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 We do not cite page or line numbers in the current version, but occasionally refer to the page and line numbers given by the reviewers.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.

108. Comment:

Page 17 – Scope and Mandate – The three risk management questions: I question whether "impact" is the correct way to evaluate the risk assessment. As I feel impact looks beyond just what the number is but also at economical and regulatory effects. Therefore, I am reviewing the document more from the standpoint of whether all aspects where taken into consideration to develop a useful risk assessment tool for policy decisions.

108. Response:

No response is necessary.

108. Comment:

Page 18, lines 40-47, Page 19, lines 1-11 – Discussion is given about temperature abuse and that the majority of poisonings are not linked to RTE products produced in FSIS facilities. Therefore, if we look at a policy impact by changing cooling/stabilization requirements in FSIS plants will we have a public health impact since regulatory authority is limited in restaurants or institutions. This needs to be addressed maybe more in the Policy Context on page 19.

109. Response:

The purpose of the risk assessment is to evaluate what would be the effect of changes in regulations on growth, not to pre-suppose that there would be no effect, as the reviewer appears to imply. The section on Policy Context is not present to comment on policies, but simply to place the assessment in context, so we have not changed the text.

110. Comment:

Page 21, lines 17-23 – Is there any data on the number of CP outbreaks linked to partially cooked foods? Also, do we have any data that shows the links to products produced from FSIS inspected facilities? My question then becomes what link from a foodborne illness standpoint can we make to RTE and partially cooked products produced in FSIS facilities. Are they doing their job at controlling CP during heat and cooling?

110. Response:

Between 1990 and 1999, no *C. perfringens* outbreaks with identified etiology and vehicle have been reported as linked to partially cooked food (in the regulatory sense), but 1 of 153 has been linked to an RTE product (Section 2.2 — we have added the qualifiers as to etiology, vehicle, and time frame to the text). However, it should be recognized that most outbreaks are not reported, nor is the etiology or vehicle always determined in those that are reported. Commenting on "doing their job" is not within the scope of this risk assessment.

111. Comment:

Page 22, Figure 2.1 – Do we have any indication of why the numbers were greater in 1998 and 1999 and what the products were?

111. Response:

We have not evaluated these questions, since they lie outside the scope of the risk assessment. The source data for these figures is readily available, as cited.

112. Comment:

Page 22, line 5 – Needs to be moved closer to Figure 2.2 and would be better as a footnote.

112. Response:

We do not understand the reviewer's concern, since this line immediately preceded Figure 2.2 on the page, and is the only text line referring to Figure 2.2 (so should not be a footnote). We have removed one extraneous blank line between the cited line and Figure 2.2, but left the line in the text.

110. Comment:

Page 24, lines 11-12 (typo – delete space between 24 and %) and Figure 2.7 – The statement and graph indicate that USDA-regulated food products were responsible for 76% of total *C. perfringens* while 24% of the food sources are unknown. However, previous data mentions that it is not linked to product produced in these plants. My concern then are CP outbreaks linked to raw meat and poultry that are processed in FSIS facilities but the CP outbreak is really due to improper refrigeration during transport, cooking, holding, or cooling by the consumer or food service operation. This information is critical for determining FSIS's role in the regulatory process for setting CP stabilization standards.

113. Response:

The typo has been corrected. This risk assessment is solely concerned with RTE and partially cooked products, so we have not investigated the reviewer's concerns about other products. Questions about FSIS's role in the regulatory process are also outside the scope of the risk assessment.

114. Comment:

<u>Exposure assessment</u>

The exposure assessment is outlined very logically and includes the various steps in processing, transporting, and re-heating RTE and partially cooked meat and poultry products.

114. Response:

No response is necessary.

115. Comment:

Page 29, lines 5-7 – Processing - more steps should be added to include what is in Figure 3.1. Initially when I read these lines I thought that the initial steps and primary heating steps had not been included. Therefore, this added information would be helpful.

115. Response:

In addition to the modification of the description of Figure 3.1 (see Response 20) we have added explicit reference to Figure 3.1 in each of the steps, so that there can be no confusion. The three branches at the bottom of Figure 3.1 are now explained under the heading "Preparation (reheating)."

116. Comment:

Page 30, Figure 3.1 – Under re-heated, the option of X-ed and spores activated probably needs to be added as another potential option since veg. cells die is an option on this side. Many re-heating steps will more than likely cause complete killing of veg cells and also spores could be activated.

116. Response:

The figure accurately shows what is done in the calculations and has not been changed. The possibility of complete killing of vegetative cells is already inherent in the calculation (and in the description of Figure 3.1). While it is quite possible that this re-heating would activate spores, the assumption here is that the food would be eaten before they have time to germinate. This immediate consumption has been made clear under the heading "Preparation (reheating)" in the short summary of the steps included in the risk assessment.

117. Comment:

Page 34, lines 8-15 – More explanation is needed in this section to explain the food categories and explain foods that were also omitted from the model and how the omitted foods would be considered from a stabilization standpoint and also in regards to risk assessment.

117. Response:

The explanation the reviewer requests is already present in Appendix A, as explained in Response 106. We have modified the text to make this clear. See also Response 178.

118. Comment:

Page 35, Table 3.1 – More explanation is needed in this table. In Food Category 1 under characteristics could the initial or final ppm of nitrite be included or defined as a range. Also, define or give a range of times and temperatures for hold holding. Under Reasoning, it would be

helpful to define highest risk process as related to CP and when the risk occurs during cooling at the manufacturer or during reheating or lack of reheating.

118. Response:

It is not possible to give specific ranges for nitrite concentrations, since they are not known. Appendix A describes fully how nitrite was taken into account, and we have added statements in that Appendix that document that CSFII data can be used to infer salt content of servings (from sodium concentrations) but do not contain information on nitrite concentrations. At the time Table 3.1 was constructed, it was not known what was the "highest risk process as related to CP and when the risk occurs during cooling at the manufacturer or during reheating or lack of reheating" — that is one piece of information the risk assessment could be used to throw some light on, if necessary. However, such considerations did not go into the reasoning behind Table 3.1, so should not be listed there.

119. Comment:

Under Category 3 – It would be helpful to have a definition/footnote in regards to the abcd letters. Another concern is that some of the products may also be used in a food service operation and possible can be hot-held. Under Characteristics and Reasoning, an actual pH range in the BBQ product would be helpful.

119. Response:

We are not sure what information the reviewer wishes to see in the footnotes, since all available information is already provided in the table. The entries in Table 3.1 are descriptive, and were used only descriptively, since the information about food servings in CSFII is descriptive only. As we state in Section 3.4, in the introduction to Table 3.1, foods categories were "further separated according to likely characteristics relevant for estimation of numbers of *C. perfringens* vegetative cells in the food as eaten, using example foods as a guide." Thus we cannot provide actual pH ranges, since we do not have that information, nor was it used in categorizing food servings. As indicated in Table 3.1, all that can be done in these categorizations is to indicate the likely fate of food servings, not determine them with certainty. It is not possible to determine whether particular food servings in the CSFII (to which these categorizations were applied) with certainty, so we cannot be sure which foods are hot-held, or what fraction of foods likely to be hot-held were in fact hot-held.

120. Comment:

Under Category 4 – The pH range would be helpful under characteristics. It would also be helpful to have a range of times and temperatures for hot holding for 4acd.

120. Response:

As above (see Response 119), we do not have such information. The categorizations were based on descriptions only, not on measurements.

121. Comment:

Page 36, lines 36-38 – Needs to be a little more clear in regards to the salt content.

121. Response:

We have added the phrase "assuming all sodium is from sodium chloride" to state exactly what was done.

122. Comment:

Page 37, lines 8-9 – More explanation is needed to explain what is meant by a heat treatment, are you referring to steam pasteurization, steam vacuuming or even an acid rinse that would occur at a slaughter plant. If so have any studies looked at the reduction of CP in meat due to the above intervention strategies?

122. Response:

We are referring to the heat treatment required as a lethality step for raw meat used in RTE foods (and all the RTE foods included in the risk assessment are treated with such a heat step). The reviewer appears to be confusing this with surface cleaning steps applied to meat possibly for other purposes. To clarify, we have changed "likely kills all vegetative" with "is intended to kill all vegetative," to emphasize that the lethality step is referred to. There are many studies that have examined the reduction in CP in meat due to such interventions, but these are irrelevant to the risk assessment since the lethality step is not in question.

123. Comment:

Page 37, lines 39-44 – The USDA/FSIS baseline survey looked at incidence rate of CP in raw products and did not focus on heat treatments, this is slightly unclear when the heat treatment is mentioned. Also, did Hall and Angelotti show a high or low initial level of CP in products?

123. Response:

We have tried to remove any ambiguity about the USDA/FSIS baseline survey by stating "including a heat step in the analysis method" in place of just "including a heat step." As stated, Hall and Angelotti did not enumerate CP, so we do not know the levels. The method they used could have detected one cell in their 25 gram sample, but it was entirely a yes/no procedure. If the reviewer is referring to the prevalence they found, that is shown in Table 3.2.

124. Comment:

Page 38 – It would be helpful to add text to explain why the other three studies were chosen and what data was able to used to help with the model. One concern is that the studies have higher initial end point temperatures and it is hard to determine if any "come-up" times were also taken into account. Many RTE meat and poultry products have staged thermal processing schedules/cycles that would allow for additional lethality or even potential outgrowth. Was this taken into account?

124. Response:

The other three studies were chosen because they were all that remained to provide information, and their design appeared adequate to provide the information required. We have modified "Six studies were evaluated" to "Six studies were located and evaluated" to indicate the limited pool of studies available. We are not sure of the comparison point when the reviewer states "the studies have higher initial end point temperatures." The studies are those that give us information on the spore content of the raw meat products; we require that the initial lethality step be sufficient for effectively complete killing of vegetative cells.

