Itemized Response to Comments

for

Risk Assessments of *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Egg Products

INTRODUCTION

An independent, external peer review of these risk assessments was conducted under contract with SAIC in October 2004. In December 2004, the Office of Management and Budget (OMB) released a final Bulletin of peer review standards for risk assessments conducted by Federal agencies. While these risk assessments were peer reviewed prior to the issuance of the Bulletin, the review was conducted in accordance with OMB requirements. The review was adequate based on the complexity of the risk assessments and the Agency's anticipated use of the information in decision-making. SAIC selected five experts (see appendix I) to conduct independent reviews of the risk assessments and each reviewer dedicated approximately 40 working hours to the project.

Each peer reviewer was asked to focus on his/her area of expertise. Three of the reviewers were selected for their expertise in public health, food safety, and microbiology. The charge given to the peer reviewers was to focus on the following issues: 1) Is the report clearly written? 2) Does it follow a logical structure and layout? 3) Does the background information sufficiently and accurately capture the current state of knowledge regarding *Salmonella* and egg safety? 4) Have all of the assumptions used in developing the assessments been clearly stated? 5) If so, is the rationale for these assumptions valid? Two of the peer reviewers were selected for their expertise in risk assessment modeling. These reviewers were asked to focus on questions such as: 1) Have the assumptions been appropriately modeled? 2) Does the model follow a logical structure and layout? 3) Are there programming errors within the model? 4) Are there ways to optimize the model? (see appendix II)

Comments on the risk assessments were also received from stakeholders under docket number 04-034N. Comments were submitted by the following persons: Howard Magwire, *United Egg Producers, Alpharetta, Georgia*; Hershell Ball, *Michael Foods, Gaylord, Minnesota*; Charles Beard, *U.S. Poultry and Egg Association, Tucker, Georgia*; and Brian Joyer, *Sparboe Companies, Litchfield, Minnesota*.

Itemized Response to Comments from Peer Reviewers

REVIEWER #1

Comment #1: The FSIS draft risk assessment of the public health impact of Salmonella enteritidis in shell eggs and Salmonella spp. in egg products is described in an extensive report about an interesting and sometimes innovative, complex 'farm to fork' risk model, with an endpoint in human illness.

This review focuses on some of the modeling aspects of the risk assessment and concentrates on the shell egg model. It was not possible to address all the modeling issues encountered in the report. Many of those are worked out in the annexes. Unfortunately the accompanying spreadsheet models were not much of an aid, as they have not been built in a very user friendly fashion, with too little documentation. Below, I first give a list of comments on the report and models, first in general and then referring to pages, tables and figures, concentrating on chapters 3,4, and 5, the annexes A and E, and the shell egg spreadsheet model. Next, I specifically comment on the evaluation criteria.

Microbiological risk assessment is complex and still in development. There are hardly any 'standard solutions' to the problems encountered. From experience I know it is far easier to comment on a risk assessment than to perform one. Many, quite often arbitrary, choices have to be made (in data selection, modeling techniques, basic assumptions). Doing so, it is crucial to explicitly write up the assumptions and to concentrate on answering the risk management questions. In this respect I think this risk assessment is well performed and successful.

When reading the report and annexes, it is clear that several people have been involved in this work. As a consequence terminology used is not consistent throughout, and the link between parameters in main text, annex and spreadsheet model is not evident. References are not specific enough. To understand the study, one often has to thumb through the report and this is time consuming.

Reply: It will be important in future endeavors to build the risk assessment in a linear fashion to avoid this problem. This would also make it easier to cross-reference items in the text which will make eventual editing easier. We have sought to improve the clarity and cohesiveness of the revised version.

Comment #2: Especially for a non-American reader the use of both °F and °C is irritating. This is not the international standard.

Reply: All temperature values are now listed as °C.

Comment #3: p 39. Add S1 in the fig 3-1.

Reply: S1 and S2 are intermediate *outputs*. Figure 3-1 shows *inputs*: starting bacteria, growth before pasteurization, pasteurization, growth after pasteurization, cooking, number of servings. The figure has been revised to avoid confusion.

