Peer Review Comments and Responses

for

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

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by

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Introduction

This document provides responses to comments provided by five external peer reviewers to a draft version of the risk assessment entitled "A Risk Assessment for *Clostridium Perfringens* in Ready-to-Eat and Partially Cooked Foods; PEER REVIEW DRAFT WITH MODIFICATIONS" dated 12 April 2004 on the title page and 15 April 2004 in the page header.

The reviewers examined the earlier draft version of the document, so the page, line, and section numbers cited by them do not match the current page, line, and section numbers of the risk assessment report. In these responses, we use the section numbering of the current draft (dated June 21 2004), except where explicitly quoting from the review draft examined by the reviewers (and the quote includes a reference to a section number). We do not cite page or line numbers in the current version, but occasionally refer to the page and line numbers given by the reviewers.

We provide the reviewers responses verbatim. After every paragraph of their general comments, and after every one of their particular comments, we provide a response, set off in double-indented style. All comments and responses are numbered (from 1 to 234) but are not otherwise labeled. Cross-referencing within this document is by response number.

Reviewers

To review the *C. perfringens* Risk Assessment report and model, expertise in the following primary areas was deemed to be required: *C. perfringens* microbiology, Food Safety, Food Processing and Modeling. Five reviewers were chosen for this task. Two model reviewers were chosen due to the complexity of the modeling efforts. Dr. Edmund Crouch was asked to provide names of those with the appropriate expertise to review the model.

The Risk Assessment Division of FSIS recruited the reviewers through SAIC. The identity of the reviewers was withheld from Dr. Crouch until after this comment and response document was completed.[1] The reviewers were, in alphabetical order by last name, Kathryn J. Boor, Kenny Crump, H. Christopher Frey, Kelly Karr Getty, and Ronald G. Labbe. The numerical order of reviews below is unrelated to this listing of names.

Evaluation Criteria

Reviewers were asked to respond to the following set of evaluation criteria to facilitate the organization and presentation of their comments.

1) Evaluate whether the C. perfringens risk assessment answered the specific FSIS risk managements questions:
    a. 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 2-$\log_{10}$?
    b. 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-$\log_{10}$?

[1] One reviewer disclosed his status, but not his identification number, to Dr. Crouch prior to the writing of this response document.

   c. What would the relative growth of C. botulinum (relative to the growth of C. perfringens) be for each of these stabilization standards?
2) Identification of data and critical evaluation of evidence
   a. Have all key studies and data been identified?
   b. Have the data been correctly interpreted and emphasized?
   c. Please address the validity and appropriateness of all input data in the model.
3) Overreaching logical structure of the risk assessment.
4) Biological plausibility of the assumptions.
5) Are the mechanics of the model consistent with known biology?
6) Review and analysis of model:
   a. Appropriateness of modeling techniques (model mathematics and equations)
   b. Example the methodologies used in the risk assessment for estimating parameters from the data
   c. Examine/check the data analyses (spreadsheets) for compliance with the methods and overall accuracy
   d. Examine/check the source code for overall accuracy
7) Have the risks been appropriately characterized?
8) Does the risk assessment identify and characterize the following:
   a. Key sources of variability and uncertainty
   b. Critical assumptions
   c. Important data gaps
9) User-friendliness of the model: Is the model documentation adequate to allow individuals to conduct "what-if" calculations and alter sensitivity parameters?
10) Clarity of risk assessment report: Has the report been written and presented in such a way to clearly communicate the important issues, how they were resolved, and the results?

Reviewer Number 1

1. Comment:

Overall impression:

I have read and considered the entire risk assessment document prepared for *Clostridium perfringens* in Ready-to-Eat and partially cooked foods. I am of the strong opinion that the model that has been developed in this document is as robust as possible, given the extreme paucity of appropriate data needed for analyses of this nature. Based on the data considered, the quantitative conclusions regarding the impact on human illnesses of changes in stabilization requirements for the foods in question are logical and defensible. However, as will be described below, the large gaps in our knowledge of the prevalence, ecology, physiology, and growth characteristics of pathogenic strains of *C. perfringens* in relevant food matrices contribute a great deal of uncertainty to the quantitative aspects of the model, which, in my opinion, are only partially ameliorated by the Monte Carlo simulations. The authors very clearly state the assumptions that were used to prepare this document, and have identified the limitations in the data and in the analyses that I believe to be most critical. In my opinion, in the absence of considerably more targeted research on *C. perfringens*, I do not believe that it will be possible to significantly improve upon the model as presented. Regarding the questions posed below, I will address those that fall within my area of expertise.

1. Response:

No response is necessary.

2. Comment:

Peer Review Evaluation Criteria for the *C. perfringens* Risk Assessment

1) Evaluate whether the C. perfringens risk assessment answered the specific FSIS risk management questions:

a. 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)?

b. 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)?

Questions 1a and b are appropriately addressed together, as the data and approach used to address these questions were essentially the same.

The major pieces of information necessary to address this question are (i) how many *C. perfringens* must be consumed to make a person ill, and (ii) estimation of the quantitative effects of changes in stabilization conditions on numbers of pathogenic *C. perfringens* strains. The authors used (at least partial) data from 4 independent studies (p. 130-131) to estimate the

number of microbes that must be consumed to cause illness. These data were clearly limited, as described by the authors. To address (ii), the authors had to make many critical assumptions. Among the most critical of these, in my opinion are that the limited data available on *C. perfringens* are representative of the true prevalence and distribution of total numbers of *C. perfringens* spores and vegetative cells that are present in vulnerable food products. We also lack a clear understanding of the prevalence and distribution of *C. perfringens* strains that cause human illness, as well as of the growth characteristics of pathogenic strains in relevant food matrices. The authors had to make assumptions regarding factors that affect *C. perfringens* numbers in food processing systems, including a lack of effect of partial cooking on vegetative cells or spore concentrations in meat products, and a lack of effect of various factors (e.g., pH, salt concentration, nitrite concentration) on *C. perfringens* spore germination. The extremely large error bars about the central estimates of illnesses associated with bacterial growth largely reflect many of the biological uncertainties that are present in the model (e.g., Figure ES-1). Clearly, experimentation would help to improve our confidence in the assumptions made in each of these areas, and would likely help to shrink the error bars. With the notable exception of the likelihood that the model generated underestimates the number of illnesses due to *C. perfringens* growth during hot-holding because the model treats each food serving as independent from all others (see p. 151), in my opinion, the majority of the biological assumptions mentioned above that contributed to the quantitative assessment of changes in stabilization conditions on numbers of human illnesses were conservative in nature. As a consequence, changes in stabilization conditions that would lead to increases in *C. perfringens* numbers are unlikely to lead to increases in *C. perfringens*-associated human illness beyond those predicted by the model.

2. Response:

We agree with the reviewer that "Among the most critical of these, in my opinion are that the limited data available on *C. perfringens* are representative of the true prevalence and distribution of total numbers of *C. perfringens* spores and vegetative cells that are present in vulnerable food products."

As indicated by the reviewer, the underestimate for illnesses caused by growth during hot-holding is noted in the document, and no further information has come to light that would allow a better estimate of the rate of such illnesses. However, that rate is, to all intents and purposes, independent of the growth allowed during stabilization of RTE and partially cooked foods.

As few assumptions as possible were made, and we agree with the reviewer that some were conservative, such as assuming a lack of effect of partial cooking on vegetative cells or spore concentrations in meat products, and that the overall effect was conservative. It is not quite so clear that the assumptions of lack of effect of various factors (e.g., pH, salt concentration, nitrite concentration) on *C. perfringens* spore germination are necessarily conservative, since the average effect of some omitted factor might conceivably be to enhance germination.

The overall bias to conservatism arose from the nature of missing information, and we have tried to evaluate the sizes of the most important biases. In most cases, assumptions were made that were the simplest plausible ones that we could

think of, given the lack of available information on which to base any alternative. Specifically for partial cooking, we lacked information of the effects on vegetative cells in practical situations, so had no basis for any alternative assumption than no effect. Only one category of food servings (category 3b) was identified as partially cooked, and such food servings contributed only 1% of the estimated illnesses at the highest examined growth of 3.5 log10 during stabilization, and less at lower growths. Thus the overestimate made by assuming no effect of partial cooking on vegetative cell numbers is minimal.

3. Comment:

c. What would the relative growth of C. botulinum (relative to the growth of C. perfringens) be for each of these stabilization standards?

In the absence of additional appropriate data, I believe that this question remains largely unresolved. Having said that, in my opinion, the analyses conducted answered this question to the best extent currently possible given the lack of specific data essential for appropriately addressing this question. As described on pages 152-153, currently existing data are inadequate for addressing this question with any level of confidence.

3. Response:

We agree with the reviewer. Some further progress may be possible, however, by examining the relative growth of *C. perfringens* and *C. botulinum* using "typical" cooling curves, although such analyses would not capture worst-case possibilities.

4. Comment:

2) Identification of data and critical evaluation of evidence.

a. Have all key studies and data been identified?