As previously stated, the initial lethality step is not at issue; all vegetative cells are assumed to be killed during the lethality step in processing meat for RTE foods. It is not clear whether the reviewer is referring to additional lethality and potential outgrowth of vegetative cells originally present in the meat, or derived from spores that germinate somewhere in the process. If the former, we are not interested in those vegetative cells at this point, since they are killed at some subsequent time. If the latter, the additional lethality/outgrowth is considered part of the "growth during stabilization" term in the model.

125. Comment:

Page 39 – In the Greenberg study, it is good that samples of meat were available but a 3 gram sample size is slightly limiting considering mostly 25 g samples are currently taken. Was the small sample size taken into consideration in regards to potential false negatives?

125. Response:

The Greenberg study was used only qualitatively, to obtain the shape of the concentration distribution particularly at high concentrations. The detection limit (which depends on the meat sample size) is practically irrelevant for this purpose. However, it should be noted that the 3 gram sample size referred to is the quantity that was cultured (and this convention is adopted throughout the text), not the meat sample size initially taken. This quantity cultured is unusually large, so that the detection limit was unusually low in the Greenburg study (as stated in the text). While 25 gram samples of meat may be initially taken, the whole 25 grams

is not cultured. In all the literature examined, some small sub-sample, usually in the range 0.1 to 1 gram, was ultimately cultured.

126. Comment:

Page 40, Figure 3.2 - More information is needed to explain the figure better such as number of samples of what type of product (raw, heat treated, meat, poultry, hot dogs) were samples ground, whole muscle…Also should it be number of colonies per 3 g sample or per gram, what is the unit.

126. Response:

The reviewer appears to be misconstruing the purpose of Figure 3.2. Its purpose is not to convey the concentrations observed in Greenburg *et al.* 1966, nor in what products (both of which are adequately described in the cited reference, and, in fact, in Table 3.2 and the text), but simply to illustrate the adequacy of fitting a gamma distribution to the upper tail of the concentration distribution For this purpose the material requested by the reviewer is irrelevant, indeed misleading, since we are showing a comparison of the observed and predicted frequency of counts (pure numbers); it does not matter in what the counts occurred (they could in principle have been in samples of different sizes, for example). We have therefore added the phrase "illustrating the adequacy of fit of a gamma distribution" to the caption.

127. Comment:

Page 40, 3.4.3 – In the studies it would be helpful to explain the samples as related to the heating parameters to determine links between meat and poultry cooking cycles.

127. Response:

We have added the phrase "prior to the sampling and analysis" at the end of the sentence "All three studies included heat steps corresponding closely to those expected for RTE foods" to clarify this point.

128. Comment:

Page 42, Table 3.4 – The parameters of the heat treatments for the different products would be helpful to add to the footnotes.

128. Response:

The heat treatment used was the same for all products and was described in Section 3.5.3. We have added the sentence "In all cases the same procedure was applied to all samples" there to make the common heat treatment clear.

129. Comment:

Page 43, lines 6-10 – Although, the studies give estimates at fairly high heat processing temperatures, more research is needed to explain different processing schedules and addressing integrated lethality and how this may have an effect on the numbers.

129. Response:

The reviewer appears to be confusing the stabilization step in the model with the heat treatment step. While these two may be intertwined in actual practice, the model treats them separately. We have added the phrase "(that is, post heat treatment but prior to stabilization)" after "the data of Kalinowski *et al*. (2003), Taormina *et al*. (2003), and Eblen *et al.* (2004) were used to estimate the initial levels" in an attempt to make this clear.

130. Comment:

Page 44, lines – I have a concern that the cured and uncured data was mixed together as cured products have both salt and nitrite and these products were excluded in the model. I would consider maybe just dropping the cured and would agree with keeping everything together as the range may also represent variability of CP from plants and due to varying thermal process schedules.

130. Response:

Taormina et al. (2003) specifies which results are from cured meats and which are from uncured meats. Most of the raw meat samples used in this study have been cured in an unspecified way. However, normal curing methods are not expected to affect unactivated and ungerminated spores. The cured raw meat samples were diluted 10-fold in peptone water, stomached, and filtered to produce a homogenate that was cooked, so the concentrations of any curing agents present in the cooked homogenate samples would be unlikely to affect activation and germination of spores. Finally, the results of Taormina *et al*. were used only as an upper bound on the estimates of concentrations, since Taormina *et al*. did not confirm presumptive *C. perfringens* colonies. We therefore do not share the misgivings of the reviewer, and the analysis has not been changed.

131. Comment:

Page 45, Figure 3.3 – More explanation is needed and should also at least indicate CFU/g of meat and poultry? Also why is USDA/FSIS included instead of Eblen (is this a mistake). The USDA/FSIS data does not include heating steps.

131. Response:

We have added the phrase "concentrations in meat and poultry" to the end of the caption. The reviewer does not specify what further information is missing from the explanation, and we are at a loss to know. We have corrected the notation

USDA/FSIS to Eblen *et al*. (Eblen *et al*. are with USDA/FSIS, and the original document had a different citation involving USDA/FSIS).

132. Comment:

Page 46, lines 26-28 – It would be helpful to put the prevalence in servings based also on a typical serving size of meat and then to an entrée or to at least give the number of ounces = 100 grams.

132. Response:

We have added the sentence "The weighted average quantity of meat per serving evaluated in this risk assessment is 69.5 grams (2.45 oz.); the prevalence in servings with that quantity of meat is about 1.30%."

133. Comment:

Page 47, 3.6.1 – Examples of partially cooked products would be helpful. The upper range temperature is also needed. This information would aid in better determining if the prevalence and levels of *C. perfringens* in partially cooked products should be the same as identified in raw meat products.

133. Response:

As stated in Table 3.1, the only food codes in the CSFII database that could be identified as partially cooked were chicken patties (see also Appendix B, where all the food codes are listed). We have added a cross-reference back to Table 3.1. We do not know the upper temperature range, nor is it as helpful as the reviewer suggests because partial cooking may involve temperature gradients throughout the product.

134. Comment:

Page 51, lines 12-14 – Relate serving size back to ounces and then give an example of how much of a serving may be needed to make a person sick.

134. Response:

The conversion to ounces has been added. It is not possible to state "how much of a serving may be needed to make a person sick," since that would also depend on both the concentration within the serving (which concentration varies from serving to serving) and the strain of *C. perfringens* involved (which also varies from serving to serving). Moreover, at best only a probability value could be specified, not a definite statement as to whether someone would or would not get sick. At this point in the discussion, we also believe that any attempt to make such a statement would be out of place, since dose-response is not evaluated until Section 5. We have therefore not included any such attempt here.

135. Comment:

Page 53 – I am concerned that many of the studies related to spices are fairly old and that spices are treated much differently to control pathogens. Is there any information on treatment of spices in regards to current manufacturing practices? I feel that this is somewhat covered better in section 3.7.3

135. Response:

We share the reviewer's concern, which is why Section 7 (Research Needs) contains the recommendation to investigate current type A, CPE positive *C. perfringens* spore concentrations in spices. We did not locate published information, other than those referenced, on any relationship between *C. perfringens* concentrations and treatment of spices, or that evaluated the current prevalence of various treatments. The cited page reference was a discussion of available studies, while the cited section (now Section 3.8.3) discusses how the analyses of as-measured concentrations are used in the risk assessment, so the coverage is appropriate.

136. Comment:

Page 54 – I agree that it is best to combine the spices as for the most part, industry generally as spice companies create blends for specific products. However, may need to state the potential for over or under estimating due to the actual spices in a specific product.

136. Response:

Section 4 explicitly includes a statement to this effect, namely that "Combination of spices into the groups selected here adequately represents the spice concentrations in diverse spices."

137. Comment:

Page 59, lines 29-39 – Please include the actual water activity values.

137. Response:

Only qualitative information is required at this point, so a forward reference to Section 3.11.5.5 has been added, since the same experiments are discussed there in more detail with this quantitative information.

138. Comment:

Page 61, lines 33-37 – I'm a little concerned that only one study related to heat sensitive/resistant strains involved meat and the data showed a large fraction of spores germinating. Is the data adequate to really analyze strain differences and germination rates?

138. Response:

As indicated in Section 3.9.4 discussing the use of the results the reviewer cites, and in the Research Needs of Section 7, we share the concerns of the reviewer. The data are not adequate to analyze these differences, so we made a best estimate and used sensitivity analysis.

139. Comment:

Page 66, line 20 – Delete extra .

139. Response:

The stray period has been deleted.

140. Comment:

Page 75, Table 3.24 – More explanation of the table is needed. A footnote explaining the mathematical symbols would be good for both Table 3.24 and Table 3.23.

140. Response:

The mathematical symbols are explained in Sections 3.11.1 and 3.11.2, and a note has been added to that effect immediately following each of the tables cited. Equation 3.21 has been clarified by adding the definition of the symbol f to equation 3.15. It takes two full sections to adequately explain all the symbols, so footnotes for this purpose are impractical. The general methodology used for obtaining the tables has been explained in the added Section 3.3

141. Comment:

Page 76, lines 1-6 – Explain why this data was evaluated and also indicate that the storage temperatures are not at all typical for many meat products.

141. Response:

We have renamed Section 3.11 to "The growth of *C. perfringens* and *C. botulinum*" and modified the sub-sections accordingly, with an explanation in Section 3.11.1 that the growth of *C. botulinum* is required to respond to one of the original questions posed (see Section 1.1). The temperatures cited by the reviewer relate to growth of *C. botulinum*, not to storage temperatures, and commenting on storage temperatures here (in a section entirely on growth rates as functions of temperature and other parameters) is inappropriate.