Comment #4: p 40. 'variability present in their estimates' ?? What is that? State explicitly that all distributions in the exposure model (until serving) are variability per egg.

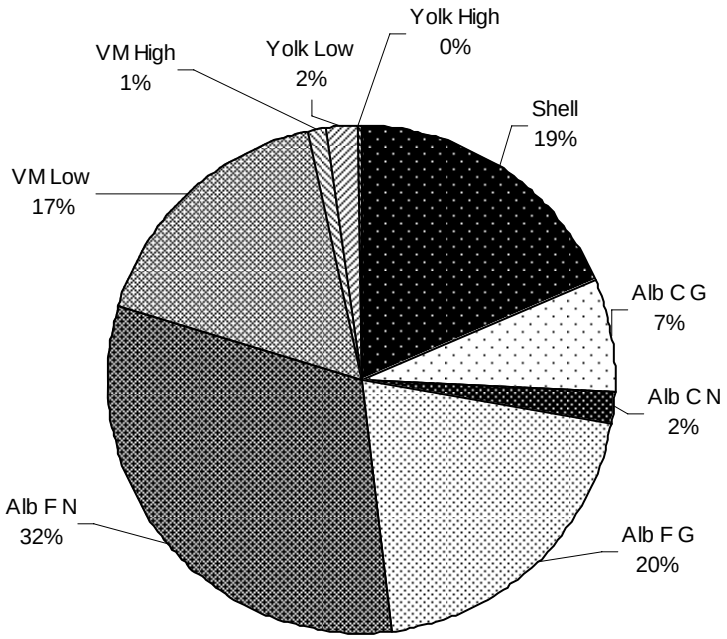
Reply: Changed from "In general, the values for S_0 are estimated using probability distributions to represent the variability present in their estimation" to "The values for S_0 are estimated using probability distributions to represent the variability in bacteria per egg."

Comment #5: p 44-45. When referring to Annex B, refer to a page or equation. The Weibull parameters could not be found there. Why use distributions for K and $M(t)$ if you only use the mean in the model anyway? The end result (E) is a number (EV means Expected value, I guess). I calculate a different value for $E(M(t))$ using a Uniform (0,20) W (=3.1).

Reply: $M(t)$ was calculated by taking the average of values calculated for (1, 2, 3 . . . 20) weeks post-molt. A uniform from 0 to 20 does give 3.1. On the other hand, hens do not achieve full production immediately following molt, so the likelihood of collecting any egg is less at that time. The effect of increasing or decreasing $M(t)$ can be seen in the sensitivity analysis.

Comment #6: Table 3-2 A pie chart may help to see the relative fractions.

Reply: A pie chart is now included:



Comment #7: Table 3-3. The initial bacteria estimate should be an integer. e^Normal(2.6,1.3) is not.

Reply: This change did not make a significant difference in the results. e^Normal(2.6,1.3) has been kept in the model.

Comment #8: p 47. What is the biology behind the assumption of starting with 1 cell in VM and Yolk high? Why 1 and not 10, 3 or 11?

Reply: A value of 1 was used for convenience. The rationale behind this decision was that because rapid growth of cells is expected to occur, the number of cells at the beginning of growth would not significantly affect the results.

Comment #9: p 51. 'is added to Equation introduce' ???

Reply: Revised to read “is added to Equation 3.7 to introduce.”

Comment #10: p 52-54. Why do the selection of egg production facility (step 1) and egg contamination location probabilistically (Monte Carlo)? One can also do each combination separately, and then adjust the outcome for the results of the different scenarios by taking the relative frequencies. This will give better insight in the results for the different facilities and locations and simplify the Monte Carlo model. (Just take more runs)

Reply: This is similar to the approach taken for egg products in which each product is modeled separately. The current approach facilitates sensitivity analyses, but the suggested approach will better resolve lower frequency events. We used a programmed simulation that provided separate estimates for each egg contamination location.