Yes.

4. Response:

No response is necessary.

5. Comment:

b. Have the data been correctly interpreted and emphasized?

The authors have done an admirable job of working with extremely limited data. In all cases, authors justified which data were used, and which were not, and have identified their assumptions, gaps, and limitations. While it is relatively easy to quickly come up with a long list of data that one would like to see in an analysis of this nature, the investment to create those data would likely be larger than the resulting public health impact would justify.

5. Response:

The analysis was restricted to risk assessment. It would be possible to use the analysis or an extension of it to perform a cost-effectiveness or cost-benefit analysis for investments to create various missing data, but that is beyond our current remit.

6. Comment:

c. Please address the validity and appropriateness of all input data in the model.

As described above, the limitations in available data have forced the authors to make many assumptions regarding *C. perfringens* numbers and behavior in food matrices. In virtually all cases, the authors have chosen to use assumptions that are more likely to over-estimate rather than under-estimate *C. perfringens* numbers in foods under the conditions considered (e.g., see p. 93, lines 27 – 29 and pages 153-155). This conservative strategy should contribution to minimizing public health risks.

6. Response:

We agree with the reviewer that overall effect of the assumptions is probably to produce conservative estimates. Page 93 lines 27-29 described how growth during storage is handled in the model if storage temperatures rise above T_{min}, the minimum temperature for growth: "This process is modeled in the risk assessment by assuming that vegetative cells in RTE and partially cooked foods are ready to enter the exponential phase of growth with no delay period, and applying the growth rates obtained in Section 3.10 for the duration of storage," and pages 153-155 discussed the "what-if scenarios" of overgrowth by psychrotrophic spoilage organisms and consumer detection of spoiled foods.

We have tried to list in Section 4 the modeling assumptions made that might be important (the assumption about growth during storage is listed there), and have analyzed the potential effect of some assumptions in the "what-if" scenarios of Section 6.5.

7. Comment:

3) Overreaching logical structure of the risk assessment.

The risk assessment is logically assembled. I will make specific suggestions for improved clarity of presentation in a separate section at the end of this document.

7. Response:

No response is necessary here. See below for responses to specific suggestions.

8. Comment:

4) Biological plausibility of the assumptions.

5) Are the mechanics of the model consistent with known biology?

I perceive questions 4 and 5 to be similar in nature, therefore will address them together. Given the limitations of the existing data, and the need to protect public health, many assumptions were required to complete this work. The assumptions are clearly stated in the text and appendices, with the most important listed on pages 127-128. All stated assumptions are biologically plausible and consistent with current knowledge.

8. Response:

No response is necessary.

9. Comment:

6) Review and analysis of model:

a. *Appropriateness of modeling techniques (model mathematics and equations),*
b. *Examine the methodologies used in the risk assessment for estimating parameters from the data,*
c. *Examine/check the data analyses (spreadsheets) for compliance with the methods and overall accuracy,*
d. *Examine/check the source code for overall accuracy.*

This question is outside of my specific expertise.

9. Response:

No response is necessary.

10. Comment:

7) Have the risks been appropriately characterized?

In my opinion, the *C. perfringens*-associated risks likely to result from changing stabilization parameters for RTE and partially cooked foods have been thoroughly identified and appropriately characterized.

10. Response:

No response is necessary.

11. Comment:

8) Does the risk assessment identify and characterize the following:

a. Key sources of variability and uncertainty

b. Critical assumption

c. Important data gaps

Yes, to all three criteria.

11. Response:

No response is necessary.

12. Comment:

9) User friendliness of the model: Is the model documentation adequate to allow individuals to conduct "what-if" calculations and alter sensitivity parameters?

Outside of my specific expertise.

12. Response:

No response is necessary.

13. Comment:

10) Clarity of risk assessment report: Has the report been written and presented in such a way to clearly communicate the important issues, how they were resolved and the results?

The report is clearly written. The authors used minimal jargon and the results are presented in a logical fashion. In the next section, entitled "specific comments", I will address items that I believe should be addressed to further improve the final report.

13. Response:

No response is necessary here. See below for responses to specific comments.

14. Comment:

Specific comments:

p. 12, lines 7-10. The authors state that a secondary purpose of this report is to examine whether steps taken to "limit the potential effect of contamination…". This report does not address contamination of foods with either *C. perfringens* or *C. botulinum* (except in describing the limited prevalence data that currently exists). The focus of this report is on factors that prevent germination/outgrowth of these organisms. This point should be clarified in the executive summary.

14. Response:

Agreed, the sentence may be misleading. We have altered the executive summary to read: "A secondary purpose was to examine whether steps taken to limit the germination and growth of *C. perfringens* occurring in raw ingredients of RTE and partially cooked foods would also be adequate to protect against germination and growth of similarly occurring *Clostridium botulinum* bacteria."

15. Comment:

p. 12, line 33. Missing period after RTE foods

15. Response:

The period has been inserted.

16. Comment:

p. 15 and 16, lines 13 – 19 Figure ES-2. The description of Figure ES-2 is not clearly written. *C. perfringens* growth is not shown in FOUR circumstances as described in line 13, but rather, three. The legend states that the figure shows how "growth rate" of *C. perfringens* and *C. botulinum* differ, but really, all that is shown is that *C. botulinum* appears to grow better at lower temperatures and less well at higher temperatures than *C. perfringens*. It would be better to be specifically descriptive in the executive summary, rather than assuming that the reader will draw the same conclusions by reading this legend. Also, while the growth media for *C. perfringens* are given, it is not given for *C. botulinum*, so it is not possible to know how to make the appropriate comparisons between the organisms from the data presented.

16. Response:

Four has been changed to three.

The legend of Figure ES-2 and Figure 3-4 has been changed to be more specific both as to what is shown in the figure and the media used — "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." This figure shows somewhat more than the reviewer suggests, in that the curves shown are fitted to and summarize substantial amounts of data.

The description in the executive summary has been altered to read:

"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 can grow 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 and higher temperatures."

17. Comment:

p. 19, line 41. Define RAD. Also good to state here by whom the risk management questions were presented to RAD.

17. Response:

The acronym has been entirely removed, since it occurs only once. The questions were presented by The Office of Policy, Program & Employee Development (OPPED) of FSIS, and that information has been added.

18. Comment:

p. 22, Figures 2.1 and 2.2. Y axes should be labeled "number of outbreaks"

18. Response

The labels of Figures 2.1, 2.2, 2.3, 2.5, and 2.6 have been corrected to show number of outbreaks (plural).

19. Comment:

p. 23, Figures 2.4 and 2.5. Y axes should be labeled "number of cases"

19. Response

The label of Figure 2.4 has been corrected to show cases (plural). Figure 2.5 shows outbreaks (see previous response), as indicated in the text.

20. Comment:

p. 30. Figure 3.1 should be revised for clarity. It is not clear what an "X" in a box means. The boxes in the "heat step(s)" line are not intuitively easy to understand. It appears that the boxes on the left refer to the outcome of vegetative cells being heated, and that the boxes on the right refer to outcome from spores being heated. If that is the case, then why are there spores in the left-hand boxes, and what does the arrow pointing to the left represent? Are the vegetative cells in the right-hand boxes meant to represent those resulting from spore activation by heat? Why is "(put at beginning of storage)" listed after "germination during storage"? What is the box with the X in it in the "cook" step?

20. Response

Figure 3.1 has been revised, and the reader referred to the text for further descriptions. The text description now confirms that the boxes on the left indicate what happens to vegetative cells, and those on the right what happens to spores, that arrows indicate activation/germination of spores to vegetative cells (after

vegetative cell killing); so there are never any "spores in the left-hand boxes" or "vegetative cells in the right-hand boxes". The "(put at the beginning of storage)" was unnecessary in the figure and has been deleted. As explained in the text, an X indicates complete killing of vegetative cells.

21. Comment:

p. 36 line 32. As "j" is not a number, probably better to state "spice" designated by j, rather than number j

21. Response

The reviewer is perfectly correct: "*j*" is a symbol that in principle can represent an arbitrary index, not necessarily a number, although in practice here, j represents an integer in the range 0 to 3. To clarify, the definitions below equation (3.1) have been augmented with:

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

and all occurrences of "number *j*" have been replaced by "indexed by *j*"

22. Comment:

p. 66, line 20. Extra period and spaces

22. Response

They have been removed.

23. Comment:

p. 67, line 8. Should be "conditions"

23. Response

The "s" has been added

24. Comment:

p. 71, line 32. Should specify that personal communications were with Juneja.

24. Response

The parenthetical observations has been modified to "(personal communications, 2003, with L. Huang, H. Marks, and V.K. Juneja)"

25. Comment:

p. 76, Figure 3.4. Not clearly described or labeled. See comments above for p. 15-16.

25. Response:

The legend of Figure 3-4 has been changed to be more specific both as to what is shown in the figure and the media used — "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." This figure shows somewhat more than the reviewer suggests, in that the curves shown are fitted to and summarize substantial amounts of data.

