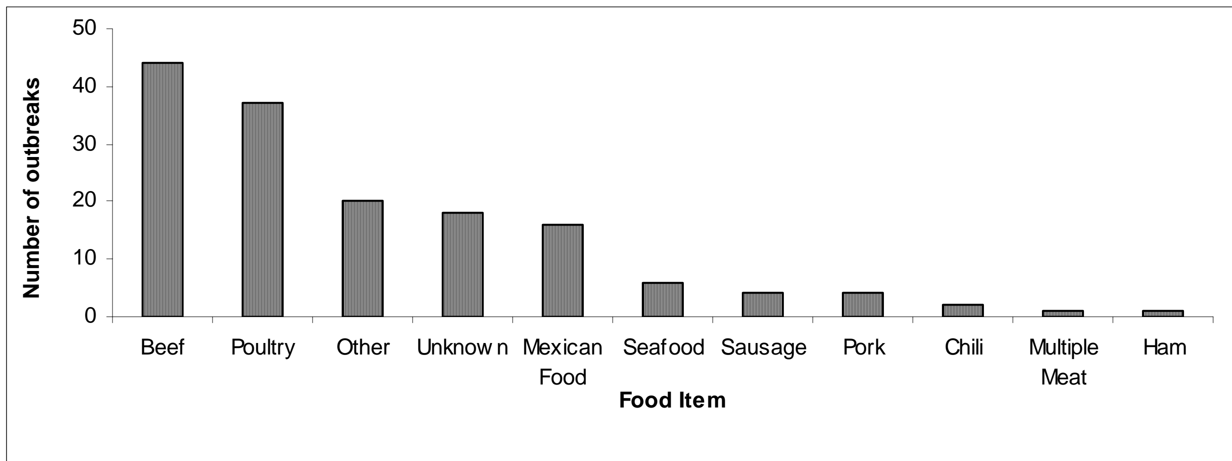
Figure 2-4 Geographical distribution (state) of *C. perfringens* cases (1990-1999).



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).

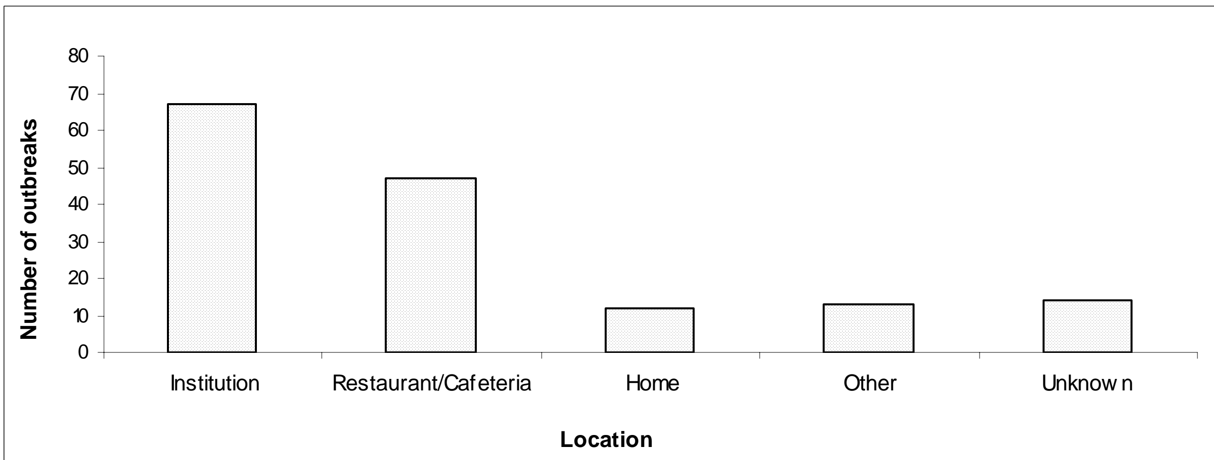
This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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%).

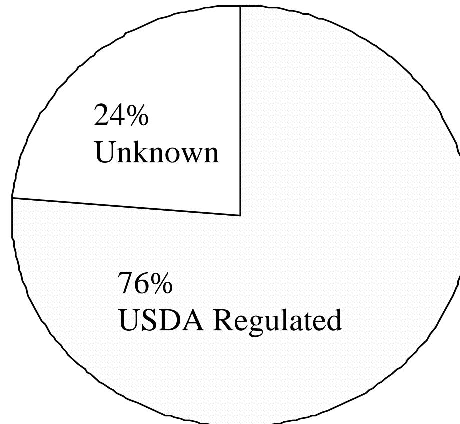
Figure 2-6 Location of *C. perfringens* outbreaks (1990-1999).