Comment #11: p 57. The square representing observed are not good symbols here, they obscure the visual impression of the goodness of fit.

Reply: The symbols representing observed data have been changed from filled squares to open squares. This improves visual impression of the goodness of fit.

Comment #12: Table 3-14 is not easily linked with Annex E. Variable names are in capitals in the table, not in the Annex. *D,G,G,K* should be *d,f,g,k* . This table is confusing, not clarifying. Please refer exactly to the Annex.

Reply: This has been corrected.

Comment #13: Table 3-16: Likewise: When referring to Annex E be more precise. This Annex is 54 pages with tables and equations.

Reply: This has been corrected.

Comment #14: Table 3-16: Why is *R* assumed to be 5? (important assumption, see comments on Annex E) What is N_t in eq. for $\beta(t)$?

Reply: Other values for *R* are considered in the sensitivity analysis. N_t was changed to S_{t-1}.

Comment #15: p 73. The choice for the Baranyi model is a good one. And Eq. 3-17 seems a good choice.

Reply: No response needed.

Comment #16: Complex equations in table 3-17 and 3-18 are not well-printed in the document, I cannot read them.

Reply: It looks like the equations for several variables are not shown. Tables describing the equations are now included in the document.

Comment #17: p 93. Fig 3-24 should be a bar graph, because the numbers are discrete (I hope). Fig 3-25 is redundant and can be left out.

Reply: Fig 3-24 is unchanged. Fig 3-25 has been removed.

Comment #18: Fig 3-26: As the x-axis is continuous, the y-axis can and need not be read. Leave out numbers here.

Reply: We have left the scale designators on the y-axis in some of the graphs because some reviewers of the document found it helped them visualize relative differences in values.

Comment #19: p 94. Modeling of G1 is very complex and important. Presenting only 1 figure of the results is too little to understand what happens.

Reply: For further details about modeling G1, see the Annex titled "Modeling growth of *Salmonella* Enteritidis in eggs."

Comment #20: What about the differences in facilities and egg locations? Was it worthwhile to do all this complex modeling?

Reply: In-line facilities have been modeled differently in the revision (pre-processing storage is no longer included in the model). Experimental data indicate cooling constants differ for eggs at the center of a pallet vs. those on the periphery. These cooling constants affect growth of *S.* Enteritidis in eggs.

Comment #21: Fig 3-30. Giving x-axis values in terms of log reduction does not make it simpler for the reader. The numbers refer to table 3-26 and 3-27, but the results do not

completely agree (why 0.527, not 0.5302 for 12.00?) Where is sunny side up (1.8 log reduction) in the figure?

Reply: The results do not completely agree with the inputs because they are the results of a Monte Carlo simulation. “Sunny side up” is now included up in the figure.

Comment #22: Fig 3-31 should be bars too (?), certainly not a smoothed curve. If it is the input, why should I want to read this figure? Why is Fig 3-22 not sufficient? This is clearer figure anyway.

Reply: Fig 3-31 has been removed.

Comment #23: Fig 3-32: What is the line and what are the dots? Does the line represent anything? Are there classes? Are indicators (1,2,3) upper limits, medians? Is 10^11 not more than MPD?

Reply: Fig 3-32 has been revised to improve clarity.

Comment #24: Figs 3-34 and 3-35 don't add anything. They should be left out.

Reply: Fig 3-34 and Fig 3-35 have been removed.

Comment #25: I don't understand what figs 3-38 and 3-39 represent. Dependency of G1 (and G2) to T and t, or simulations results? How do they help me to understand what is going on?

Reply: Fig 3-38 and Fig 3-39 reveal that growth is dependent on time and temperature. Growth takes place only if eggs are stored at too high a temperature for too long a period. We agree, however, that the figures may be more confusing than helpful. They have thus been removed.

Comment #26: Why not add 99% to fig 3-40? What does the distribution after home storage, prior to cooking look like? (See Risk characterization comments below)

Reply: This value has been added.