The discussion of Section 3.11.2, where the data used for Figure 3-4 are introduced, has been supplemented by specifying the laboratory medium used for *C. botulinum* growth (reinforced Clostridial medium (RCM) supplemented with oxyrase enzyme). Section 3.11.3 has also been supplemented by replacing the reference "(Figure 3-4)" with "(Figure 3-4 plots the Ratkowsky growth-rate versus temperature curves with parameter values estimated from the data)"

26. Comment:

p. 78, line 17. Prefer a more precise and scientific wording than "they look almost exactly straight"

26. Response:

We prefer the wording exactly as shown and have not altered it. There is nothing unscientific about describing exactly what was done, and this observation did not require precision, which was supplied by the result of the Shapiro-Wilk test, given just before the observation. In applying the Shapiro-Wilk test it is essential to also qualitatively examine the distribution shape to ensure that we are not misled by a shape to which the Shapiro-Wilk test is not sensitive.

27. Comment:

p. 87, line 8. "that" is repeated twice in the same sentence

27. Response:

The typo has been corrected.

28. Comment:

p. 89, line 23 and throughout this section, including tables. Use of "decade/day" is unusual. Should this be "log reduction/day"?

28. Response:

The reviewer is too kind. "Decades/day" is incorrect. It has been replaced by "$\log_{10}$ reduction/day"

29. Comment:

p. 93, line 18. Should be "methods"

29. Response:

Agreed. This has been corrected.

30. Comment:

p. 93. The 4 categories of foods should be defined here in the text, around line 36. The description could be very brief, such as the description present on p. 186-7, and could refer to the appropriate spot in the appendices for further detail.

30. Response:

The following summary has been added: "Food categories were defined in Section 3.4 and in more detail in Appendix A — briefly the categories are: (1) foods with 2.2%–3% salt in the presence of nitrites; (2) foods unlikely to be reheated before consumption; (3) foods likely to be reheated before immediate consumption; and (4) foods served hot but not necessarily prepared for immediate consumption."

31. Comment:

p. 99, figure 3.12. The lines in the figure are not labeled, so the reader cannot currently distinguish between them.

31. Response:

This has been corrected to label the paired and unpaired measurements.

32. Comment:

p. 99, lines 11-12. The meaning of the sentence starting "While the food remains below…" is unclear.

32. Response:

The sentence has been replaced by "During such reheating the number of *C. perfringens* vegetative cells may initially increase, so long as the temperature of the food remains below 53.5 °C."

33. Comment:

p. 112, line 10. Is a word missing between "average" and "selected"?

33. Response:

The paragraph is confusing. It has been re-written to be clearer, as follows: "The number of food servings reported to be eaten by a sample person (and selected for use in this risk assessment) was divided by the number of days for which that person was surveyed to give the individual's servings per day (of the servings selected in this risk assessment). This value was multiplied by the person's single day sampling weight, all of these values were added together, and the sum was divided by the sum of all the sampling weights to give a weighted average servings per day of 0.677 for the sampled population (again, this refers to the servings selected in this risk assessment). Multiplying this value by the U.S. population (281,000,000, from the 2000 census) and the days per year gives a total national, annual number of servings of foods selected in this risk assessment of 69,600,000,000."

34. Comment:

p. 112 lines 16-17. While a difference of 5 billion may seem small relative to a total of 69 billion servings, it is not small. The criteria by which it was deemed "small" should be clearly stated.

34. Response:

The phrase has been rewritten to clarify that the comparison is with the total uncertainty in the assessment: "and the difference (about 7%) from the first estimate indicates that the relative uncertainty in this number contributes a small fraction of the total uncertainty in the risk assessment."

35. Comment:

p. 112, line 21. The numerical figure for the 80% of servings that are assumed to represent RTE or partially cooked foods (55.7 billion) should be stated here. Otherwise, it's not crystal clear that this is the same figure as that given on p. 143, line 15.

35. Response:

The phrase "(that is, 55.7 billion servings)" has been added.

36. Comment:

p. 152. Correct spellings of "victims" and "recorded" in footnote #81

36. Response:

The spellings have been corrected.

37. Comment:

p. 154. Correct spelling of "independently" in footnote #83

37. Response:

The spelling has been corrected (this was footnote #85, not #83)

38. Comment:

p. 158, line 6-7. A typographical error?

38. Response:

This was an error. A placeholder was inserted to indicate an incorrect statement, and we failed to write the correction. Sections 6.6.1 through 6.6.14 were extremely (in some cases incomprehensibly) terse and did not adequately describe how the numerical entries in Table 6.6 were calculated. This has been corrected.

39. Comment:

p. 159, lines 2-4. These sentences seem to be missing some words.

39. Response:

Sections 6.6.1 through 6.6.14 were extremely (in some cases incomprehensibly) terse and did not adequately describe how the numerical entries in Table 6.6 were calculated. This has been corrected.

Reviewer Number 2

40. Comment:

Review of *Clostridium perfringens* Risk Assessment

Overall assessment

My review focused upon the overall structure of the modeling process, the statistical and mathematical methodologies used to implement in the model, and the use of data in the model. I am not familiar with the literature and have made no effort to evaluate whether the literature has been adequately reviewed, or whether the best available data have been used in the risk assessment.

40. Response:

No response is necessary.

41. Comment:

My overall assessment of this report is very positive. Addressing this issue in a quantitative fashion is an extremely complex problem. Reviewing the literature, pulling together all of the data, building a biological and statistical framework for incorporating these data into a risk assessment, developing and implementing methods for estimating parameters -- often from data published in a less than desirable form -- and putting these data together in a coherent fashion so as to address the pertinent issues, was a Herculean task. This report shows evidence of a huge amount of careful thought and effort. The methods employed are generally state-of-the-art, and, in some instances, innovative. The overall modeling framework appear to be logical and well thought out. The statistical and mathematical methods used are appropriate. In some cases special effort was expended to use suitable, but somewhat nonstandard, methods to accommodate data summarized in the literature in a less than desirable form. The mathematical modeling of the growth of *C. perfringens* under various conditions was highly sophisticated. Insofar as I was able to work my way through them, the statistical and mathematical calculations were correct. The methods used to summarize the results were appropriate. The writing, is for the most part, clear and well-organized. The assumptions and limitations underlying the analysis are discussed.

41. Response:

No response is necessary.

42. Comment:

There are numerous ways in which the report could be improved. I will offer specific suggestions in my detailed comments below. The analysis is, of necessity, very complicated, and difficult for the reader to "get his hands around". The report could benefit from a clearer

presentation of the steps in overall modeling approach and from tables and figures summarizing the model, the various distributions used in the assessment, etc.

42. Response:

We agree with the reviewer to a large extent. We found presenting the amount of required information daunting, and attempted to do the best job possible within reason. We value the reviewer's remarks and have responded to specific recommendations below.

43. Comment:

The modeling approach was somewhat spotty, being highly sophisticated in some areas and relatively crude in others. It seems possible that some of these might make an important difference in the risk assessment if addressed differently.

43. Response:

We agree with the reviewer to a large extent, but argue necessity. Some areas have been sufficiently studied to allow the use of sophisticated analyses, while others have barely been mentioned in the literature. We attempted to match the depth our analysis with the limitations of the available data. We have explained the approaches adopted in more detail, and the reasons for them, in Sections 3.1 and the new Section 3.3. However, we agree that in some areas we could have missed important effects (as is always true with any such analyses), although we attempted, as in the case of overgrowth by other organisms at low temperatures, to perform some sensitivity analyses. We respond below to specific recommendations.

44. Comment:

Any risk assessment of this type will invariably have uncertain and weak components, particular when trying to evaluate the "uncertainty" in our knowledge. The amount of "uncertainty" present is usually uncertain. This assessment uses mainly objective methods, based upon statistical methodology, to quantify uncertainty through the development of uncertainty bounds for the output. Although this approach has the very desirable feature of objectivity, it does not necessarily incorporate what often are seemingly some of the most important sources of uncertainty. These include uncertainty in the adequacy of the statistical model, and the relevance of the experimental data to the different (non-experimental) circumstances to which they are being applied. The present report does not adequately address this issue. The overall method used to address uncertainty needs to be explained more carefully. At present, without wading through examples of data analysis, there is no clear picture of how uncertainty is addressed. The authors should consider a broader context for uncertainty, whether their methods are adequate to address major sources of uncertainty, whether additional uncertainties, not presently accounted for, can and should somehow be quantified – perhaps more informally – and at the least provide more discussion of this issue.

44. Response:

We largely agree with the sentiments of the reviewer, and attempted to meet such objections by listing what we considered major limitations that are not quantitatively addressed in Section 4. What the reviewer calls here the "relevance of the experimental data to the different (non-experimental) circumstances to which they are being applied" corresponds, we believe, to what we called the "representativeness" assumptions listed in Section 4 where we have specifically stated that the modeling does not incorporate such uncertainty. We did, however, fail to incorporate in our list what the reviewer brings to our attention here, namely the adequacy of the statistical models adopted. We have attempted to repair that failure by adding the adequacy of statistical models as an entry to a further sub-section in Section 4 that covers other limitations.