USDA-regulated food products were responsible for 76% of total *C. perfringens* outbreaks while 24 % of the food sources are unknown (Figure 2-7).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).

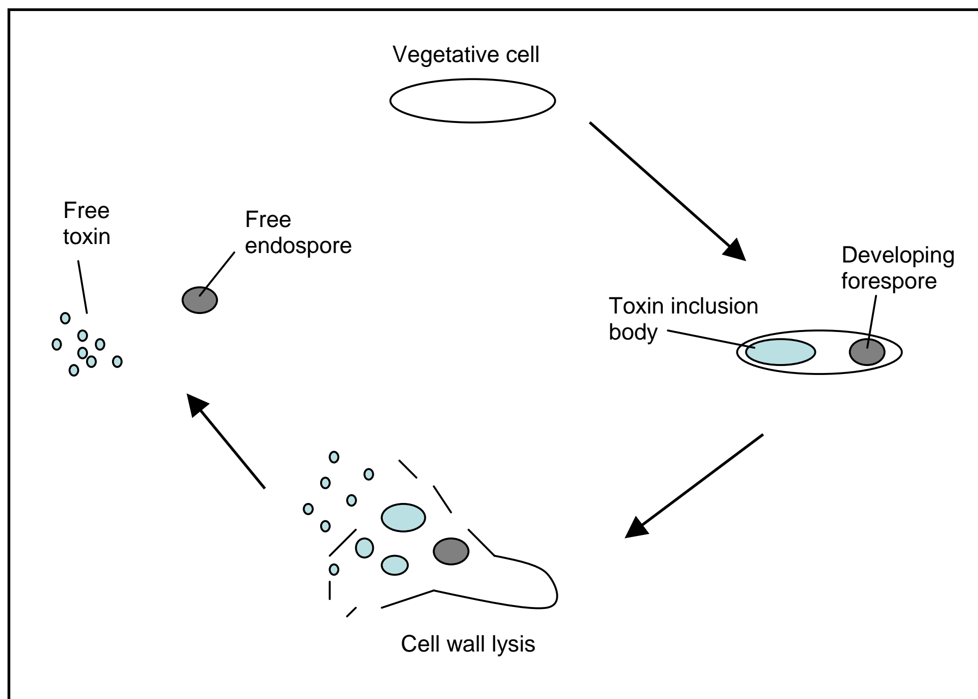
This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).



This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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[6] 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[7] 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

---

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

every such serving may be treated differently before finally being eaten, so we have to account 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, 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,[8] 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.

---

[8] 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 stabilizaiton after initial cooking of foods, so is not a prime focus of the risk assessment.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 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):