142. Comment:

Page 76, Figure 3.4 – Similar comments as were addressed in the Executive Summary. Hard to determine treatments in the graph and was the *C. botulinum* in a specific meat or poultry product.

142. Response:

See Responses 16 and 25.

143. Comment:

Page 80, lines 19-21 – May want to also include statement that many RTE products are also cooked in casings or packages that also provides an anaerobic environment.

143. Response:

This information has been incorporated.

144. Comment:

Page 81, lines 32-38 – Is the uncertainty standard error now lower for products containing nitrite and salt compared to those with only low levels of salt as in the other Categories?

144. Response:

No. The discussion cited by the reviewer obtains an estimate of 0.582 ± 0.042 for the relative growth rate in the presence of nitrite. This relative factor (with its uncertainty) is applied to the growth rate estimated in the absence of nitrite (including its uncertainty). The phrase "This factor will be applied to all Category 1 foods" has been modified to "This factor is applied to the estimated growth rates of *C. perfringens* in all Category 1 foods" to make this clearer.

145. Comment:

Page 81, lines 42-43 – Explain the effect in more detail, increase or decrease and by approximately how much.

145. Response:

We have replaced the description with: "In their study, Juneja *et al.* (1996b, see Section 3.11.5.2) evaluated the effect of salt, temperature, sodium pyrophosphate, and pH in a laboratory broth medium. Salt appeared to be significant in various interaction terms in a model for lag phase duration (estimated from fitting Gompertz curves to experimental growth data)." As stated in Section 3.11.5.3, "the application of these results to RTE and partially cooked foods is questionable," so we did not pursue the analysis any further. In particular, we did not perform an analysis similar to that in Section 3.11.5.2, precisely because of the large disparity in lag phase durations between the broth medium measurements and the durations seen in cooked meat media. We also did not attempt to evaluate the sizes of the effect in the broth medium; attempting to use the model coefficients provided by Juneja *et al*. gave results that we considered meaningless at zero sodium pyrophosphate concentration.

146. Comment:

Page 83, lines 6-28 – I have a concern that pH can't be addressed. What about products such as fermented sausages that have low pHs below 5.0, are they less of a risk from CP because of the pH, salt and nitrite levels? Also, how do you explain the reasoning for risk in the BBQ products that potentially are more acidic? Maybe pH doesn't effect the delay phase but what about overall growth? The low pH may also be a factor in why products with higher salt and nitrite were excluded from the risk assessment.

146. Response:

Products such as fermented sausage would be considered shelf-stable (see Table A-3) because they are dried, retorted, or jar-packed, and omitted from consideration in this risk assessment as described in Section A.3.4. We noted that all BBQ sauces, tomato pastes and a number of other sauces supplied to manufacturers have rather consistent pH values (3.6-4.3) but the amount of these sauces used with various meat products is quite variable.

The relevant sentence of Section 3.11.5.4 has been modified to "An analysis of their published estimates of exponential growth rates (see Section 3.11.5.2) showed no significant effect of *pH*" to clarify where we document our analysis of the Juneja *et al*. (1996b) data showing no significant effect of *pH* on growth rates during the exponential phase. Products with higher salt and nitrite concentrations were specifically excluded from the risk assessment because they do not support *C. perfringens* growth, see Section A.3.6.

147. Comment:

Page 84 – Water Activity – I would agree with the assumption on water activity, it overall has little effect and most meat products still have fairly high water activity levels. Another factor that has been associated with water is the moisture-to-protein ratio in fermented products and jerky. This was also thought to be another safety hurdle. Research on *E. coli* O157:H7 has showed that even if the MPR is lower *E. coli* O157:H7 can still survive. Is there any research that shows a similar trend with CP.

147. Response:

Shelf-stable foods like fermented products and jerky were excluded from the risk assessment because they do not support *C. perfringens* growth, see Section A.3.4. We have therefore not investigated moisture-to-protein ratio in such foods. Survival of *C. perfringens* vegetative cells in such foods would not be important, so long as there is no growth; and spores are almost certain to survive.

148. Comment:

Page 85 – Maximum vegetative cell density – The studies need to be explained more to determine whether they were studies where inoculation of meat was purposefully done and these

were the levels that were obtained on just raw meat. It is hard to determine when the counts were taken and how is the cured aspect taken into account.

148. Response:

The descriptions of the experiments are given in the locations referenced (Sections 3.11.2 and 3.11.3). These descriptions have been enhanced to indicate the use of inoculated media, and the selection of appropriate times for analysis of counts. The "cured aspect" has no effect on the analyses, but some account was taken of this in the selection of the most appropriate estimates of growth rates (Section 3.11.3 and 3.11.4)

149. Comment:

Page 85, line 32 – Explain…and chilled (possibly twice), give an example.

149. Response:

The phrase has been replaced by "and has been stabilized"

150. Comment:

Page 87, lines 2-4 – Explain where this information came from and what are some of the parameters for cooling and stabilization. Also include examples where products such as processed meats are cold showered.

150. Response:

The phrase has been changed to "several hour" from "six hour." We have no surveys of information on cooling and stabilization parameters. We have added a parenthetical phrase "(although more rapid cooling processes are in use in some cases)" to take account of the reviewer's concern.

151. Comment:

Page 87, line 8 – delete second that

151. Response:

It has been deleted.

152. Comment:

Page 87, line 15-17 – I disagree that blast freezing should not be considered as important for products in Category 3. For food entrees and items such as chicken patties, blast freezing or individually quick frozen techniques are utilized for most of these products. These methods provide excellent stabilization and as measured by some of the research may also provide a method for killing CP cells. If this is taken into consideration, these products are much less at risk for outgrowth over 1-log during stabilization.

152. Response:

We do not deny that blast freezing or individually quick frozen techniques are excellent for stabilization. We agree with the reviewer that such methods "may" also provide a method for killing CP cells. What we accurately state is that the relationship between available research and the techniques used in industry is not clear; and we were unable to model the effect of industry techniques. This is clearly indicated in Section 4, Limitations of the Exposure Model. The overall effect of incorporating any effect for some Category 3 foods would be negligible, since the predicted fraction of illnesses due to Category 3 foods is so small (see Table 6.3).

153. Comment:

Page 87, line 40 – Check on the type of product "ground cured whole-muscle ham"

153. Response:

The description is correct. Taormina *et al*. (2003) state that the material referred to as "whole-muscle ham" was raw whole-muscle ham that was cured then ground prior to inoculation with *C. perfringens* for their experiments.

154. Comment:

Page 88, lines 14-29 – The two studies by Juneja are slightly unclear and more explanation is needed.

154. Response:

We cannot discern what aspects of these summaries the reviewer finds unclear, or what we have omitted, since they are practically identical to the other summary descriptions also provided. We have not altered them, since the summaries provide all the information we consider necessary. The cited original studies may be examined for more details, and our analysis is provided in the workbook CP_cold_storage.xls accompanying the risk assessment.

155. Comment:

Page 90, Table 3.29 – Explain "decades/day"

155. Response:

See Response 28

156. Comment:

Page 93, Lines 2-9 – These values are slightly higher than normal refrigeration and more explanation is needed in regards to how they fit into the model.

156. Response:

The temperatures mentioned do not correspond to storage temperatures, but to temperatures at which growth of *C. perfringens* vegetative cells has or has not been observed. The paragraph has been slightly re-phrased to make this clearer.

157. Comment:

Page 105, Figure 3.15 – Change the lines if not printing in color.

157. Response:

We have examined all the figures in the document, and changed them so that color is not the only distinguishing feature between any lines on them. In addition, we have attempted to ensure better clarity in the captions.

158. Comment:

Page 106, Figure 3.16 – Change patterns of lines or indicate by pointing to lines.

158. Response:

See Response 157.

159. Comment:

Page 107, lines 21-30 – Microwave cooking and even oven cooking will have variability and many times what may happen is that a person will heat a product and the middle will be slightly colder and then upon mixing have a fairly uniform product but from a microbial standpoint heating was not uniform. Might want to figure out a way to address this situation.

159. Response:

We agree. Heating patterns are likely to be complex for both oven and microwave heating, and we have also neglected the time between heating and consumption. We have modified the paragraph to indicate this, and also added a comment that the low sensitivity of the results to heating times suggests that such effects are probably not important for the risk assessment.

160. Comment:

Page 110, Figure 3.19 – The legend for Cat. 4c is hard to read.

160. Response:

See Response 157.

161. Comment:

Page 123, lines 20-25 – It is mentioned that the parameter values are with fixed temperatures. Is there any way that the model will take into consideration staged cooking cycles?

161. Response:

The reviewer is referred to the following section (Section A3.2.5) where the extension to varying temperatures is examined.

162. Comment:

Page 129, line 42 – Please explain the sentence "Only doses higher than 10^8 cells were administered. How does this relate to per serving size or the measurement of cells per g or ml?

162. Response:

The cited sentence is in a paragraph describing the available studies on human health effects caused experimentally by administering doses of *C. perfringens*. It is not related to serving size or measurements of cells per g or per ml., but is purely descriptive of the human experiments. We have modified and extended the sentence to read, "In these human feeding studies, all the administered doses were higher than 10^8 cells, so the effect of smaller doses must be conjectural."

163. Comment:

Page 134, 5.2.2 – How do these studies relate to food and what may be the significance?

163. Response:

We have changed the introductory paragraph to Section 5.2.2 to clarify the reasons for discussing these studies. It now reads, "As mentioned in Section 5.2.1, data from four studies were included in dose-response modeling. However, some of the six studies identified also included data acquired by administering strains of *C. perfringens* which are not expected to cause disease, or that were otherwise unusable in dose-response modeling. The reasons for excluding human feeding data from such studies discussed in the following paragraphs." Given the few human studies available, we believe it is necessary to document why some were excluded from the analysis.