We agree that the report as reviewed does not clearly explain the overall methods used to address uncertainty. We have extended Section 3.1 and added a further Section 3.3 specifically for this purpose.

We also agree that some further discussion of a broader context for uncertainty is in order, and have modified Section 6.2 to be "Uncertainty estimates" in general, starting with Section 6.2.1 "Uncertainties not incorporated in the model" to emphasize that broader context before going on to Section 6.2.2 with "Uncertainties incorporated in the model" that is the Section 6.2 of the reviewed document. In particular, we draw attention here to the "what if" scenarios that informally quantify the potential sizes of some of these unincorporated uncertainties.

45. Comment:

These general comments and suggestions will be addressed more specifically below, and organized around the peer review evaluation criteria. Although I am very impressed by this effort, because of the complexity of the issues involved and the analysis, I would not feel comfortable at this point "buying into the results".

45. Response:

The reviewer appears uncomfortable with "buying into the results," but does not specify precisely what is meant by this, or how, or if, we should proceed forward to meet his objections (if, indeed, it is possible to meet them). The results of this risk assessment obviously cannot, because of the limitations of the analysis and the uncertainties involved, represent the final word on the subject of the effect of *C. perfringens* on the occurrence of diarrhea in the US population. However, such comments apply to practically any risk assessment; and our intent was not to obtain any such final word — rather, we are satisfied if our attempt "sheds considerable light" (see specific comments below) on the questions asked. To proceed, we respond to the reviewer's specific comments below, and perhaps the reviewer's discomfort might be assuaged by our responses to these and the other reviewer's comments, and by the modifications made in the text.

46. Comment:

Peer Review Evaluation Criteria
1 a,b) Evaluate whether the risk assessment answered the specific FSIS risk management questions regarding the impact upon the probability of human illness if the allowable growth of C. perfringens is raised from 10-fold to a) 100-fold or b) 1000-fold.

The report definitely sheds considerable light on these questions. However the methodology used to address this specific question seems somewhat simplistic relative to much of the remainder of the report. The report addressed this question by evaluating the effect of raising the amount of growth during stabilization from a fixed value of 10-fold to fixed values of 100-fold or 1000-fold. However, this likely is not an adequate representation of the regulatory context. Presumably a regulation regarding an allowable growth would specify a maximum value of *C. perfringens* not to be exceeded, which would be enforced somehow, possibly using periodic sampling of *C. perfringens* in food before and after the stabilization process. There would be variation from situation to situation in the actual growth, and the median growth would likely be less than the regulatory limit. Rather than modeling the growth as a fixed value equal to the proposed maximum allowable amount, a more realistic evaluation of the effect of various regulations would require a more detailed modeling of the regulatory enforcement process and the practice of food processors in response to regulations. The approach to this in the current draft report likely tends (disregarding the effect of all other steps in the risk assessment) to overstate the number of *C. perfringens,* and consequently possibly the probability of disease, while underestimating the uncertainty.

46. Response:

We largely agree with the reviewer. However, as is stated in Section 3.12, there are essentially no data available that allow any handle on the actual growth potential currently achieved in preparation of RTE and partially cooked foods. We agree that "a more realistic evaluation of the effect of various regulations would require a more detailed modeling of the regulatory enforcement process and the practice of food processors," but we do not have any such detailed modeling nor the data with which it might be built. Getting that information and building such a model would involve an effort substantially larger than the entire effort for this risk assessment so far.

The format of any proposed regulation has not been explicitly defined, although it is likely to be in the form of a limitation of the maximum allowable growth. Currently, regulation specifies that the maximum growth be less than 1 $\log_{10}$, but that is not and cannot be enforced or monitored by sampling — the concentrations and prevalence of *C. perfringens* spores are too low, and the variability too large, for any practical sampling program to be effective — and the same would be true of any alternative regulation. Instead, what is done is to predict the growth potential given a cooling curve, using a model, and take action if the potential growth exceeds the 1 log10 level. There are very limited published data on cooling curves that have been observed in various situations, but we found no information on the variation in these.

We have built the model in such way that were the variability in growth known, it could be simulated. We just have no information on that variability. As the next best thing, we have modeled the effect of fixed growths; and this has now been made more explicit.

We have also added the following paragraph to the executive summary to describe how and why the risk assessment evaluates fixed amounts of growth during stabilization.

"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 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)."

The analysis of growth of *C. perfringens* from spores performed in Section 3.11 does allow an evaluation of the minimum variability that could be achieved in growth rate for a fixed cooling curve. Thus we can evaluate the variability expected for a fixed cooling curve designed to achieve a particular growth, and that evaluation is under way.[2]

47. Comment:

1 c) What would be the relative growth of *C. botulinum* (relative to the growth of *C. perfringens)* for each of these stabilization standards.

Because of the limited data on *C. botulinum,* the report exercises appropriate caution in not drawing firm conclusions on this issue.

47. Response:

However, indeed a firm conclusion is drawn that such a prediction is not possible without further information. This is substantially different than not drawing a firm conclusion.

[2] This evaluation may be somewhat compromised by the likely stochastic variability in initial cell divisions, that may affect the variability in delay times to be expected at low spore densities. The discussion in Section 3.11.4 has been corrected to indicate that most illnesses are simulated to arise from servings with initial spore densities considerably lower than 100 CFU/g, so the delay time variability may be underestimated.

48. Comment:

2) Identification of data and critical evaluation of evidence

I am not familiar with the literature and did not attempt to evaluate the selection of data.

48. Response:

No response is necessary.

49. Comment:

3) Overreaching logical structure of the risk assessment

The overall structure appears to be appropriate. However, it suffers from lack of a clear presentation.

49. Response:

We have attempted to be clear, precise and succinct. Clearly we have not entirely succeeded, so we respond to any specific comments below.

50. Comment:

4) Biological plausibility of the assumptions

The biological assumptions inherent in the mathematical equations appear reasonable. I have not evaluated the biological reasonableness of the various parameters estimated from the data. I also have not systematically considered the biological relevance of the experimental conditions in the various studies to the situation being addressed in the risk assessment.

50. Response:

No response is necessary.

51. Comment:

5) Are the mechanics of the model consistent with known biology?

All mathematical models are wrong, but some are useful. The assumptions inherent in the modeling appear reasonable (and lead to useful models).

51. Response:

We take this as a compliment.[3]

[3] The reviewer's phrase "All mathematical models are wrong, but some are useful" appears nowadays to be a proverb. A possible source is Box, G.E.P. (1979), Robustness is the strategy of scientific model building, in

52. Comment:

6) Review and analysis of the model

My specific comments are mostly in this area and are listed below.

52. Response:

We respond to specific comments below.

53. Comment:

7) Have the risks been appropriately characterized?

The output of the risk assessment, as presented in the risk characterization section, was adequately summarized.

53. Response:

No response is necessary.

54. Comment:

9) User friendliness of the model

I did not find a copy of executable program and did not investigate its user friendliness.

54. Response:

This is unfortunate and we are unsure as to why no copy was provided. We believe that any risk assessment of this nature should be accompanied by all the original data, all the analyses performed (in this case, the spreadsheets used), and the programs used (here, because the main program is not standard, we provide source codes also; the spreadsheets also contain substantial amounts of programming, and there is a set of spreadsheet function add-ins that is used).

55. Comment:

10) Clarity of the risk assessment report

The writing is generally clear and the report shows evidence of considerable effort to communicate important issues. However, this is an extremely complex analysis, and difficult to present in a clear and understandable fashion. The report would benefit greatly by a more detailed non-technical description of the overall framework before launching into the description of the data and detailed analyses. As the model is currently presented it is very difficult to obtain

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Robustness in Statistics, (Launer, R.L. and Wilkinson, G.N., eds), pp. 201–236, Academic Press, New York, NY; "ALL MODELS ARE WRONG BUT SOME ARE USEFUL" is a subheader in the chapter on page 202.

some "feel" about whether the results are reasonable. As a first step, it would be helpful to summarize the distributions for the parameters appearing in Equations 1.1 – 1.3.

55. Response:

We have modified the general description (Sections 3.1 and 3.2), and added a section that describes the overall approach used to estimating parameter values (Section 3.3). It is difficult to make a summary of the distributions in equations 3.1 to 3.3, since a large fraction of the document is devoted to describing them. As stated in Response 56 (below), we have strengthened the links between quantities introduced in Equation 3.1 to 3.3 and the text where they are each described. As for obtaining a "feel" for the results, this is extremely difficult; we have not ourselves been able to obtain such a "feel", primarily because the results depend critically on extreme values of the storage temperature distributions (so, for example, one cannot readily perform mean value calculations to obtain estimates for the results).