Comment #27: p 110, table 3-30: What does "see commercially prepared bread" refer to?

Reply: This was a mistake due to a formatting error in cross-referencing. It has since been corrected.

Comment #28: p 111, eq 3.22 Here the Weibull is introduced, which is already mentioned in table 3-1.

Reply: The Weibull is not explained in the table. This, coupled with the fact that we introduce a modified form of the Weibull warrants a brief description at this point in the text.

Comment #29: Fig 3-46 cannot be true. Numbers of bacteria per serving are integers. If log $SS_0 = 0$ there is 1 SE bacterium in a serving. But what if it is -1? 0.1 bacterium is none. I don't know how to interpret this figure. (If 0.1 is the probability of a bacterium, or the expected number, I don't know why I should want to know this.) Actually, Table 3-30 says SS_0 is a sample from Poisson, and thus an integer. ??) Same is true for figs 3-47, 3-48, 3-49. Bacteria per serving must be an integer.

Reply: Each of these figures represents expected values; therefore, when the draw is taken bacteria are represented by integers.

Comment #30: Fig 3-52 Use different colors and explain what is what. Why compare with the expected value? You could also give the % of products where the number of bacteria SS_0 per serving is larger than the log reduction. This indicates the % of products with surviving bacteria.

Reply: This figure has been redone to give the % of products where the number of bacteria SS_0 per serving is larger than the log reduction.

Comment #31: Table 3-43 is far more instructive than [table] 3-42. I could do very well without the latter, as we have figs 3-56 to3-62.

Reply: Table 3-42 has been removed.

Comment #32: This annex describes an essential and innovating part of the risk assessment: the modeling of growth of Salmonella enteritidis in eggs. This is rather complex and unfortunately the annex is not well structured. It needs this structure because the complexity requires all the readers' attention. There is no clear overview of the contents (TOC!), the logic behind the modeling (choices) and the results. Table E-1 is not very clear. The question why simpler modeling is not possible is not well addressed. Graphs of growth curves resulting from the modeling would be of help, but are missing. (Just fig 3-12 is not enough. It should be explained better which models are used for which part of the curve.)

Reply: The annex has been revised.

Comment #33: p E-10 An example of sloppiness a subheading gets number 1., but there are no other numbers.

Reply: This has been corrected.

Comment #34: p E-13 It is (thus) assumed that Rat is constant and = 5 because it is found to range between 3 and 10. That is not clear. Why not make it variable? (But considering fig 5-10 it is not very relevant...)

Reply: The effect of the *Rat* is evaluated in the sensitivity section of the risk characterization by setting the value of *Rat* at 2 for a simulation and then at 10 for another simulation.

Comment #35: p E-15 first paragraph: symbols are missing (maybe a computer problem)

Reply: This has been corrected.

Comment #36: The JEMRA work on SE in eggs is published (WHO, FAO 2002).

Reply: The reference has been updated.

Comment #37: I guess (?) the expected values of alpha and beta are the ones uses in the model described in this report (page 146). The uncertainty is not taken into account (I guess) because uncertainty is not taken into account in the exposure assessment either. This should be stated and stressed explicitly; it is the most important thing of this whole chapter.

Reply: Table 4-4 has been supplemented by an explanation that the expected values are used to generate the baseline. The effects of assuming lower and upper bounds are shown in the sensitivity analysis in the risk characterization.

Comment #38: I would like to see uncertainty intervals in Table 4-10, as this is relevant in comparing these numbers with the outcome of the risk assessment.

Reply: These have been included in the revised table.

Comment #39: p 163 The DR function should be given here. Just because it is discussed at length, it is NOT clear what you use here.

Reply: Included in the revision are the dose-response equation and parameters at the most likely values.

Comment #40: p 164 IE is not a number but a rate, i.e. the expected number of illnesses. This causes quite some confusion all over the report.

Reply: Naming this value has been difficult. We began by calling it the probability of illness, but that's not appropriate because it can exceed 1. "Rate" doesn't work either because it implies a time component. The value is referred to as "illness frequency" in the revision.