164. Comment:

Page 136, lines 9-18 – Explain in regards to serving size and sickness and what is the CFU/g or mL.

164. Response:

We do not understand the reviewer's concern. We discuss here the theoretical possibility for a *single* bacterial cell to cause illness. Serving size and CFU/g are thus irrelevant to this discussion.

165. Comment:

Page 136, lines 40-46 – Do most studies show that outbreaks are linked to usually one strain?

165. Response:

The reviewer brings up a point that we investigated but did not document in the risk assessment; however it is sufficiently important that it should be documented. We have assumed throughout the risk assessment that illnesses are caused essentially by monoclones, or (probably) by closely related clones, since all the growth models are essentially of growth from monclonal cultures and the dose-response models are for single clones (this assumption is documented in Section 5.4 on assumptions about the dose-response curve). We assume that illnesses are the result of clonal growth in individual servings. There are a few recent studies that address this issue by comparison of the relationship of more than one isolate taken from foods and/or patients involved in outbreaks. A short discussion of this point has been added to the Hazard Characterization section, citing the papers involved (Ridell *et al*. 1998, Lukinmaa *et al*. 2002, and Miwa *et al*. 1999), and a reference back to that section has been added at the location cited by the reviewer.

166. Comment:

Page 141, Figure 5.3 – Change lines if not being able to print in color.

166. Response:

See Response 157.

167. Comment:

Page 142, lines 20-27 – Is there anyway to address the potential difference between broth versus meat in regards to infecting a person and the potential to get foodborne illness?

167. Response:

As noted in footnote 70, it was not possible to discern any effect of medium of administration in the available data. Such an effect may nevertheless exist, which is why we included the possibility in the list of assumptions (Section 4).

168. Comment:

Page 147, line 5 – delete a at the end

168. Response:

This typo has been corrected.

169. Comment:

Page 150, Figure 6.4 – Explain this figure more and what food Categories are taken into account.

169. Response:

The figure is explained in Section 6.3.3 (preceding the figure). We have added slightly to that explanation to try to make it clearer, and have added a footnote to indicate that, although all categories of foods are included in the simulations, the number of illnesses simulated is too small to obtain a reliable breakdown by category.

170. Comment:

Page 150, line 10 – Should this number be in degrees Celsius since other temperatures are?

170. Response:

We have mostly used °C throughout the text, but switched to °F here because the original measurements were recorded and reported in °F. The same is true for Figure 6.5.

171. Comment:

Page 151, 6.4 – How will the policy ultimately relate to public health? Do we have a good estimation of what RTE and partially cooked products are solely produced in FSIS facilities and if the policy will address only control by FSIS will the numbers truly increase? The risk model addresses stabilization for all situations of RTE and partially cooked foods and I can't get a clear understanding of the focus on the situation for FSIS inspected and regulated RTE products.

171. Response:

The reviewer's questions here all relate to policy decisions. However, the risk assessment is not concerned with policy. It does not discuss or put forward any policy viewpoints, nor is policy supposed to be discussed anywhere within it. Section 6.4 is purely a response to questions posed. It is thus not surprising that the reviewer cannot get a clear understanding of FSIS policies, since these are not addressed in the risk assessment.

172. Comment:

Page 152, 6.4.2 – More information is needed in regards to any predictions, what about looking even at *C. sporogenes* for additional trends as they relate to *C. botulinum*. It is still reasonable to state that overall control and an adequate process will have an impact on various pathogens.

172. Response:

We have not examined *C. sporogenes* and make no predictions whatever about that organism; nor was any such prediction within the remit of this risk assessment. With regard to *C. botulinum*, we have strictly answered the question posed, and not attempted to speculate about factors that we have not investigated.

173. Comment:

Page 158, 6.6.8 – Explain more the type of oven.

173. Response:

No specific type of oven is discussed; indeed "oven" versus "microwave" is used as a convenient synonym for slow versus rapid heating, without or with heat shock respectively (see Section 3.14.1). The dichotomy between oven types has been slightly re-written throughout the assessment to emphasize that the real dichotomy is between heating rates.

174. Comment:

Page 159, 6.6.11 – Is there a potential for products in Category 3 to be hot-held?

174. Response:

There is the potential for some servings that are placed in Category 3 to have been hot held. The categorization of servings, and their treatment in the risk assessment, is necessarily incomplete (since we do not have survey information on how the servings were actually prepared). The object of the categorization was to place servings in their most likely category, in an attempt to adequately evaluate different food handling methods (see Section 4, Limitations of the Exposure Model). We used criteria of product design and package size. We reasoned that single serving frozen meals, designed to be heated and served, would not be as likely to be hot-held as a large family or restaurant sized casserole pan of product such as roast beef slices in gravy.

175. Comment:

Page 160, lines 33-35 – Unclear in regards to the 1% value and how it is used.

175. Response:

The cited lines have been corrected to state that 1% of food servings placed in categories 1 and 4 were assumed to be hot-held, and Section 3.15.2 has been corrected to include category 1 as well as category 4. Also, to ease the reader's burden, cross references back to the relevant section of the document have been added to all the entries in the list of Section 7.

176. Comment:

Page 161, 5. – I question the approach used to determine this number and needed more information about the experiments to get a better idea of how the data was used/analyzed.

176. Response:

See Response 62. Also, to ease the reader's burden, cross references back to the relevant section of the document have been added to all the entries in the list of Section 7.

177. Comment:

Page 161, lines 44-45 – Should pork also be included?

177. Response:

The sentence has been corrected to replace "beef" with "meat."

178. Comment:

Appendix A

Overall, it wasn't until I read Appendix A that I realized that various products containing high salt levels, low pHs, low water activities, and nitrites were excluded from the risk assessment. This information needs to be in the Executive Summary. There as been some controversy over whether various products like those mentioned above need to closely follow Appendix B cooling guidelines. This information also needs to be included in the discussion about the various Categories in the main document and would be good to include in the Table to at least state that the various products were not considered at risk for CP outgrowth and were excluded from the model. It is also important to note that foods that are sold raw or uncooked were excluded from consideration. However, it is unsure that these products were not somehow considered from a public health standpoint where problems of CP are due to improper heating, cooling and hot-holding of meat and poultry products in food service or at home.

178. Response:

The executive summary has been augmented, augmenting the sentence "This is done using a computer program to perform Monte Carlo simulations" with ". . . on meat-containing food servings selected from the Continuing Survey of Food Intakes by Individuals (CSFII) (USDA, 2000). The selection of servings was

made to limit analysis to those servings considered capable of supporting growth of *C. perfringens* (omitting, for example, shelf-stable foods and foods high in salt and nitrite)."

The risk assessment does not enter any policy discussions, for example "over whether various products like those mentioned above need to closely follow Appendix B cooling guidelines"; the policy context is adequately discussed in Section 1.2.2.

A summary of the Appendix A procedures for selecting foods has been added at the beginning of Section 3.4, including a description of the exclusion criteria (including the exclusion of raw foods).

We agree that raw foods may be important from a public health viewpoint, but this risk assessment is solely for RTE and partially cooked foods, so raw foods are not considered anywhere here.

179. Comment:

Page 180, Table A-3 – Under Sausage, change to fermented/direct acidified. Under Slim Jim include fermented. Under Stick may be better to state Snick Sticks and include fermented/direct acidified.

179. Response:

No changes have been made to Table A-3. This lists the search terms that were actually used to locate potential foods for exclusion. The term "acidified" does not occur in any descriptor of included foods, so including "direct acidified" would not alter the outcome. "Slim Jim" is all-inclusive; any sub-headings is identified. The search term "Stick" would locate "Snick Sticks."

Reviewer Number 4

180. 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,b. The assessment properly answered the probability of human illness if allowable growth of *C. perfringens* is raised from 1 or 2 log. However the error factors are vast (Fig. E5-1). Considering the types of foods involved for this assessment and the fact these types are rarely involved in *C. perfringens* outbreaks (Table 3.1) due to inherent "hurdles" (curing salts, heating step) it is reasonable to conclude that the "errors" in practice would reflect the best-case scenario, i.e., fewer illnesses/million servings than the central estimates shown (Fig E5-1)

180. Response:

We agree with the reviewer's conclusion. We have in several places been conservative, in the sense of overestimating risks. We believe we have identified these places in Section 4 and Section 7.

181. Comment:

c. It is not possible to extrapolate to *C. botulinum* for reasons given in the assessment. Further foodborne disease outbreaks due to *C. botulinum* Type A and B are not commonly associated with the types of foods addressed by this Assessment.

181. Response:

No response is necessary

182. Comment:

2. Identification of data and critical evaluation of evidence: The authors have identified the prevalence and key characteristics of *C. perfringens* including enterotoxigenicity of isolates from foodborne disease outbreaks. One omitted study (Applied and Environmental Microbiology vol. 69, pg 1642, 2003) indicated the absence of enterotoxin-positive isolates from a variety (131) of food samples examined (forty non-enterotoxigenic C. perfringens isolates were obtained)

182. Response:

The cited study (Lin, Y-T., and Labbe, R. 2003. *Applied and Environmental Microbiology*, 69(3)1642–1646), examined 133 retail food samples from Western Massachusetts, finding 39 samples with between 3 and 292 CFU/g *C. perfringens* vegetative cells, and one further sample with >1,100 CFU/g. 85 of the samples (with 29 positive for *C. perfringens* vegetative cells) were meat or poultry

products, and a further 20 samples (with 5 positive for *C. perfringens* vegetative cells) were instant soups and dry seasonings (the remainder were fish and vegetables, so not relevant to the risk assessment). None of the *C. perfringens* detected were positive for the *cpe* gene. It was not specified whether the meat samples were raw, cooked, or otherwise processed, although by implication the meat and poultry was probably raw.