56. Comment:

Detailed Comments

The parameter that controls the growth of cells during stabilization is critical and needs to be clearly identified. More generally, there needs to be a closer connection between the model (Equations 3.1 to 3.3) and the detailed development of their estimates. E.g., the parameters in the model need to be referred to using their mathematical symbols.

56. Response:

We have expanded the discussion of G_c, the parameter that controls the growth of cells during stabilization (Section 3.12). We have strengthened the connection between each of the terms in Equations (3.1) through (3.3) with the detailed development of their estimates by ensuring that each section referred to contains the symbols indicated in those equations (usually in the header of the section) and a description of how the value of the quantity represented by the symbol is both estimated from data and calculated in the program.

57. Comment:

After giving the basic model equations (3.1 – 3.3), the report describes briefly the Monte Carlo procedure, using general statements like "Choose a sample from the uncertainty distribution for each term used in Equations (3.1) through (3.3) for N" and "Choose a sample from the variability distribution of each term on the right hand side of Equation (3.3) for N". This description needs more explanation. E.g., no mention is made of at what step the food type is selected and how this is accomplished. The only figure used in describing the model (Figure 1.1) was not comprehensible to me.

57. Response:

We have modified somewhat the generic description of the Monte Carlo procedure, hopefully in a way that makes it more useful. We are at a loss to understand what the reviewer means by "no mention is made of at what step the food type is selected and how this is accomplished," since the first statement of the inner loop is precisely "Select a RTE or partially cooked food serving from the CSFII database (USDA, 2000)." We do not specify precisely how this is accomplished in this generic description, since that takes Section 3.4 and Appendix B to describe; and we do not document implementation details of the program used, through lack of sufficient time and funds and since those sufficiently interested will read the code for themselves.

58. Comment:

It would be very useful to have a more general discussion of the parameters and distributions, perhaps including a table summarizing uncertainty and variability distributions. More general explanation of the meaning of an "uncertainty distribution", contrasted with a "variability distribution", along with a description of the general process used to develop each.

58. Response:

We have modified and extended slightly the introductory material to Section 3 (in particular Section 3.1), but have not attempted a table summarizing all the distributions, since the actual distributions used are of secondary importance in a description of the process. Section 3.3 has been added to describe the general process used to develop the distributions.

59. Comment:

There needs to be a section showing the results of the exposure assessment. This section should contain tables that summarize distributions for the key parameters. If distributions for parameters in equations 3.1 – 3.3 are defined in terms of distributions for more basis parameters, some summary of the resulting distributions of the parameters in these equations should also be provided. This section should also summarize the resulting exposures, categorized by food group, and possibly in other ways.

59. Response:

We disagree that there is any such need. The selection of intermediate values in Equations (3.1) through (3.3) was done for pedagogic purposes to allow concise and precise description (in the original draft, there was just one equation), and the values obtained for them are not useful for the end point of the risk assessment. One could request that all possible intermediate values should be similarly summarized, but we see no point in cluttering the risk assessment with unnecessary intermediate results, particularly if they shed no particular light on the results of the risk assessment. This is such a case. The intermediate results

that are the "result" of the exposure assessment are of no particular interest here, and do not rise to the level that requires their display.

60. Comment:

The limitations of the uncertainty distributions need to be considered. It appears that they are calculated mainly so as to reflect only the statistical confidence regions for parameters estimated by fitting models to data. This approach has the advantage of providing a data-driven basis for obtaining numerical estimates. However, it is surely an underestimate (perhaps a huge underestimate) of the real "uncertainty". It does not reflect the uncertainty in the relevance of the data to the parameters being estimated (e.g., experimental data likely do not adequately reflect "accidents" during production runs and storage). I would think these uncertainties are often far greater than the uncertainties represented by the statistical confidence regions. The paper should discuss these limitations more thoroughly, along with possible ways to obtain better uncertainty estimates (informed guesses?).

60. Response:

We largely agree with the sentiments expressed by the reviewer, and believe that we have both considered and sufficiently emphasized them. We have listed in Section 4 and Section 5.4 many of the limitations imposed by lack of data, and attempted to emphasize the importance of non-data-driven uncertainties in these sections, in the new Section 6.2.1, and in the analysis of "what-if" scenarios (Section 6.5). As we understand the terms, the "relevance of the data to the parameters being estimated" is what we call the "representativeness assumptions" in Section 4.1. The research necessary to obtain better uncertainty estimates is summarized in Section 7 (Research Needs).

61. Comment:

The modeling appears to assume essentially that every serving is obtained from a separate production run by the producer, that is, it ignores the dependence of samples from the same batch. This will cause the uncertainty in the number of cases to be underestimated.

61. Response:

The modeling does indeed assume independence of servings. However, except in the case of hot-holding (where the dependence is induced by mixing of servings at the time of hot-holding, not at the time of production, although of course there may be an overlap in the servings so mixed), we believe that this should introduce little additional uncertainty. What sort of "dependence of samples from the same batch" would cause increased uncertainty? This would occur only if such dependence (a) caused the measurements of *C. perfringens* in raw products to misrepresent the distribution of concentrations in servings, or (b) induced a correlation between servings eaten effectively simultaneously by a single individual *and* the doses delivered were in a non-linear part of the dose-response

curve. Sample masses for the measured concentrations were typically of the same or smaller size than servings, so (a) is unlikely. It is plausible that individuals might effectively simultaneously eat servings from the same batch that had identical histories, but for the same product such an occurrence is already recorded as the consumption of a larger serving, all parts of which are already (in the modeling) assumed to have an identical history. Only if different products from the same batch are eaten simultaneously is there a problem; but it is unlikely that different products are produced from the same batch.

62. Comment:

I haven't had sufficient time to evaluate each parameter in detail, but my impression is that the approach is somewhat spotty, with a high level of and sophistication applied to some parameters and relatively crude methods applied to others. I suggest that the authors reevaluate their overall methodology for all parameters for consistency. I will use the maximum vegetative cell density to illustrate my concerns. This appears to be an important parameter, according to the sensitivity analysis, as increasing the MLE value from 8 $\log_{10}$ to 8.5 $\log_{10}$ caused a 40% increase in the MLE number of cases. The data used to estimate this parameter come from four recent studies (involving four different growth media) by Juneja and coauthors. These data were apparently not summarized in the report – at least I did not find such a summary. The description in the report discusses censoring of data by the original authors, which was sustained in the risk assessment, and does not increase one's confidence in the data. The reasons for censoring include "suspected overgrowth". I don't know what that refers to, but it suggest questions regarding the representativeness of the data used, since it seems possible that a experiment showing "overgrowth" might be important for estimating maximum cell density. A model was fit to these data, and one of the parameters estimated was C_m, the maximum vegetative cell density. Even though individual experiments collected data at several different temperatures were used in the various experiments, a single C_m, independent of temperatures, no attempt was made to account for potential differences in C_m at different temperatures reported in each study. The standard errors of the C_m estimates were not reported, nor were they used in developing an uncertainty distribution, which seems to be at odds with approaches used with some other parameters. Instead, only the four C_m estimates -- 9.9 log10, (experiments of Juneja et al., 1999) using a broth medium; 7.6 log10 (experiments of Juneja et al., 2001) in cooked cured beef; 8.07 log10 (experiments of Juneja and Marks, 2002) in cooked cured chicken; and 8.03 log10 (experiments of Huang, 2003) -- were reported. Based on these four values, and apparently without any additional formal analysis, it was assumed that the maximum cell density in <u>all</u> foods is 8 $\log_{10}$, with a variability of 0.5 in the $\log_{10}$ scale. Of the four estimates upon which this is based, one (9.9 $\log_{10}$) is four standard deviations away from the assumed central value, which would suggest that values that extreme should only occur with probability 0.00003 rather than one in four. Unless there is reason for disregarding the value from broth (If so, it should have been presented in the report.) it seems to me that there is room for considerable debate both with regard to the central value of 8 $\log_{10}$ and the variability of 0.5. The sensitivity analysis presented on this variable was helpful, but I question whether increasing the assumed central value by only 0.5 $\log_{10}$ was sufficient to portray the uncertainty in this variable. E.g., it appears that using the most extreme of the four estimated values (9.9 $\log_{10}$) would cause an enormous increase in the number of cases.

62. Response:

As we stated above, in responding to the general comments, some areas have been sufficiently studied to allow the use of sophisticated analyses, while others have barely been mentioned in the literature, and we attempted to match the depth our analysis with the limitations of the available data. In this respect we have not attempted to be consistent, indeed cannot be, since the data are lacking. The reviewer discusses the maximum cell density, for which we did not perform an extensive analysis. This quantity is discussed in Section 3.11.5.6, from which the reviewer appears to have obtained most of the information cited here. The four growth experiments mentioned (Juneja *et al.*, 1999; Juneja *et al.*, 2001; Juneja and Marks, 2002; and Huang, 2003) are discussed in Section 3.11.2; the results in each case are too extensive to be given in the risk assessment, but are given in full in the accompanying Excel workbook, so no summary was considered necessary.