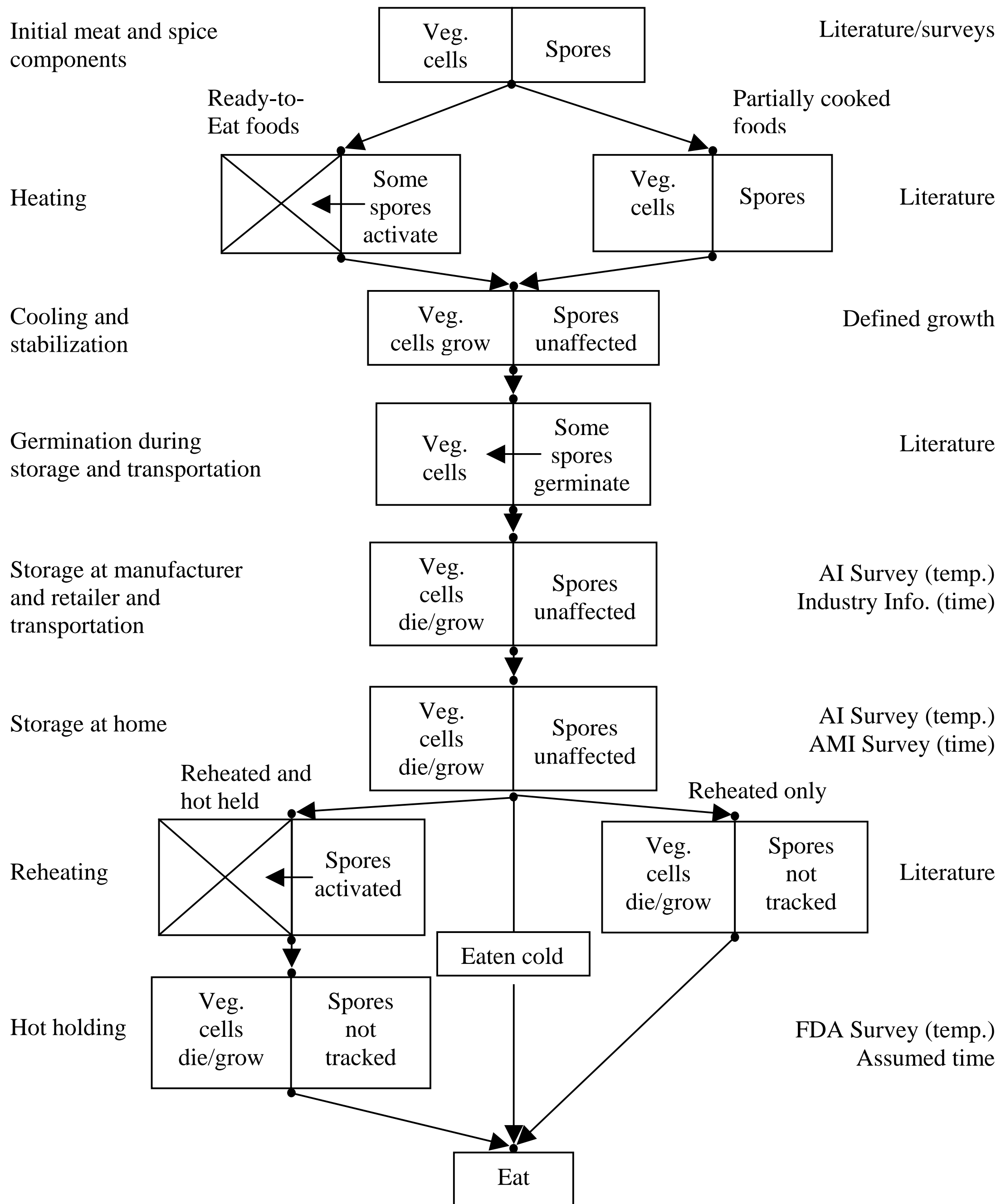
- *Processing (chilling and secondary cook steps and associated chilling)*. Fully or partially cooked foods are prepared from raw materials, cooked, then cooled and stabilized (possibly with more than one cooking and stabilization step). These processes are labeled “Heating” and “Cooling and stabilization” 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

- 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:

$$\begin{aligned} n_g &= B(n_s, g_s) \\ n_p &= B\left(\left[(n_s - n_g)\, l_s\right], g_p\right) \end{aligned} \qquad \textbf{(3.2)}$$

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:

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

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[9] (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.[10]

The Monte Carlo procedure can then be described as:

[9] 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.
[10] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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

- Choose a sample from the uncertainty distribution describing each of the inputs[11] 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 a 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[12] required of the calculations. The uncertainty loop may be performed only

[11] 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).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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

---

[12] 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 (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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

way that the profile likelihood of the transformed parameters are approximately normal.[13] The 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,

[13] We proceeded by plotting the profile likelihood as a function of the transformed parameter value, with the transform parametrized 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 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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
- 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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		Hotdogs, franks	- 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 and are made via the highest risk process.
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 CP 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 CP.
	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 CP.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

	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.[14] 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).

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 (corresponding to spore concentrations in raw meat, modified by the fraction that are activated to germinate during heat-treatment) represents a primary source of *C. perfringens* for such foods. An extensive analysis was thus applied to the estimate for this concentration.[15]

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

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

Table 3.2 *C. perfringens* in meat products.

Reference	Season samples collected	Region	Lethality step[a]	Presumptive CP[b] colony confirmation	Products evaluated	Results
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 log10 CP spores/g. 0/197 samples with >2 log10 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 log10 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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[16] 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 distribution shape.