The prevalence and concentration results of this study are not directly usable in the risk assessment, since we were concerned with concentrations of *C. perfringens* vegetative cells and spores in raw meats entering processing plants for RTE and partially cooked foods, not in retail stores; and there are potentially different opportunities for contamination at the two types of location. Moreover, the geographic coverage of this study is limited (and the time frame is not specified). However, the prevalence and concentrations of vegetative cells appear similar to those in the studies examined (Table 3.8), although we have not attempted a formal analysis. The instant soup and dry seasoning results cannot be used in the risk assessment, or even compared with the values used in the risk assessment, without knowledge of the number of each type tested (only the sum is presented).

The absence of *cpe* gene in all samples tested might be usable to refine the estimate of the fraction of *C. perfringens* that are type A, CPE-positive in food samples. However, the observed fraction of *cpe*-positive isolates (0/29 for meat and poultry products; 0/5 for instant soups and seasonings) is consistent with the results used in the risk assessment (Table 3.19), and the added information would be low because of the small number of isolates. We have not added these results to the analysis, because the small information gain is outweighed by the effort involved.

183. Comment:

7. The risks have been appropriately characterized.

183. Response:

No response is necessary.

184. Comment:

10. The report clearly communicated the important issues.

184. Response:

No response is necessary.

Reviewer Number 5

185. Comment:

Review of "A Risk Assessment for Clostridium Perfringens in Ready-to-Eat and Partially Cooked Foods"

The peer review evaluation critera for the study on "A Risk Assessment for Clostridium Perfringens in Ready-to-Eat and Partially Cooked Foods" include the following:

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-$\log_{10}$?
   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?

The main focus of this review is on the following criteria: 1, 3, 6a, 6b, 7, 8, and 10. Criteria 2 is not a main focus of this review because the reviewer is not an expert in C. perfringens, and therefore is not in a position to comment on whether there might be studies or data that were not identified by the authors. However, some comment is provided on aspects of this criterion. Criteria 4 and 5 were not included in this review because the reviewer is not a biologist. Criteria 6c, 6d and 9 were not addressed mainly because of lack of adequate time given the short time frame to provide comments. For example, the time required to examine line-by-line computer code was beyond the time resource available. Moreover, the authors could report on the results of efforts to verify the model and the computer implementation of the model. Some comments along these lines are given in the following.

185. Response:

We have not specifically attempted to verify the model. Unfortunately, the data to do so do not currently exist, and their existence would, to some extent, negate the necessity for modeling — such data would include measurements of *C. perfringens* concentrations in foods during and after processing, and as it is transported from manufacturer to retailer to consumer. All the information that we could locate has been used to estimate parameters of the models, and we have attempted to list critical assumptions of the modeling in Section 4 (for the exposure modeling) and Section 5.4 (for the dose-response modeling).

Verification of the computer implementation of the model has also been very limited. We are relatively certain that the computer model does what we intended, since the code is written in highly modular form using pre-tested modules where possible, and we have tracked and verified the computations on individual servings throughout the model during debugging activities. The code for selecting servings[4] was checked by counting the result of selecting approximately 100 billion servings, and performing a chi-squared test for comparison with the expected numbers. Almost all other distributions are standard distributions, which have been individually tested. During this process, we updated some of our code for these distributions to use the fastest

[4] This uses binary search in the cumulative frequency table for servings, but each search is speeded up by pre-selecting a limited range for each search (this occurs during initialization of the program). The speed-up is by a factor of about two.

implementations that we could locate in the literature.[5] The underlying 64-bit linear congruential pseudo-random number generator is not the best now available, but was retained since it appears to be adequate for the task.[6]

To improve the possibility of others locating errors, our entire source code is included along with the risk assessment.

186. Comment:

Criteria 1: Does the risk assessment answer the specific FSIS risk management questions?

The report clearly states, on page 17 in Section 1.1, the three FSIS risk management questions. In Section 6.4, the first two of these questions are clearly answered. The analysis is highly responsive to and focused upon the first two risk management questions. Despite the complexity of the model, the answers to the risk management questions are relatively easy to summarize, especially in response to the first two questions as is done in Section 6.4.1.

186. Response:

No response is necessary.

187. Comment:

In addition to the quantitative answers to the questions, the report discusses qualitative factors that could lead to biases in the estimates, such as abusive hot-holding. It would be helpful if the authors would make a post-hoc estimate of uncertainty that takes these considerations into account, if at all possible.

187. Response:

See Response 233, where this specific comment is addressed.

188. Comment:

The authors argue that the third risk management question cannot be answered based only on information regarding C. perfringens, since estimation of growth of C. botulinum is not directly proportion and requires additional information. The rationale for not providing a more detailed answer to the third risk management question appears to be compelling, implying perhaps the need for more resources to adequately deal with this question, since a perhaps similar or parallel assessment of C. botulinum would likely be required.

[5] Speed is essential in this code, and various speed-up techniques have been applied throughout. The number of simulations required is unusually large for such simulations, because of the low incidence of illnesses.

[6] In particular, the cycle length is adequate to prevent repetition even for the hundreds of billions of servings simulated in the uncertainty analysis.

188. Response:

See Response 3.

189. Comment:

The authors perform a useful assessment of "what-if" issues pertaining to spoilage organisms and detection of spoilage by consumers. Thus, although some of the difficult to model or unmodeled factors might imply a higher risk estimate, consumer detection of spoilage and discarding of spoiled food would argue for a lower risk estimate. The "what-if" analysis requires assumptions, of course, and the assumption of the probability to dispose of food servings as a function of temperature appears to be highly speculative in its specific quantitative estimates, although intuitive plausible in terms of the general trend. The authors might comment on the plausibility of the assumption they make in this regard. Could similar "what-if" analyses be conducted for the issues raised in Section 6.4.1 pertaining to abusive hot-holding and the probable bias in reporting?

189. Response:

The reviewer is correct, and we have added the following cautionary paragraph to Section 6.5.2: "We emphasize that Equation (6.3) is almost completely speculative, and is used solely for this "what-if" analysis. No reliance should be placed on the particular shape of this curve, since only at the upper end (around 8 $\log_{10}$ cfu/gram) have we any evidence for potential detection of contaminated food (see Footnote 90)."

It would be possible to add what-if scenarios for hot-holding, but they do not advance the primary aims of the risk assessment, since the number of predicted hot-holding illnesses are essentially independent of the growth during stabilization (with our assumption that heating prior to hot holding is sufficient to kill all vegetative cells).

190. Comment:

Criteria 2: Identification of data and critical evaluation of evidence.

The report provides a well-structured presentation of information regarding hazard identification in Chapter 2, including information on effects, incidence, epidemiology, summary information of specific outbreaks, and clinical presentation of C. perfringens Type A food poisoning. The evidence presented regarding hazard identification establishes that C. perfringens poses a hazard and provides the key characteristics of the hazard, including steps in pathogenesis, especially susceptible subpopulations, and especially important scenarios of exposure (e.g., temperature abuse in institutional and restaurant settings) that are of importance to structuring the risk assessment. The main hazard is diarrheal illness, but this illness can combine with other complications to cause more severe effects, especially for elderly.

190. Response:

No response is necessary.

191. Comment:

Table 3.1 provides a rationale for the identification and selection of food categories that are included in the risk assessment. The categories are based upon time-temperature histories expected. Thus, foods are grouped into a category if they will have similar heating and hot-hold patterns, even though the foods may be different in terms of whether it is beef, chicken, or other examples. This categorization seems reasonable from a modeling perspective.

191. Response:

No response is necessary.

192. Comment:

From the CFSII database, information is obtained regarding the mass of the serving, meat constituent fraction of the serving, and fraction of the serving that is a particular type of spice. The salt content is also obtained and is used to modify growth rate estimates.

192. Response:

No response is necessary.

193. Comment:

The authors do a good job of explaining why some studies were excluded and others included as a basis for input information to the risk assessment. There is extensive citation of literature and databases. The authors are very consistent in clearly identifying which studies were considered for a particular model input, why some studies were excluded and why others were included as a basis for quantifying inputs to the model, and how the quantification was done.

193. Response:

No response is necessary.

194. Comment:

Criteria 3: Overreaching logical structure of the risk assessment.

The risk assessment follows accepted practice in terms of overall structure. The major risk assessment steps of hazard identification, exposure assessment, hazard characterization, and risk characterization are made clear by the structure of the document. Each of these four major steps has a dedicated chapter.

194. Response:

No response is necessary.

195. Comment:

Key factors considered in the risk assessment include types of RTE and partially cooked foods eaten, serving size, frequency with which they are eaten, and the number of C. perfringens cells in each serving. There is variation in the characteristics of each serving, including how they are treated. There is also uncertainty because of lack of knowledge. Variability and uncertainty are quantified using Monte Carlo simulation.

195. Response:

No response is necessary.

196. Comment:

Variability is quantified using probability distributions. For example, the concentration of C. perfringens spores in raw meat varies from time to time and from place to place. Each serving of RTE or partially cooked food differs in size and composition.

196. Response:

No response is necessary.

197. Comment:

The text on page 28 is not entirely clear as to how uncertainty is separately characterized compared to variability. The text here seems to be written for a general audience, but as a result suffers from lack of clarity and could benefit from clearer use of technical terms. For example, the authors often use the term "parameter", which is confusing because this term is used both to refer to inputs to a model as well as parameters of a model (the latter are constants that are typically selected based upon some type of calibration process). For example, a gamma distribution for variability has two parameters, which can be estimated by fitting a distribution to data. In contrast, quantities such as the size of a serving, are inputs to the model, not parameters. The authors are strongly encouraged to consider the use of terms "input" and "parameter" as referring to two different types of quantities. A glossary of some terms might also be helpful.