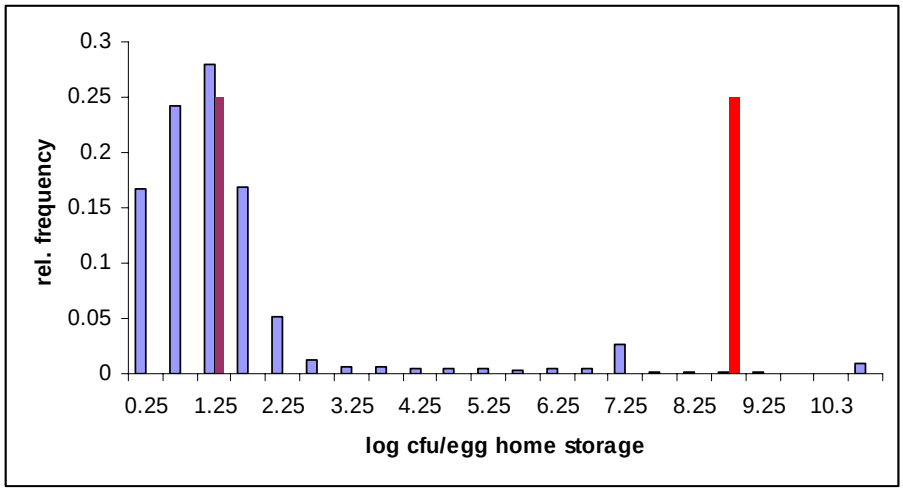
Comment #41: Table 5.1 is not informative at all.

Reply: Table 5.1 has been removed.

Comment #42: p 166. Why are mean numbers given in table 5-2? The baseline is an exposure assessment result which is not given in the exposure assessment results chapter. The means presented in the table are much larger then the 95% percentile value of the distribution, just because the distributions are so skewed. This means that these means are imprecise, and will get larger with increasing number of iterations. They are not very representative. I would like to see graphs with shifting distributions, to understand what is really going on. What is happening to the median? The geometric means?

Reply: Figure 5-1 has been added to address the reviewer's above comments; it shows the potential for illness associated with the distribution of bacteria at the end of each stage.

Comment #43: Actually, when working with the spreadsheet, it took me a long time to find out that it looks as if there are more baselines (??), and this is the one without any pasteurization. This baseline shouldn't be called baseline, but 'no pasteurization'. For home storage you get the distribution below (see figure) at log scale, with the red bar representing the mean and the purple one the median. The mean is at the 98.8 percentile of the distribution and thus not very precise.



Reply: A sentence has been added to explain that the term "baseline," as used in the shell egg model, refers to a scenario in which no eggs have been pasteurized. There is a very small percentage of shell eggs that are pasteurized but these are not included in the report. Rather, the effect of pasteurization on any proportion of shell eggs can be calculated given the no pasteurization and 3 or 5 log pasteurization scenarios.

The mean of the number of bacteria in an egg can be strongly influenced by just a few highly contaminated eggs. Thus, a separate simulation using a different seed could have a significantly different mean. But the value of interest is not the mean of bacteria in an egg; it is the frequency of illness. At high levels of contamination the frequency does not

change as much as compared to lower levels. This is shown in the hazard characterization chapter.

Increasing the dose from 0 $\log_{10}$ bacteria to 1 $\log_{10}$ bacteria increases the frequency of illness 810%. Increasing the dose from 7 $\log_{10}$ bacteria to 10 $\log_{10}$ bacteria, however, increases the frequency of illness 15%. It is this frequency of illness that is the endpoint of the risk assessment. It is stable within the limits stated (about 6% standard error of the mean.)