As discussed in Section 3.11.5.6, we believe that the maximum vegetative cell density is dependent on the food medium, and that information from the four cited experiments, although providing some information, is not suitable for formal analysis. We therefore refrained from formal analysis, since attempting any such formal analysis would be misleading. Instead, we made a guess at a best estimate for typical foods (which is why the high value of 9.9 $\log_{10}$ was discounted, as explicitly stated in Section 3.11.5.6); and tested the effect of our guess using the sensitivity analysis.

We would, for this reason, reject the reviewer's attempt to impose a structure on the four values obtained in the four experiments cited — the value of 9.9 is 4 standard deviations from the value of 8 that we use only if you believe that broth is comparable in this respect with typical RTE and partially cooked foods as growth media for *C. perfringens*; we do not. We believe the broth to not be representative of such foods.

We agree that there is room for considerable debate about the value of 8 and the uncertainty of 0.5. That is why the maximum cell density was evaluated in the sensitivity analysis. Since the results were found to be sensitive to this value, and since we have very little information on it (as is true for other quantities in the risk assessment), it appears as item 5 in Section 7 (Research Needs).

63. Comment:

Page 13 "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."

Good comment, but I am wondering if more needs to be done in this area, e.g., a more critical examination of "how well the input data reflect what really happens" in specific instances, and whether some numerical guesses, based upon informed judgment, about unquantified uncertainties would be useful.

63. Response:

We do not know what more we can do. We performed a critical examination to the best of our ability on the available information. At this point, we believe that further guesses would not be all that useful — we have made some guesses, tested for their effects through sensitivity analyses, and listed research needs. We question what further information is available upon which to make "informed judgment," but when this document goes out for public comment, perhaps we shall learn of some possibilities; perhaps some data that we have overlooked or that is not publicly available.

64. Comment:

page 31, line 3

Spores that germinate during storage are assumed to have the same growth factor as vegetative cells that are present initially at the beginning of the storage process. Is this reasonable, given that they have a shorter growth period than the vegetative cells originally present?

64. Response:

We believe that this is a reasonable approximation, at least in this risk assessment. The fraction of illnesses predicted to be caused by spores that germinate during storage is very small, so the uncertainties introduced are similarly small.

65. Comment:

Equation 3.3

Why should only cells that germinate during hot holding grow during this hot holding. Shouldn't the factor G_h should also be applied to the number of vegetative cells present following preparation?

65. Response:

The reviewer is correct, and equation (3.3) has been modified to correct this error. However, there is no difference in the calculated results. The assumption made in the modeling is that the heating preceding hot-holding is sufficient to kill all vegetative cells and activate spores. The only vegetative cells present, therefore, are thus those arising from spores that germinate during the heating. This assumption is documented in Figure 3.1 and in Section 3.14.5, and it is included in the list of assumptions in Section 4, but is not sufficiently emphasized in text near equation (3.3), and there was some misleading text in Section 3.14. Equation (3.3) was previously written to correspond to the logic in the computer program; with the adopted assumption the first term on the right necessarily vanishes for hot-held foods, and there is no provision in the computer model even to input a distribution of heating temperatures for hot-held foods (which would be distinct from the cooking temperatures used for other foods, and from the distribution of holding temperatures, both of which are inputs). We have added a footnote to the

discussion of Equation (3.3) to clarify this point, and clarified the misleading text in Section 3.14. If heating prior to hot holding did not kill all vegetative cells, then the amount of growth during stabilization would have an effect on the numbers of illnesses caused by growth during hot-holding, and this information has been added to the discussion of results.

66. Comment:

Page 33, line 1 "Choose a sample from the variability distribution of each term on the right hand side of Equation (3.3) for N, ….".

Why not equations 3.1 and 3.2 as well? Don't these also have variability distributions?

66. Response:

The text should indeed indicate Equations (3.1) and (3.2) also, and has been corrected to do so. We have also clarified the description to separately indicate what quantities in Equations (3.1) through (3.3) have to be sampled (the various *n* are intermediate values in calculations, while other terms are derived from input values).

67. Comment:

Equations 3.1 – 3.3 and following

The discussion of selection of parameter values needs to be linked more closely to the equations, e.g., by using the mathematical notations for the parameter values in the text.

67. Response:

We have strengthened the connection between each of the terms in Equations (3.1) through (3.3) with the detailed development of their estimates by ensuring that each section referred to contains the symbols indicated in those equations (usually in the header of the section) and a description of how the value of the quantity represented by the symbol is both estimated from data and calculated in the program.

68. Comment:

Section 3.11, page 85

This section presumably refers to parameter G_c. So how was this parameter modeled? It appears that it was probably modeled by assuming the discrete fixed values used in the summary figures, although no where is this stated so far as I can tell. But does this really provide the information needed? if the purpose is to determined the effect of a change in a regulation upon the number of cases, a different analysis is needed. One that evaluates the number of cases resulting under the present regulation, and then the number that would result under a proposed change in the regulation. If the 10-fold limit (or some proposed increase) is a regulatory bound, then presumably the distribution of growth lies mostly to the left of the 10-fold value. This

distribution and any changes resulting from changes in a regulation would need to be considered in order to evaluate the effect or proposed regulatory changes.

68. Response:

Section 3.12 does indeed refer to G_c, and this has now been made explicit

Please see Response 46 for the response to the rest of this comment.

69. Comment:

page 86

The data on spore germination in favorable conditions without heat treatment and the corresponding analysis seem of uncertain relevance to storage of food products. What is meant by favorable conditions? Doesn't the time in favorable conditions make any difference? The distribution of the fraction germinating during storage and transport is not well justified by these data. It also seems questionable to assume the fraction germinating to be independent of the temperature, duration, or any other conditions of storage.

69. Response:

We agree that the distribution of the fraction germinating during storage and transport is not well justified by the data examined. If we had located any data available to estimate the effect of temperature, duration, or any other condition of storage, we would have made such an estimate. The lack of data led us to make a best guess at a range of values and examined the sensitivity of the result to that guess. Since the sensitivity is low, further research on this fraction is considered to be a lower priority research need in Section 7.

70. Comment:

page 86, line 9

How can we know that germination during storage and transport is a "minor contributor to risks"? If this is so, it raises another question that has bothered me about the results, but have been unable to resolve. It appears to me that, based on Equation 3.1, assuming risks from spores generating while foods are hot-held is a minor component of total risk, if spores germinating during transport are also a minor contributor to risk, then any increase in the growth of cells during stabilization is magnified by subsequent growths. Consequently, it is not clear to me how the number of predicted illnesses can less than double when the growth of cells during stabilization increases by more than two orders of magnitude (i. e., under these assumptions, why doesn't the number of cases increase more in proportional to the growth during stabilization?) Is it because of the maximum growth, which is itself, as discussed earlier, a uncertain quantity?

70. Response:

We evaluated whether various factors, like the fraction of spores germinating during storage, by evaluating the fraction of predicted illnesses (in the simulation) that are due to spores germinating during storages. Sufficient information is recorded during the simulation to extract this information; and if other

information is required, it is straightforward to modify the program to record such information. The information currently extracted is recorded in the workbook results.xls that should accompany the risk assessment.

The reviewer is here expressing a sentiment that we initially also held, until initial runs of the simulation informed us further. It is difficult to get a "feel" of the effect of various inputs without examining the simulation results in considerable detail (see the discussion of "feel" above). The explanation of the reviewer's confusion (which we initially shared) lies in what happens during storage. If the storage temperature is below T_{min} then essentially nothing happens. If it is above T_{min}, however, then the length of storage is usually sufficiently long that any initial number of *C. perfringens* vegetative cells are predicted to grow to stationary phase. Thus growth during stabilization has only a small overall effect — on that small fraction of servings with few cells that would not quite have grown all the way to stationary phase. In addition, as discussed in Section 6.3.3, as the growth during stabilization increases substantially, illnesses can be caused by concentrations of cells that arise entirely due to that growth (with no further growth during storage).

We have attempted to convey some of this information as to the primary causes of illness by adding a new Section 6.1.2 that includes the information discussed here.

71. Comment:

page 98

The modeling of temperatures may be a good illustration of concerns about the relevance of data. It seems possible that a good percentage of cases of sickness are due to "accidents" that are not reflected in the temperature monitoring data.

71. Response:

We share the reviewer's concern in that it is clear that the simulation indicates that most illnesses caused by RTE and partially cooked foods are due, essentially, to failures of refrigeration. The temperatures used for estimating storage temperatures are from relatively large, but non-random, surveys of consumers; but, as noted in Section 4, the risk assessment makes the assumption that the temperatures measured in these surveys are representative of storage temperatures for RTE and partially cooked foods. The surveys did not exclude "accidents," so to the extent the surveys are representative they include such accidents.

72. Comment:

pages 113 - 115

The authors are to be commended for taking pains to develop and use likelihood methods appropriate for handling data presented in non-standard formats.

72. Response:

No comment is necessary.