197. Response:

We agree that the issue of nomenclature is confusing, and the review draft attempted brevity with a lack of specificity; the result at the cited location was, as the reviewer notes, undoubtedly confusing. However, the problem is a little more difficult here than implied by the reviewer because of the presence of both variability and uncertainty, and the lack of standard nomenclature. Thus the parameters of the variability models are not constants, but are random varieties derived from the uncertainty distributions; only the parameters of the uncertainty distributions are constants, hence parameters in the sense given here by the reviewer. The reviewer's recommendation on the definition of parameters is thus incomplete. The size of a serving is indeed an "input" in one sense, in that the variability distribution used here is the empirical distribution; but that is not

fundamental. We have re-written the offending section of Section 3.1 to clarify these issues.

A glossary is useful if terms have accepted definitions across all fields. We have not attempted such a glossary in this risk assessment, because we found substantial cross-specialty mixing of definitions — terms mean different things to different specialists. Where possible, we have used such terms with the appropriate meaning in the appropriate places in the assessment.

198. Comment:

The use of the term "guesswork" on page 28 is somewhat questionable. Perhaps it was truly "guesswork" or perhaps the authors are intending to be extremely frank about the lack of pedigree of some of the available information. On the other hand, if there was a process of expert judgment, rather than just guesswork, it would be better to describe this process and to describe it as being based upon expert judgment (if applicable).

198. Response:

We truly meant "guesswork." There are values, such as the fraction of the selected foods that are RTE or partially cooked, and the fraction of Category 1 and 4 foods that are hot held, on which we have essentially zero information, and for which we could identify no useful surrogates (in the sense of having more information available for those surrogates). The values used were not obtained by expert judgment or any formal process; they are literally guesses.

199. Comment:

Aside from the "big picture" steps of risk assessment, the authors also describe four key steps that pertain to the exposure assessment. These steps include: (1) processing of food (pre-retail); (2) transportation and storage; (3) preparation (e.g., reheating); and (4) hot holding. This structure is consistent with information presented in the hazard identification chapter, which implies that "temperature abuse" is a key concern. Thus, the model structure enables evaluation of a variety of temperature history patterns.

199. Response:

No response is necessary

200. Comment:

Criteria 6a: Appropriateness of modeling techniques

The mathematical equations presented in the exposure assessment chapter, such as Equations 3.1, 3.2, and 3.3, appear to be appropriate. The use of integer numbers for the number of cells is appropriate. The assumption that growth or death processes are not constrained by integer values does not seem like a significant limitation of the model, and no doubt simplifies the model formulation. As the authors appropriately point out in footnote 5 on page 32, the rounding to the

next lowest integer value will have little effect on the accuracy of results if the number of cells is reasonably large (e.g., more than a few thousand). As noted in the hazard identification chapter, a substantial number of cells are required to cause an adverse effect. Therefore, this limitation is quite reasonable.

200. Response:

The initial number of vegetative cells and spores in servings that are predicted to cause illness is often very low (it is common for a single initial vegetative cell to be predicted to cause illness). The discussion of Section 3.11.4 was previously incorrect in suggesting that most illnesses arise from servings with cell densities of order 100 CFU/g, and this has been corrected. The variability between servings may thus be underestimated by the approaches taken in the risk assessment, particularly in assuming the same fixed growth during stabilization for all servings is possible. However, no alternative method of analysis currently exists — a full analysis would require replacing the deterministic growth equations with a probabilistic approach, and no experimental data are currently adequate to support the development of such an approach. We have added an additional assumption in Section 4 to indicate the existence of this potential problem, namely "The variability incorporated in the growth modeling is adequate to represent the stochastic processes that probably occur at low cell densities (particularly the likely stochastic variation in delay times)."

201. Comment:

Footnote 6 on page 33 refers to the numerical precision of the simulation. Suggest that the term "numerical precision" be used instead of "numerical uncertainty," although the point made is of course correct.

201. Response:

We have adopted the suggested nomenclature.

202. Comment:

A number of inputs are assigned distributions based upon expert judgment, rather than data analysis. For these inputs, it appears that the distributions are intended to represent variability (e.g., fraction of spores that germinate in favorable conditions). The lack of knowledge associated with specifications of these distributions are dealt with in sensitivity analysis. However, in principle, uncertainty regarding end points and modes of triangular distributions could be incorporated into the uncertainty dimension of a two-dimensional Monte Carlo simulation.

202. Response:

Indeed, that would be true, except that we have no idea about the size of the uncertainty. The "expert judgment" in these cases is limited to either evaluation of the very few studies that are available, or pure guesswork. Attempting to

invent uncertainty distributions would strain credulity, and we believe would not improve the analysis.

203. Comment:

As the authors note on page 71, further testing of the effect of the variance parameter *a* might be appropriate.

203. Response:

This would be possible, but is extremely labor intensive (it essentially requires the complete re-analysis of the growth experiments, although the spreadsheets are set up to do that). It would also not have a significant effect on the risk assessment, since the only part of the assessment that is directly affected is in the evaluation of growth from spores during hot-holding. The number of illnesses caused by such growth is independent of the growth during stabilization, so is of secondary interest to the risk assessment; and the uncertainty in the number of such illnesses is already extremely high because of our lack of knowledge of the fraction of servings that are hot held and because of the possibility for cross contamination during hot-holding.

For the main part of the assessment, only the growth rates during the exponential growth phase are used; and these are (or should be) practically independent of the estimated value of *a*.

204. Comment:

There is a complex presentation of the methods used to model growth rates, but there is not sufficient information presented (e.g., in Section 3.10.3) for the reader to evaluate the goodness-of-fit or agreement of the model with the data used to calibrate the model. A graphical comparison of the model with the data, for example, would have been helpful. The comparison of the model to other studies is useful, and the basis for the bias correction multiplier of 1.739 appears to be reasonable.

204. Response:

There are so many growth data that presentation of fits would take a huge amount of space, and provide little useful information. Examples of comparison between primary model fits and data are presented in the accompanying spreadsheet (CP_fixed_temp.xls), and all the data and predicted values are given there (allowing the interested reader to construct more graphics). Similar fits to mathematical functions with similar shapes as the primary model used here are shown in several of the original papers, and there is little to choose between primary model functional forms. In the literature generally, formal goodness-of-fit estimates to such primary models are unusual, because the fits are usually obviously within the range of variability of the experimental systems used; large deviations are usually explained or explicable by observed upsets in the experimental systems.

205. Comment:

In general, the modeling techniques appear to be reasonable, and are based upon citations to literature (e.g., with respect to issues such as growth rates).

205. Response:

No response is necessary.

206. Comment:

Page 91 – the authors should comment on the extent to which negative estimates for standard deviations of the lognormal distribution were realized. If this occurs infrequently, then this is approach is probably okay. If substantial truncation was required, then this is approach may not be the best choice.

206. Response:

The footnote describing the occurrence has been modified to say "This occurs less than 0.001% of the time for temperatures above 0 °C and less than 4% of the time for temperatures below 0 °C, and in such cases the standard deviation is set to zero. This approximation was considered adequate, because the uncertainty in death rates during cold storage contributes so little to the overall uncertainty."

207. Comment:

The approach for intra-household serving to serving variability is based upon some assumptions. The sensitivity of the results to these assumptions should be evaluated.

207. Response:

The reviewer is correct, but the primary assumptions are of a form that cannot be adequately evaluated using sensitivity analysis, since they are representativeness assumptions (and we have no alternatives to evaluate in any sensitivity analysis). This is acknowledged in Section 4 (Limitations of the Exposure Model).

208. Comment:

Minor comment: "D-value" should be defined in Table 3.31 for convenience of the reader.

208. Response:

A summary definition has been added as part of footnote b.

209. Comment:

The authors might comment on the consistency of input information for this risk analysis. In some cases, what appear to be somewhat crude judgments are made with regard to triangular distributions, while in other cases very complex calculations are performed to arrive at distributions or parameter values for specific models.

209. Response:

See Response 43.

210. Comment:

For cases in which the authors state that the uncertainty of a distribution was considered small enough to ignore, it is preferred if the authors give a quantitative criterion for a threshold of uncertainty below which the uncertainty can be ignored, and some justification for this assumption (e.g., by comparison with more important sources of uncertainty in the assessment).

210. Response:

It is difficult to respond to the reviewer's comment without a specific example. Where meta-analyses of experimental data were performed, uncertainties were always included. Where empirical data were used as inputs, they were considered representative, so the only uncertainty is incorporated in the representativeness assumption. In two cases (Figure 3-15, and Figure 3-17), fitted distributions were used in place of the empirical distributions that were considered representative, and the resulting uncertainties are so obviously trivial that no formal analysis was performed. For quantities that had to be evaluated by sensitivity analysis, no uncertainties can be assigned, as discussed in the text.

211. Comment:

Values that are treated as assumed constants, e.g., fraction of servings that are hot-held, are good candidates for sensitivity analysis.

211. Response:

That is correct, so such values are indeed treated by sensitivity analysis, see Section 6.6 in general, and Section 6.6.11 in particular for the fraction of servings of Category 1 and 4 that are hot-held.

212. Comment:

The appendices of Chapter 3 are very useful. However, these could be placed as appendices at the end of the report to have better flow of information in the main body of the report.

212. Response:

We have maintained the current layout, not through any great preference but because the effort involved in changing the layout exceed the utility of it.