Comment #44: Fig 5-1 is illustrative to show the effects of reduction. Please state explicitly what causes the increase effect PostProcess, this is important. Is it just YMB or are there more reasons? Which are they? (I expect the uncertainty of the mean and the fact that the mean is not representative for such a skewed distribution is relevant too)

Reply: This figure has proven confusing regardless of how it is presented; it has thus been removed. The mean is driving what is going on. YMB is part of it. There are also new opportunities for growth for any bacteria that are not killed. To try to clear up confusion, the revision gives the estimated number of illnesses at each step. Thus, after lay there are *X* illnesses, just before pasteurization *Y* illnesses, just after pasteurization *Z* illnesses, etc.

Comment #45: #p 167. Explain that the baseline = no pasteurization. The 0.023 illnesses per contaminated egg can be considered the end result of the risk assessment and should get a little more attention than just somewhere in a phrase. Number of illnesses per egg is a rate (illness rate), not a number.

Reply: A sentence has been added to explain that the term "baseline," as used in the shell egg model, refers to a scenario in which no eggs have been pasteurized. The end result is illnesses per egg rather than illnesses per contaminated egg. The bulk of the model, however, is devoted to contaminated eggs. The value 0.023 is multiplied by the proportion of contaminated eggs to get the *frequency* of illnesses per egg. This value is not a rate because it does not have a time component.

Comment #46: #Table 5-3. Why not just (or also) give the conversion factor?

Reply: The conversion (approximately 3.2), which is the mean number of servings per egg, is given in the revision.

Comment #47: #p 169 The estimated annual number of illnesses [a number indeed] for pasteurized shell eggs is not 200,000 and 170,000 for 3 and 5 log reductions. The text presents other numbers than the figure. Actually, the numbers are for 60% pasteurization.

Reply: Updated values are now included.

Comment #48: #Presenting both table 5-5 and figure 5-4 is redundant.

Reply: It is redundant, but we feel it useful. No change has been made.

Comment #49: #Table 5-6. Why are results for 53 and 60 F not given?

Reply: Predictions based on these values yielded more illnesses than the baseline value. Thus they could not be considered as mitigations and were not included.

Comment #50: #Table 5-7 Please express also as a mean and SD.

Reply: This was expressed with a mean and SD previously. It caused a lot of confusion in a presentation, however, and was subsequently removed. The mean is 331,554 and the standard deviation is 25,848.

Comment #51: #p174. The choice for not doing a second order MC is OK. The arguments given are good arguments.

Reply: No response needed..

Comment #52: #The Hazard characterization chapter indicates the uncertainty of human illness, not the variability. This uncertainty should be given in table 4-10.

Reply: A description of the uncertainty is now included.

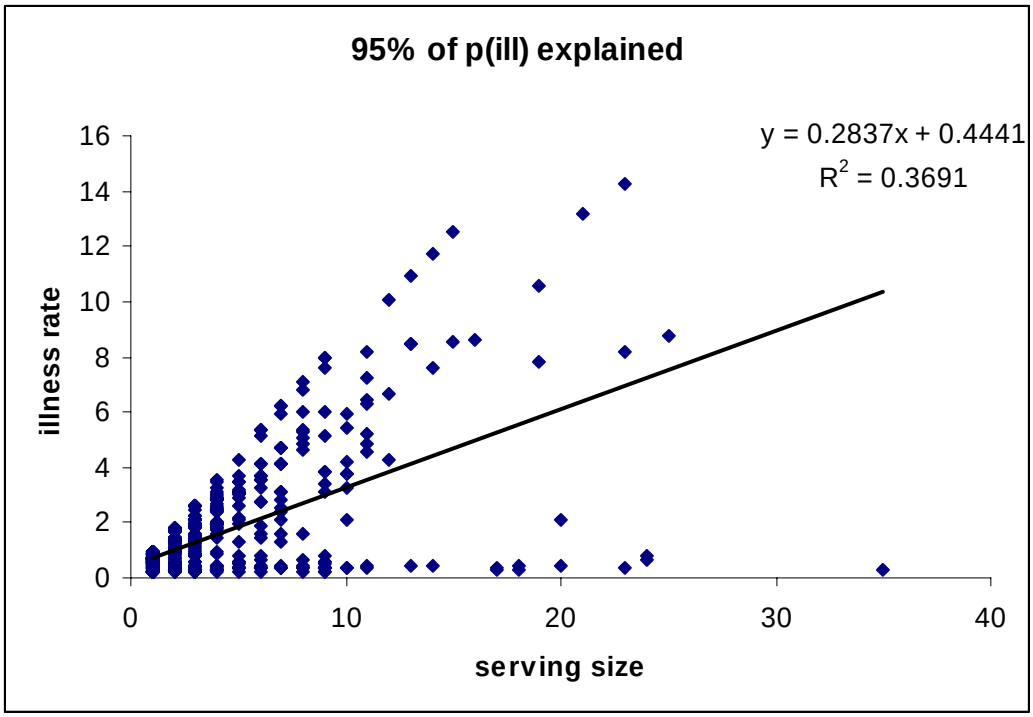
Comment #53: p175. Table 5- = 5-8 ?