73. Comment:

page 116, lines 1-10

The method used to transform variables to make them more normal should be described in detail somewhere.

73. Response:

We have added a section (Section 3.3) that summarizes the methodologies used.

74. Comment:

Page 152, lines 1-7

I'm not sure about this. So long as maximum growth hasn't been attained, there should be a dilution effect.

74. Response:

That is true, but the contingency (that maximum growth has not been attained) is important since for most of the predicted illnesses maximum growth does appear to be attained. We could test the effect by simulation, but refrained because such effects are secondary to the purpose of the risk assessment (since illnesses arising from growth during hot-holding are not affected by changes in growth allowed during stabilization). Our argument is heuristic in any case, designed to give an indication of the potential size of the effect — what actually happens will depend on details about which we know little or nothing. To better represent the uncertainty, we have modified the text to be less definite; instead of "is approximately equal to the average number of servings" we write "may approach the average number of servings."

75. Comment:

page 172, line 5

This assumption is not necessary, as various threshold models will give virtually the same answer. It also could be misunderstood, and could provide a basis for criticism of the model. I suggest further thought regarding how best to discuss this issue.

75. Response:

We are unsure what the reviewer refers to here (page 172 was located in the middle of the reference list). We guess that the reference is to the discussion of dose-response modeling (Section 5.3.1, that was on page 136). However, it was already pointed out there (and in Section 5.3.3) that the assumption about the shape of the dose-response curve for an individual strain is not very important.

76. Comment:

<u>Minor comments</u>

Before Equation 3.1

Replace "initially present" with "present immediately after initial processing".

76. Response:

We have tried to be more clear and replaced "initially present" with "present immediately after to initial processing (and before chilling, stabilization, and any secondary cooking steps)."

77. Comment:

page 85 "Growth behavior of C. perfringens is a modeling method rather than an input to the model. This is the proposed control variable for regulations, and so is modeled as an input to the risk assessment."

So, is it or is it not an input? Identify the variable (G_c?) that is being referred to.

77. Response:

We have clarified this section. See Response 46.

78. Comment:

page 118

Although the end result seems O.K., the description of g(t) seems awkward, and A3.2.6 doesn't seem equivalent to A3.2.1. Better to directly define
$g(t) = k(t) \exp[-K(t)]$?

78. Response:

This is largely a matter of taste, since the equivalence is displayed. We have left it in its current form, since that is the way we approached it.

79. Comment:

page 118, possibly elsewhere

Equation numbers in text need fixing.

79. Response:

We have fixed the equation numbers.

80. Comment:

page 118

Although adding $(1 - y)^2$ to A3.2.4 may make it easier to solve, I don't see how that can be interpreted as corresponding to the assumption that "the rate of transformation decreases

quadratically to zero as y goes to 1". It seems to me that this assumption would require introducing the term 1 – y into the first equation in A3.2.1 as well.

80. Response:

The reviewer is correct that our description does not match the equations, but the suggestion given is not the one we choose. We have corrected the description to read ". . . the rate of transformation to vegetative cells is independent of cell density, but that the survival of those vegetative cells decreases quadratically to zero as $y \rightarrow 1$"

81. Comment:

page 130, line 19

The mean outside the range.

81. Response:

The text has been corrected to read "(mean of 9.8×10^8 cells, range of 7.4×10^8 to 1.3×10^9 cells)." This typo did not affect the calculations.

82. Comment:

Page 143, line 1

Why isn't 120,000 predicted illnesses (corresponding to the 1 $\log_{10}$ standard being discussed here instead of 111,000?

82. Response:

We introduced confusion here by failing to point out the difference between the current standard and the fixed growths that we modeled. We have corrected the text to read "Assuming that federally inspected plants are meeting the current 1 $\log_{10}$ stabilization performance standard, the median estimate of 120,000 illnesses at 1 $\log_{10}$ growth obtained here by modeling falls within Mead *et al*.'s estimate," with an added footnote "The modeling is for a fixed growth during stabilization, see Section 3.12, whereas we can expect variation in growth among plants meeting a 1 $\log_{10}$ standard. The median in the latter case would be smaller than the median estimated for a fixed 1 $\log_{10}$ growth during stabilization, assuming that every plant strictly met the standard."

83. Comment:

Page 146, lines 16-20

This equation and associated numerical values needs more explanation. Also, is this g the $\log_{10}$ of the G_c on line 13, page 32?

83. Response:

We have provided some more explanation, although we are uncertain as to what was unclear. The relationship $g = \log_{10}(G_c)$ has been displayed explicitly, with a reference back to the discussion of G_c.

84. Comment:

page 115, line 6

This equation is technically not quite correct if the x1 and x2 represent the minimum and maximum of the concentrations (so are functions of the experimental data), rather than some *a priori* determined limits. This is not a serious problem but perhaps is worth a comment.

84. Response:

Agreed. We called out exactly that point on the previous page (in footnote 50), and also pointed out there that the calculation is only approximate anyway because the concentration values given are only estimates.

Reviewer Number 3

85. Comment:

Peer Review Evaluation Criteria for the *C. perfringens* Risk Assessment

1) Evaluate whether the *C. perfringens* risk assessment answered the specific FSIS risk management questions:

a. 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)?

b. 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)?

The document overall addressed the probability of human illness as related to both the allowable 2-log and 3-log growth during stabilization of RTE and partially cooked products. Unfornately, there are various data holes that can give us a true picture of what may or may not happen. The research needs to improve the risk assessment model were very accurate. To better answer the above two questions, please review my Peer Review Comments document as it individually addresses various concerns with the risk assessment model.

85. Response:

No response required.

86. Comment:

c. What would the relative growth of *C. botulinum* (relative to the growth of *C. perfringens*) be for each of these stabilization standards?

Overall, little information was found and I felt like this question was lacking in additional information needed or even looking at the approach from a general food safety aspect that an adequate process will control various pathogens.

86. Response:

The examination in the risk assessment was specifically responding to the question posed, which was expanded somewhat in oral communications. The requirement was to determine whether and to what extent controlling the growth of *C. perfringens* during stabilization (that is, implicitly by temperature control) would simultaneously control growth of *C. botulinum*. There was no request to examine general food safety aspects. No change has been made to the risk assessment in response to this comment.

87. Comment:

2) Identification of data and critical evaluation of evidence.

a. Have all key studies and data been identified?

I would like for the risk assessment to at least consider three documents that study CP control during cooling/stabilization. The references are listed below and I would be happy to provide copies as needed.

Danler, R.J. 2001. Microbial safety of vacuum packaged, cooked, chilled beef and pork. Ph.D. dissertation, Kansas State Univ., Manhattan.

Danler, R.J., Boyle, E.A.E., Kastner, C.L., Thippareddi, H., Fung, D.Y.C., and Phebus, R.K. 2003. Effects of chilling rate on outgrowth of Clostridium perfringens spores in vacuum-packaged cooked beef and pork. J. Food Protection, 66: 501-503.

Vander Wal, L.S. 2002. Microbial validation of a cook-in-bag lamb in curry sauce product. M.S. thesis, Kansas State Univ., Manhattan.

87. Response:

We will discuss the first two references together, followed by the last.

1) The Danler (2003) study was previously published in the Danler (2001) thesis. The results of the 2003 report appear to be the same as the 2001 report. The language is almost verbatim, suggesting that these two publications refer to the same experiments. We will therefore only respond to Danler (2001).

Danler (2001) reports research conducted on *C. perfringens* (CP), *C. sporogens* (CS) and general microbial flora of meats (this last study includes natural unspecified Clostridia). Though CS can be used as experimental a surrogate for CP, this is typically done for *C. botulinum* (CB). The experiments reported do not provide sufficient information to directly estimate growth rates during cooling, only the integrated effect of the growth rates over a cooling curve. In light of the availability of CP growth data, there is no need to use CS growth data, and attempting to use it would add substantially to the complexity and required assumptions in the risk assessment. Additionally, for the CS studies, a dwell time of 4 hrs or more at 82 ºC was employed; such conditions are not consistent with stabilization of RTE products.

The CS studies on chilled storage provide no information useful for the risk assessment, since all measurements were below detection limits.

The CP study within this dissertation evaluates a slightly extended stabilization range from the Appendix B compliance guidelines for meeting the current stabilization performance standard. Appendix B states:

"During cooling, the product's maximum internal temperature should not remain between 130°F and 80°F for more than 1.5 hours nor between 80°F and 40°F for

more than 5 hours. This cooling rate can be applied universally to cooked products (*e.g.*, partially cooked or fully cooked, intact or non-intact, meat or poultry) and is preferable to (2) below."

The Danler (2001) study finds that the performance standard can be met for both beef and pork by extending 130°F and 80°F by half an hour. However, again, these studies provide only single point estimates of an integral of growth rate over a particular cooling curve, and are not useful for use as inputs to the risk assessment.