213. Comment:

In comparison to the complexity of the model, the results that answer the risk management questions appear to be relatively simple. The authors might provide some context to the reader, perhaps in a summary at the end of each chapter, or in Chapter 6, as to the parts of the model that are really the most critical, and regarding parts of the model that turn out to be unimportant. As the reader goes through Chapter 3, for example, there is perhaps a presumption that each equation is equally important. When building a model, it is often tempting to do the best job possible on each component, even though some components may matter little to the final answer. Conversely, however, some components may be of such critical importance that additional resources should be devoted to their formulation. Some clear messages long these lines regarding priorities for model refinement would be useful.

213. Response:

The reviewer is completely correct with respect to the overall results; and indeed, while building the model we did attempt "to do the best job possible on each component, even though some components may matter little to the final answer," primarily because we did not know at the time which parts might turn out to be important, and because it is always very difficult to not do the best job possible once one gets started on doing any sort of job.

We have provided some indication of where additional resources need to be devoted in Section 7, but the main need is for additional data, not for additional effort on model formulation.

We give some guidance as to the most important parts of the model for the overall results in the new Section 6.1.2 (see also Response 70). However, we caution against going too far in this respect. For example, we provide breakdowns of results by various sources in Section 6.3, and some of the breakdowns show very small component contributions; however, such contributions may be important for some reason to FSIS, so we are loathe to prioritize modeling resources too strongly without knowing exactly what components may subsequently be of interest.

214. Comment:

Criteria 6b: Methodologies Used for Estimating Parameters from the Data

An example of parameter estimation is described on pages 44-45, in the case of the concentration of C. perfringens in cooked meat in RTE foods. In this case, the scale parameter was set equal for distributions fit to data of each of three different studies. However, the shape parameter was allowed to differ in two cases – one parameter value was estimated based upon a combination of

two studies, and another parameter value was estimated based upon a third study. The rationale for the two values for the shape parameter is to support uncertainty analysis.

214. Response:

The reviewer is not quite correct as to the rationale. The actual rationale for different distributional parameters was because the two distributions were expected to be different, since one was for a larger group of organisms than the other. It then turned out that the scale parameters of the distributions tested as equal. This happenstance was convenient, in that it allowed relatively easy enforcement of the required inequality in the uncertainty analysis by enforcing an inequality in the two shape parameters; but it is incorrect to say that the rationale for choosing two values is to support the uncertainty analysis.

215. Comment:

Uncertainties in the parameters of the gamma distribution were estimated using likelihood estimation and transformations in order to obtain normal error structures. The maximum bound of the shape parameter estimated from the third study was used as a constraint on the uncertainty distribution for the shape parameter. It appears to be the case that the upper bound for the shape parameter, a_T, also has an uncertainty distribution. This would be appropriate, since the knowledge of a_T is limited by the finite data set from which the estimate is made.

215. Response:

As now explained in the new Section 3.3, the transformations were not so much to obtain a normal error structure as to obtain an approximation to the likelihood function that could be rapidly sampled and that included the main features of the likelihood function, including the correlation structure particularly near the maximum likelihood.

216. Comment:

While the transformation to obtain normal error structures is plausible, a concern nonetheless is whether a back-transformation properly preserves the dependence between distribution parameters. The authors should provide a graphical comparison of the probability bands for the distribution of variability versus the original data in order to confirm that the simulation adequately captures the characteristics of the data while also providing a plausible estimate of variability.

216. Response:

As explained above, and in the new Section 3.3, the transformations were chosen not so much to obtain normal error structure, but to obtain approximations to the likelihood function that could be rapidly sampled and that preserved the main features of the likelihood structure, including the correlations structure, near the maximum likelihood. The transformations were obtained in such a fashion that the profile likelihoods for the individual parameters were very close to normal

(usually the correlation coefficient between the square root of the logarithm of profile likelihood deviation from the maximum likelihood and the transformed parameter value was larger than 0.998), so probability bands for marginal distributions should be as accurate as is possible with likelihood approximations. The correlation structure was then approximated by approximating the structure of the likelihood function in the transformed variables by a multinormal distribution. This approximation was performed by numerically approximating the information matrix, with the numerical step sizes for derivative estimation chosen to be approximately equal to the standard deviations of the marginal distributions. This ensured that correlations out to around 1 standard deviation of the marginals were reasonably accurately incorporated.

To examine the adequacy of the approximations, we examined 2-dimensional arrays of differences between the normal approximations and the likelihood deviation from the maximum likelihood, and found that the differences between these surfaces generally were less than a few percent, although we have not attempted to convert the deviations into differences in approximate probability intervals.

The reviewer calls for graphical displays of these differences (which would require, at minimum, 3-D plots of approximations to probability bands) to be included in the text, but we believe that such relatively obscure technical data are not appropriate in this text. In addition, the approach adopted was not designed in any attempt to obtain highly accurate fiduciary intervals, but to incorporate with reasonable accuracy the major structure of the likelihood function.

217. Comment:

Since a similar methodology is employed for other inputs (e.g., C. perfringens vegetative cell concentrations in partially cooked food), the same comments apply.

217. Response:

See Response 216.

218. Comment:

An alternative to the use of likelihood estimation with transformations is to employ bootstrap simulation as a method for estimating sampling distributions for the distribution parameters, as well as the dependencies between the sampling distributions. For example, Frey and Rhodes (1998) illustrate example results for gamma distributions that demonstrate the complex inverse and nonlinear dependence between the uncertainties of the distribution parameters. As noted in that example, a correlation coefficient is not an adequate measure of dependence. In this risk assessment, the implications of the back-transformation with respect to proper characterization of dependence between the distribution parameters should be tested and visualized.

218. Response:

We considered using bootstrap analyses, but rejected it as being too computationally intensive in this situation. The structure of the data is sufficiently complex that bootstrap analysis would require highly individualized analysis methods for almost every experiment examined, each requiring specialized code to be written for parameter estimation.

Frey and Rhodes (Frey, H.C., and Rhodes, D.S. 1998. Characterization and simulation of uncertain frequency distributions: effects of distribution choice, variability, uncertainty, and parameter dependence. *Human and Ecological Risk Assessment* 4:423–468) examined a parametric bootstrap for the gamma distribution and showed the expected inverse and non-linear dependence in the joint distribution of parameter values. This inverse non-linear dependence is expected since in the parametric bootstrap all sample sets (from which estimates of parameters are made) come from the originally estimated gamma. The shape and scale parameter estimates a and b can thus be expected to satisfy $ab \sim$ constant, since that product is just the mean of the estimated distribution, which will approximate the mean of the originally estimated gamma. In such circumstances it is often possible to obtain better approximation to correlation structures by re-parameterizing (in this case, for example, to a and ab) to remove such trivial correlations.

We examined this possibility in several cases, and chose parameterizations accordingly. For the gamma distribution, re-parameterization in terms of $\ln(a)$ and $\ln(b)$ reduces the major non-linear, inverse, correlation to a trivial linear correlation. As we mention in Response 216, we have examined the approximation between likelihood function and its approximation; but we consider such comparisons to be too highly technical to be included in the text.

219. Comment:

In general, the authors should either report the results of goodness-of-fit tests, provide visual comparisons of the fitted distributions and the observed data to which the distributions were fit, or some combination of the two. This would also serve as a quality assurance check that the fitted distribution is in fact an appropriate representation of the data.

219. Response:

Where goodness-of-fit tests were possible, we have reported them. For many of the data, however, standard goodness-of-fit tests (such as distributional or chi-square tests) cannot be used because of the extreme sparseness of the data (*e.g.* the *C. perfringens* concentration data). Developing goodness-of-fit tests in such cases would have been a major enterprise in itself, and not fruitful in that they would necessarily not be powerful. We note that the reviewer does not provide any example where we have omitted a goodness-of-fit test that the reviewer thinks could be supplied. Similarly, where possible we have graphically shown the comparison between the data and the fit. In other cases, no such graphical comparison is possible because of the nature of the reported data — again, the *C.*

perfringens concentration data are a good example, where we have very few reported data each consisting of numbers of samples with given numbers of observed cells, or a more complex set of reported data with multiple possible interpretations (see Table 3.4 for example).

220. Comment:

Similar to the last comment for the preceding criterion, one can potentially get lost in the details of parameter estimation and lose the context as to which inputs matter the most and which are relatively unimportant. The authors might give the reader some hints along these lines by indicating which parameter estimates are later shown to be critically important and which are later shown to be relatively unimportant.

220. Response:

See Response 213.

221. Comment:

Critera 7: Have the risks been appropriately characterized.

Chapter 7 focuses on risk characterization. There are few data with which to compare model predictions of risk characterization. The authors compare model results with an estimate reported by Mead et al. (1999) and argue reasonably convincingly that the model estimate is in reasonable agreement.

221. Response:

No response is necessary. (A typographic error; it is Chapter 6 that focuses on Risk Characterization.)

222. Comment:

The numerical precision of the Monte Carlo simulation is shown to be adequate for purposes of estimating illnesses per million servings for different growth rates during stabilization.

222. Response:

No response is necessary.

223. Comment:

A comparison of Figures 6-2 and 6-1 reveal that the range of uncertainty inherent in any estimate of illnesses per million servings is greater than the variability in the median estimate of this quantity among the different growth levels considered. This implies that uncertainty is large relative to variability in the risk characterization.

223. Response:

This is not quite correct. Figures 6-2 and 6-1 show the uncertainty, but the variability (between servings) has been averaged out. The variation with growth during stabilization is not variability in the sense used in the risk assessment, since the growth during stabilization is common to all servings. While the uncertainty is larger than the variation with growth during stabilization, we can still draw conclusions about how the number of illnesses varies with growth during stabilization; but our absolute knowledge of the number of illnesses is very uncertain.