[16] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

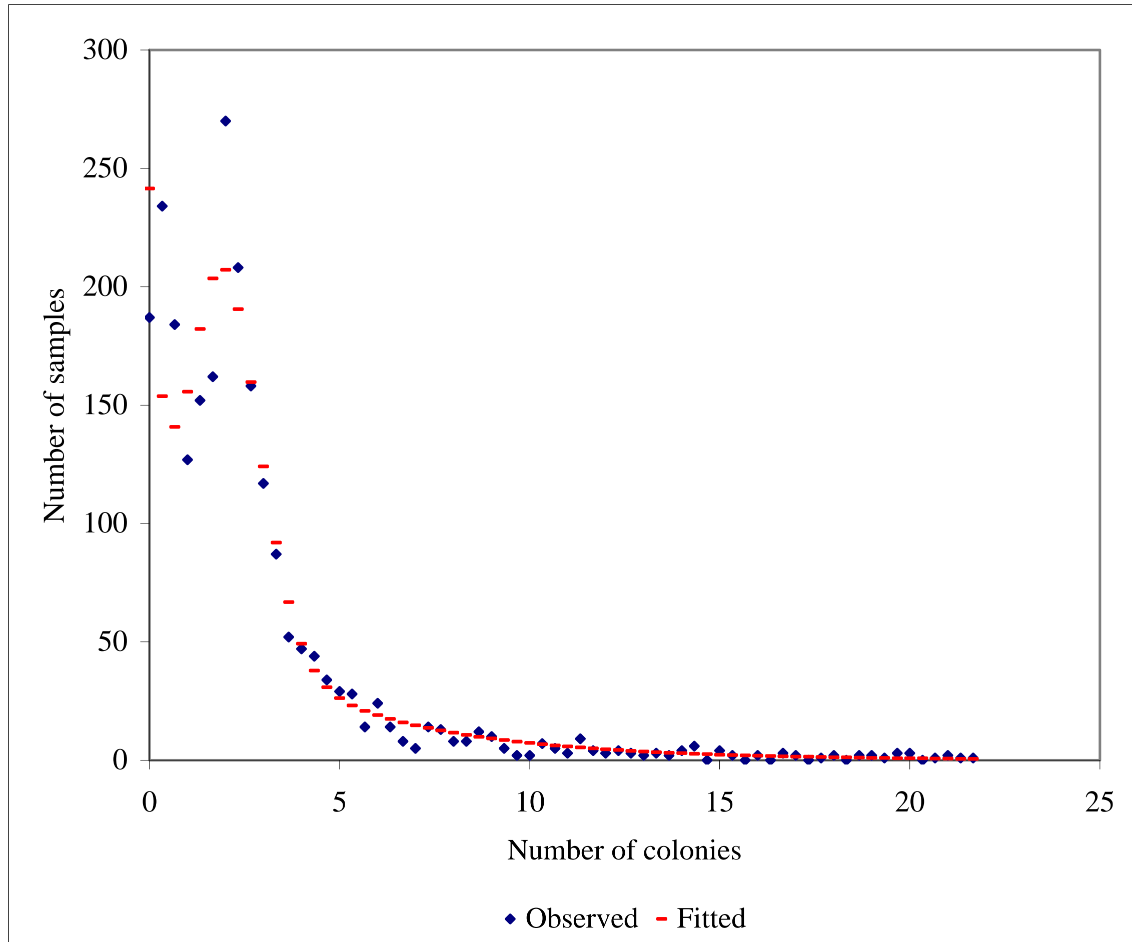


Figure 3-2 Approximate observed numbers and fitted expected numbers of samples versus numbers of colonies observed for Greenberg *et al*., 1966, illustrating the adequacy of fit of a gamma distribution.

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. 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).

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.[17] 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 $3n$, since each plate corresponded to 1/3 g of the original meat product. Kalinowski *et al*. (2003) use $3.3n$ 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 CP. The second had 48 black colonies. Of 12 of these tested, 5 were confirmed as CP giving the estimate of (5/12)*48 = 20 CP (Personal communication, R. Kalinowski, August 2003). The resulting uncertainty in actual colony count is taken into account in the analysis described in Appendix 3.1.

[17] 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..

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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,[18] 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).

[18] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 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

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

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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).