Reply: Yes.

Comment #54: The correlation analysis should be explained better. What (I think) it does is correlate the variability in an input parameter (a distribution selected by the modeler, representing variability per egg) with the variability in the output (i.e. the output should have a distribution). But the number of human illnesses is not a distribution but a number (It does not vary per egg, it is a number for the whole population eating eggs). So probably the output is something else. Is it illness rate per serving or per egg? In that case it should preferably be per egg, because all the distributions (numbers of bacteria etc) are per egg.

The correlation is with illness rate (or frequency may be preferred) per egg.

Serving size is an interesting item anyway. Although the correlation analysis does not show a correlation with serving size, serving sizes are important for the end result (the illness rate). The few cases with IE = p(ill) > 1 contribute most to the final result, and they are associated with larger serving sizes. If, in the shell egg spreadsheet, you select the simulation iterations that contribute 95% of the total I_E (i.e. the 805 out of 50000 with the highest values for p(illness)), there is a correlation:



So serving size is important.

Reply: This is an important and interesting observation and is included in the revised report.

Comment #55: p179. Please formulate the conclusions of the analyses in words. What should I conclude?

Reply: An explanation is found at the conclusion of the sensitivity analysis to describe the drivers of the model.

Comment #56: p 180: the diamond is 0.0000069? Please help me.

Reply: This value is clarified in the revision.

Comment #57: I cannot read the x-axis in fig 5-9, 5-11, 5-13, 5-14. Especially for the latter that is a great pity. (SD of servings per egg should have quite some effect (?)) These figures need to be repasted. The upper bound of mean of servings results in twice as many illnesses while the upper bound of the SD results in nearly three times as many.

Reply: The X axes on these figures were made unreadable when converting the Word document to a .pdf file. They have since been corrected.

Comment #58: p 190 and Table 5-15.Very interesting! I would appreciate more discussion on this. As the models are rather complex, it is difficult to see which is the essential difference between deterministic and stochastic. If you know this, you can