224. Comment:

Section 6.3 is critically important because this is a form of sensitivity analysis to help identify the key sources of variability in the assessment. This analysis provides useful insight into the roles of meats versus spices, and regarding the contributions of each food category.

224. Response:

No response is necessary.

225. Comment:

Criteria 8a: Identification and characterization of key sources of variability and uncertainty

Section 6.3 provides insight regarding key sources of variability in the assessment, at least in terms of the proportional contribution of different factors to the total risk estimates.

225. Response:

No response is necessary.

226. Comment:

Section 6.6 reports on a sensitivity analysis that appears to be focused on inputs that were assigned point estimates or probability distributions based on judgment (e.g., uniform or triangular distributions). The authors appear to use a local sensitivity method, in which each input is perturbed individually while others are left unchanged. However, it could be the case that other inputs for which distributions were assigned were allowed to vary according to their distributions. This could be made more clear in the text. It is not very clear as to how the sensitivity was actually measured, in terms of the categories "t, n, and a" mentioned in the last column of Table 6.6. Although the text on page 152 implies that the methodology is further discussed on the following pages, it was not very clear as to how a quantitative sensitivity measure was obtained in all cases. Also, in some cases, it was not simply a perturbation of an input with respect to a central value, but rather a change in the distribution of the input that was

assumed (e.g., heating time in a microwave oven). It is not clear how this fits into the framework of Equation 6.4.

226. Response:

See also Response 234. We did indeed perform a local sensitivity analysis as suggested by the reviewer. The details of the sensitivity analysis were very abbreviated, and have been considerably extended in each sub-section of Section 6.6 to detail exactly how the results of Table 6.6 were obtained in each case. It has been made clear that each such sensitivity analysis requires a complete Monte Carlo run to obtain the change in estimated number of illnesses per year. In cases where a whole distribution was changed, there are two possibilities. In the first case, it was demonstrated that the result depends primarily on a particular statistic of the distribution (*e.g.* the mean), and the sensitivity analysis was performed on that statistic. Second, for cases where the sensitivity is small (*e.g.* heating time in a microwave oven), only a gross measure (in this case the mean heating time) was used as the sensitivity parameter.

227. Comment:

Section 6.6 should have a summary of the most sensitive inputs, based not only on the results of Table 6.6 but on authors' judgment as to the relative ranges of the inputs. There could be a stronger link between the results of Section 6.6 and some of the recommendations in Chapter 7.

227. Response:

See Response 213.

228. Comment:

It would have been useful if the authors could have performed a sensitivity analysis based upon the Monte Carlo simulation results in order to identify key sources of variability and key sources of uncertainty, using techniques such as correlation coefficients, regression analysis, or analysis of variance (ANOVA). Although there is an analysis in the risk characterization section that apportions the risk to specific causes, it would be helpful to have some idea as to which sources of variability are more important than others. For example, where is it worth spending the next dollar to get more data to better characterize variability in a model input?

228. Response:

There is no "key" source of variability; variability is the natural variation that is present, in this case between servings. The final results that we examine have no variability, since it is averaged out in the final measures of interest (the numbers of illnesses) — the variability contributes to the numerical imprecision only, which we make negligibly small by using sufficient samples. Were we interested in other final measures that involved variability, such an analysis might be of interest.

However, it would be possible to use the techniques identified by the reviewer to identify the relative contributions of various sources of uncertainty, and to perform an evaluation of the cost-effectiveness of obtaining better data to reduce uncertainties (this is practically the same thing as better characterizing variability). The program is not currently set up to evaluate uncertainty correlations of the nature discussed, but we will investigate the implementation of such an analysis. At the moment, it is not clear whether the measures obtained would, in fact, be of substantial assistance, because the major uncertainties are those that have not been quantified and that are related either to representativeness of the data or to completely lack of knowledge about certain parameters.

229. Comment:

With regard to uncertainty, given that the range of uncertainty for a given growth level is larger than the variation in number of illnesses per million servings when comparing growth levels, there could be a clear, quantitative ranking as to which quantified uncertainties in the model are contributing most to uncertainty in the risk characterization. This information could help support the recommendations in Chapter 7. Of course, the authors would appropriately want to incorporate unmodeled issues or other qualitative sources of uncertainty into the discussion.

229. Response:

The reviewer is, we think, somewhat confused over the interaction between uncertainty, variability, and the growth during stabilization (see also Response 223). While the uncertainty in numbers of illnesses is large compared with the variation that occurs in the number of illnesses as the growth during stabilization increases, that uncertainty does not obscure in any way the variation of number of illnesses with growth. To get this point across, we have tried to improve the discussion following Equation 6.1 by adding the sentence, "The corresponding equation then shows the variation with growth at this percentile of the uncertainty distribution" at the end of Section 6.2.2. As the reviewer recognizes, the relative rankings of the recommendations in Chapter 7 are based not only on the relative contribution to uncertainties, but also on some idea about the relative difficulty and cost of obtaining the required information.

230. Comment:

As noted in some previous comments, in addition to prioritizing key sources of variability and uncertainty in model inputs and parameters, it is useful to prioritize the importance of different parts of the model.

230. Response:

See Response 213.

231. Comment:

Criteria 8b: Identification and characterization of critical assumptions

No external contamination with C. perfringens is assumed, as stated on page 33.
The properties of spores are assumed to be independent of the spice (page 36).
Because of lack of data, there is lack of stratification with respect to factors such as distinction between beef, chicken, and pork, and between whole muscle vs. ground meat, or cured vs. uncured products. The authors point out that this tends to underestimate the total uncertainty and could lead to inaccurate estimates of exposure.

231. Response:

All the assumptions listed here are now (see Response 232) included in Section 4, and all of these particular assumptions come under the heading of representativeness assumptions.

232. Comment:

In general, it is suggested that the authors do a search through the document for words such as "assume" or "assumption" and be sure that each such example given in the main body anywhere in the report is also listed in Chapter 4. For example, on page 57 "assume that C. perfringens in spices are present entirely as spores" but does not seem to be in Chapter 4. Rather than enumerate all such cases here, it would be easy for the authors to do the search through the document as suggested here. In general, the authors do an excellent job of identifying assumptions throughout the document, and it will be helpful to have all of them collated into Chapter 4. The authors may wish to create additional subcategories in Chapter 4 to make it easier for the reader to review the key assumptions.

Chapter 4 precedes the hazard and risk characterization chapters, which involve additional assumptions. Perhaps Chapter 4 could be moved to later in the document, and incorporate all key assumptions from all components of the assessment.

232. Response:

Such a search is an excellent idea, and one that we have followed. Not all assumptions are listed explicitly, since some are best treated generically (*e.g.* assumptions about normality of error distributions in various analyses), but we believe that all assumptions are now included at least generically in the list. Specific assumptions about the values of inputs that are examined in the sensitivity analysis have been excluded. We have also followed the reviewer's suggestion and expanded the subcategories of assumptions.

We have not, however, mixed the listing of assumptions in the exposure and hazard characterization sections. It is standard in risk assessments such as this to keep these two sections separate, so we have maintained that separation even in the assumption lists.

233. Comment:

The listing of key assumptions in Chapter 4 should be in some kind of priority order. Ideally, the priorities should be based upon quantitative sensitivity analysis for those inputs for which variability and uncertainty were quantified and for which assumptions were embedded in the estimates of variability and uncertainty. For inputs, parameters, or model structures that involved more highly qualitative judgments, or for unmodeled issues that may be of substantial importance, judgment should be used to provide a priority listing. For example, it would be helpful to categorize assumptions as being of major importance, moderate importance, and minor importance.

233. Response:

We agree that it would be desirable to place these assumptions in priority order, but decline the attempt. We believe that we would be sufficiently wrong sufficiently often that such an attempt would be more likely to mislead than to enlighten. It was only by examining the results of the modeling that certain "obvious" properties of the inputs about which we have substantial information became apparent in hindsight (for example, that most of the predicted illnesses arise because, essentially, of broken refrigerators). We are practically certain that the same lack of foresight would apply to our list of assumptions; the more so because of our substantial lack of knowledge that required the assumptions in the first place.

234. Comment:

Criteria 10: Clarity of the risk assessment report

In general, this report was well written and appears to be comprehensive. The assessment objectives are clearly stated and analyses are presented based upon the objectives. The limitations of input information are carefully described and key assumptions are conveniently listed in Chapter 4. The interpretation of data appears to be reasonable. The modeling methodologies appear to be acceptable, although there could be alternative approaches. One area in which the work could perhaps be improved is with regard to the sensitivity analysis. It appears that the authors performed essentially a local sensitivity analysis using a method that is appropriate for linear models, but that may not be reliable when applied to nonlinear models. The method appears to be a variant of differential sensitivity analysis. Furthermore, the effects of possible interactions among inputs is not addressed. A statistical method such as ANOVA would be more robust and might reveal insights regarding not only the main effects of individual inputs, but also interaction effects of various kinds.

234. Response:

We agree that the sensitivity analyses could be extended in the way suggested; however, extensions could be made in most areas of the analysis, and the question arises as to whether such extensions would be useful for the primary purpose of the assessment. The sensitivity analyses were performed in order to gain some insight into which are the most important of the inputs about which there is very

little information; and the differential approach applied does precisely that. It is true that there may be interactions between inputs (indeed, for most of the inputs we expect approximately multiplicative effects on the number of diarrheas, because of the structure of the model). However, quantitative or even qualitative estimates of such interactions are of no additional value at this point *for the primary purposes of this risk assessment*. That is not to say that further work should not be done to clarify any such effects; but there is no need in this document (which is already sufficiently long and complex).