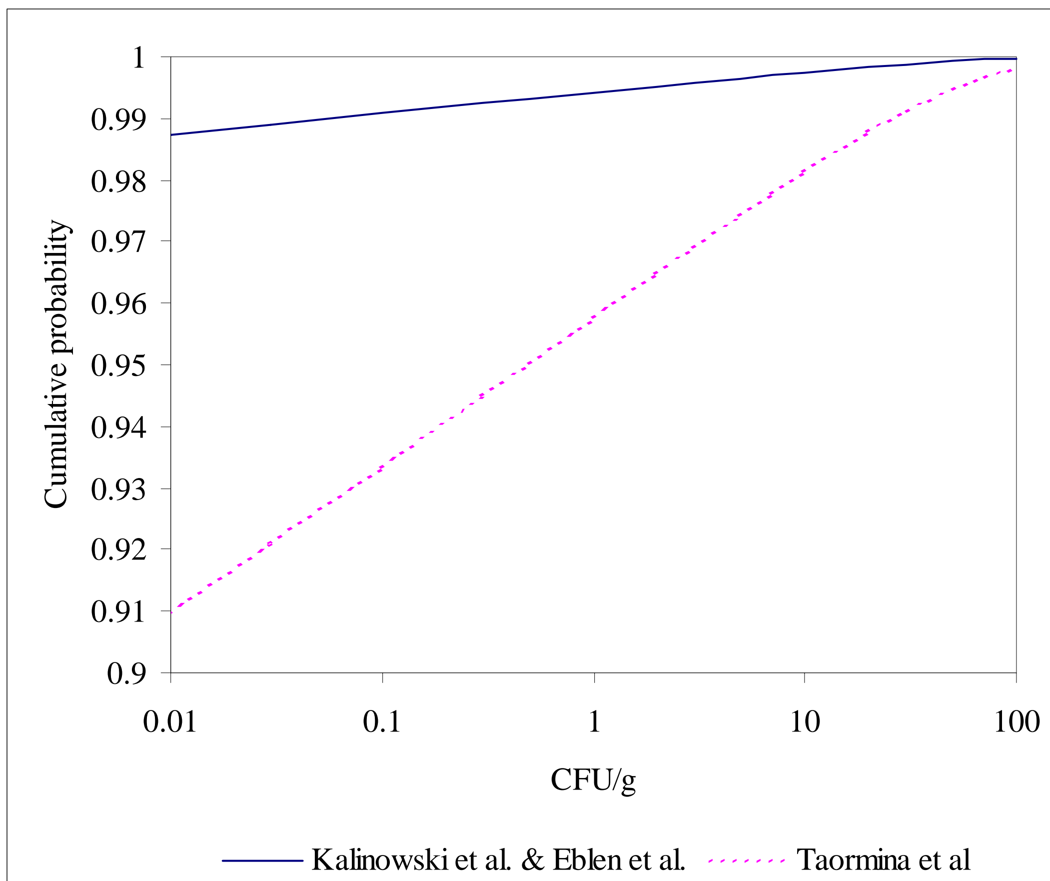


Figure 3-3 Upper end of the cumulative distributions (maximum likelihood estimates) for *C. perfringens* (Kalinowski *et al.*, 2003; FSIS, 2003) and total presumptive *C. perfringens* (Taormina *et al.*, 2003) concentrations in meat and poultry.

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.[19] 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

[19] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.

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 C_m is thus the concentration of spores that are activated to germinate into vegetative cells during initial processing involving a heat step. 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 must have been C_m/η, of which a fraction $(1-\eta)$ remains un-activated after the heat step. 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).[20]

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.

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 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 CP 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 – 2.7×10^3 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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 is 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[21] (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).

[21] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.[22] 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.

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.

[22] 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 $a_s < a_t < a_f$ is not true are censored during the calculations, to enforce the 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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 casicums, 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
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, and others	<10 – 650	0 – 89% of from 1 to 9 samples
Baxter and Holzapfel, 1982[b]	Various spices and herbs	Detection limit not specified	None detected

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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.[23] 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 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	•	•	

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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	

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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	

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 3.9.3), and applied to the estimate for the initial concentration of spores, so that

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

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.

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 is 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. For this risk assessment, no effect of water activity on germination of *C. perfringens* spores was modeled.

Evidence suggests that pH affects germination 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 at both 65 and 75 °C for 20 minutes. However, in these studies Craven (1988) quantified change in germination via reduction of optical density values rather than by enumeration. The effect of pH on germination of *C. perfringens* spores is thus unclear; moreover, pH levels of the foods examined here are not available. No effect of pH on germination was modeled in this risk assessment.

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.

This information has been peer-reviewed under applicable information quality guidelines. FSIS is distributing this information for public comment. It may be revised upon review of public comment.