A similar type of study was conducted by Vander Wal (2002). In this, CP, CS and Clostridia like organisms were investigated; and the food commodity of choice was lamb in a curry sauce. Again, CP growth was evaluated in the context of a slightly extended cooling time (by 0.5 hrs). Results indicated that CP levels did not violate the current USDA stabilization performance standard. In fact, CP levels decreased in the presence of lamb curry. The article suggests that the presence of certain spices, such as cinnamon, might have contributed to limiting growth; however, there was no investigation of the specific factors that might have contributed to such limited growth. Again, this study does not provide information that could be useful to the risk assessment.

88. Comment:

b. Have the data been correctly interpreted and emphasized?

Please refer to Peer Review Comments document.

88. Response:

No response is necessary. Specific comments are addressed below.

89. Comment:

c. Please address the validity and appropriateness of all input data in the model.

Please refer to Peer Review Comments document.

89. Response:

No response is necessary. Specific comments are addressed below.

90. Comment:

3) Overreaching logical structure of the risk assessment.

Overall, the approach was very logical and many factors were taken into effect. At times it was hard to determine where the risk assessment was going or disappointing when good points were made about outgrowth but then were dropped due to lack of information. One area that I felt was lacking was information about industry processing schedules and cooling parameters. This

information could have helped to better determine whether adequate heating and cooling processes are being done by the industry. It almost seems like the question should be what is the likelihood that current practices are allowing a greater than 1-log increase of CP during stabilization.

90. Response:

We attempted to be logical in the structure of the risk assessment, and have modified some of the writing to improve the road map through it. Where data were lacking we necessarily had to simplify the approaches taken, generally by making as best a guess as possible, examining the sensitivity of the results to the missing material, and making research recommendations as appropriate. We agree about the lack of industry processing schedules and cooling parameters. Initially, time was spent assessing existing industry process data available to us though informal contact with industry and trade associations. To characterize such data across the entire processing industry with the huge variations in products, ingredients and processes being used would have been an impossible task. Additionally, contributors put strict limitations on the use of this data. , Equally important, we cannot predict what industry would do in this regard if regulations were changed. See Response 46, and the modified version of Section 3.12.

91. Comment:

4) Biological plausibility of the assumptions.

Overall, the assumptions were quite accurate.

91. Response:

No response is necessary.

92. Comment:

5) Are the mechanics of the model consistent with known biology?

Overall, to the best of my ability the model is consistent.

92. Response:

No response is necessary.

93. Comment:

6) Review and analysis of model: *(NOT ABLE TO ADEQUATELY DETERMINE)*

e. Appropriateness of modeling techniques (model mathematics and equations),

f. Examine the methodologies used in the risk assessment for estimating parameters from the data,
g. Examine/check the data analyses (spreadsheets) for compliance with the methods and overall accuracy,
h. Examine/check the source code for overall accuracy.

93. Response:

No response is necessary.

94. Comment:

7) Have the risks been appropriately characterized?

The risks were adequately determined in the Appendix but more explanation needed to be carried through to the main risk assessment document.

94. Response:

We are unsure what the reviewer means here, since the appendices deal with Food Groups (Appendix A), Food Codes (Appendix B), Foods commonly hot held (Appendix C), and using the computer code (Appendix D). We have extended the discussions and explanation in the main document in response to all the specific comments of this and other reviewers.

95. Comment:

8) Does the risk assessment identify and characterize the following:

a. Key sources of variability and uncertainty

b. Critical assumption

c. Important data gaps

Overall, the document is very comprehensive and has included the above three points. Additional clarification is included in the Peer Review Comments document.

95. Response:

We respond to these specific comments below.

96. Comment:

9) User friendless of the model: Is the model documentation adequate to allow individuals to conduct "what-if" calculations and alter sensitivity parameters? *(NOT ABLE TO DETERMINE)*

96. Response:

No response is necessary

97. Comment:

10) Clarity of risk assessment report: Has the report been written and presented in such a way to clearly communicate the important issues, how they were resolved and the results?

Overall, the document needs to have some clarification and additional information and these points are addressed in the Peer Review Comments document.

97. Response:

We respond to these specific comments below.

98. Comment:

The team is to be commended for tackling a very comprehensive and difficult project and using data to the best of its potential and for also realizing the lack of data in specific areas. Thanks for including me in the review of this document.

98. Response:

No response necessary.

99. Comment:

Peer Review Comments of "A Risk Assessment for *Clostridium perfringens* in Ready-to-Eat and Partially Cooked Foods"

Title
perfringens should be in lower case and may want to add …and Partially Cooked Foods Containing Meat and Poultry

99. Response:

We have corrected the capitalization and adopted the change in title.

100. Comment:

Acknowledgements
Page 11, line 8 add a space between Mike Ames

100. Response:

The space has been added.

101. Comment:

Executive Summary

Page 13 – As I review this document, I will take into account the sentence on page 13 starting on line 12 and finishing on line 16 as the main goal of the risk assessment – to track contamination and outgrowth to actual preparation. However, from a regulatory standpoint we are looking at control based at the processing facility in regards to the stabilization process. Therefore, it is also very important that we answer the question of stabilization control by the process for RTE and partially cooked foods.

101. Response:

No response is necessary.

102. Comment:

Pages 13, 14 – Although examples of input are given, I would like for the writers to include how cooling/stabilization parameters were analyzed and also to at least mention two important variables (salt and nitrite).

102. Response:

We have added the following paragraph to the executive summary to describe how and why the risk assessment evaluates fixed amounts of growth during stabilization.

"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 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)."

Salt and nitrite have been added to the summary as examples: "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),"

103. Comment:

Page 14, line 31 – The risk assessment answers the questions of what will happen if growth increases during stabilization. I would like for them also to include some information in this section about the likeliness of this happening given current industry cooling parameters.

103. Response:

As explained above in response to previous comments, we do not have information on current industry cooling parameters. Even with such current information, however, we could not comment on the likelihood for particular changes without an analysis of the industry that is beyond the scope of this risk assessment.

104. Comment:

Page 15, Figure ES-1 – The graph needs to be more stand-alone, explain during stabilization in the x axis. Also include the 90% confidence interval. As a ready, I like to have the foot notes below and can read the Figure without having to refer to text.

104. Response:

The caption to Figure ES-1 has been expanded to include the indicated information: "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"

105. Comment:

Page 15, Figure ES-2 – In both the text on lines 13-19 and Figure, there is not a mention of what type of system the *C. botulinum* was in (broth or meat), please include. Also, the graph is hard to determine between the different treatments because of the small boxes, triangles, etc. It would be helpful to have Ground Beef and then a line directly to the actual line in the graph. I would still question, the conclusion about whether measures to control *C. perfringens* will have the same effects on *C. botulinum* based just on the growth curve. More data should be provided to indicate why this conclusion was formed.

105. Response:

The text and caption of Figure ES-2 have been clarified to include the required information. While it may be hard to distinguish the *C. perfringens* curves at standard magnification, they are distinguishable; and it is not necessary to distinguish them for the principal points made, so no changes have been made to the figure. The assessment does not make the conclusion that measures to control *C. perfringens* will have the same effects on *C. botulinum*, but states (see Section 6.4.2), based on the qualitative differences in the growth curves at low and high temperatures, that one cannot in principle predict the effects on *C. botulinum* growth of measures solely designed to control the growth of *C. perfringens.*

106. Comment:

Overall Executive Summary – The document needs to be increased in length. Little mention was given to the various food categories and how they were divided on how risk can be associated with the categories. In addition, no mention was given about various products that included a certain level of salt and nitrite and how they were excluded from the model/risk assessment. This is a very important part as many RTE meat and poultry products can fall under this category and could likely be treated differently in regards to risk and stabilization requirements.

106. Response:

We have increased the document length in response to the comments. The precise methods used to select the servings and assign them to categories is documented fully in Appendices A and B. We have modified the description in the text to make this clear, by altering the first sentence of Section 3.4 to read "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," and added a short summary description (in Section 3.4) of what is done in Appendix A. An extensive discussion of salt and nitrite is included in Section A.3 ("Exclusion criteria"), and the methods used to exclude foods high enough in salt and nitrite are documented there. Lower levels of salt and nitrite are explicitly accounted for in the risk assessment (e.g. at Section 3.11.5.2).

107. Comment:

The summary also needs to somehow discuss that the risk assessment is for all RTE meat and poultry products and partially cooked products produced that may or may not be under FSIS jurisdiction. (I'm a little unsure how this was analyzed).

107. Response:

No specific attempt was made to determine jurisdictional boundaries in selecting food servings, as indicated in the documentation of Appendix A. All that was attempted was to determine what food servings were RTE or partially cooked that contained any amount of meat or poultry. The only manufactured products that may not be under FSIS jurisdiction are those from the CSFII that may have less than the required 2-3% meat ingredients. However, as there may be a risk associated with these foods, they were included in the risk assessment. The largest jurisdictional dispute might actually be handling at a retail level (state and local) and these distinctions were not germane to the discussion within this document. These details would be up to the risk managers.

The discussion in the Executive Summary is accurate, and has not been changed.