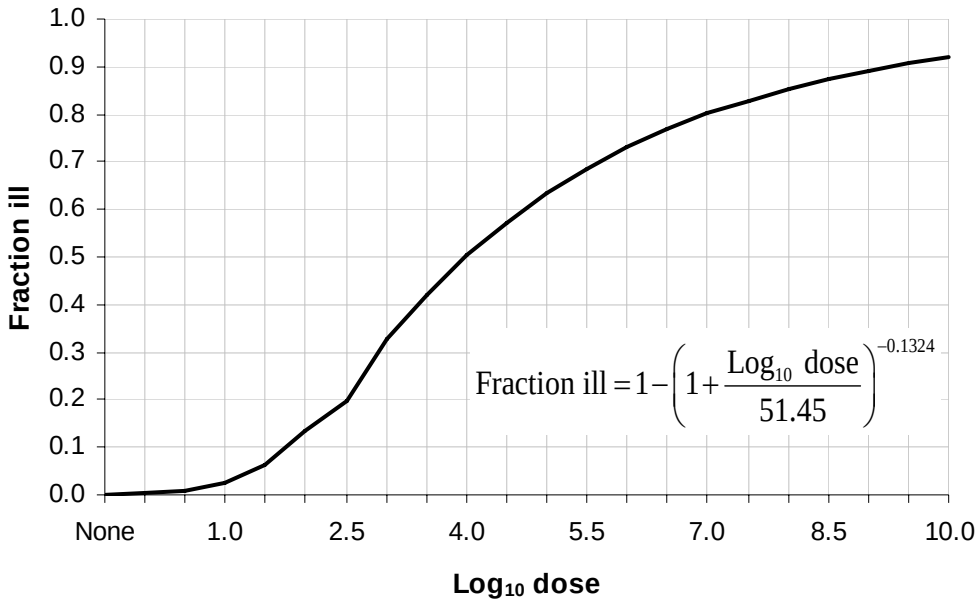
maybe simplify it again and make the stochastic model deterministic, with the probability of growth of small sized initial population as parameter. I think this result is very interesting for the international scientific community, and should not be hidden like it is now.

Reply: The essential difference appears to be the probabilistic handling of growth for small initial sized populations. Both models are fairly complex because of the need to model changes in temperature over time. Nevertheless, the stochastic model is more complex and produces a noticeable slowing in the model. We plan to submit interesting aspects of the model such as this for consideration to be published in peer-reviewed literature.

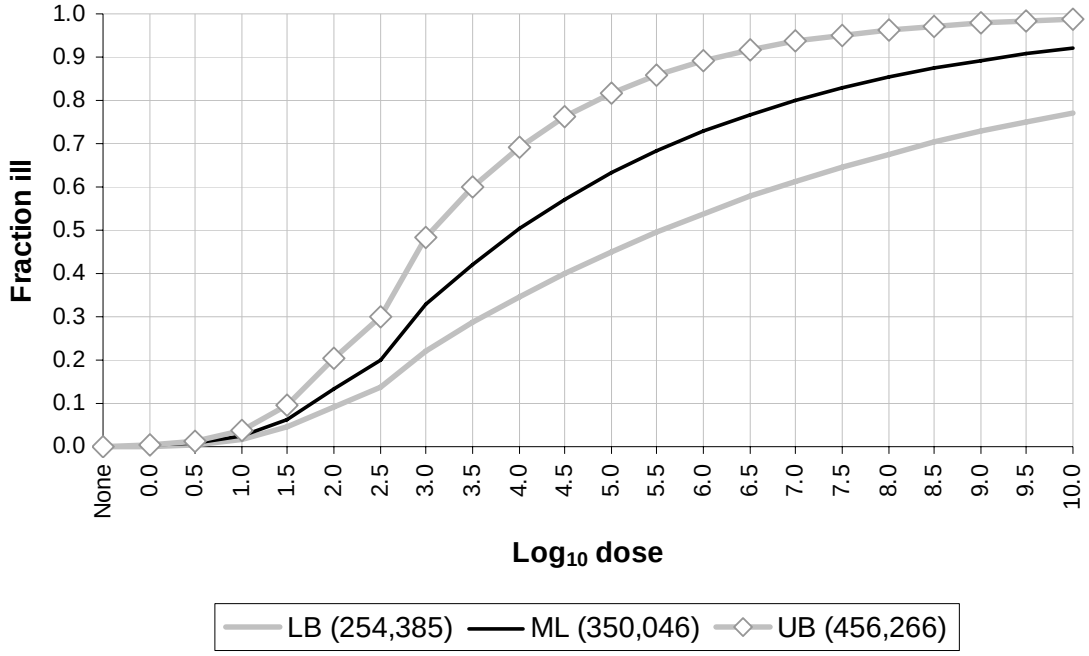
Comment #59: The sensitivity analysis of the DR model has been done (p188), but is not discussed.

Reply: We have included the estimated number of human illnesses given the two bounds for the dose response function.

The specific dose response function used for the baseline estimates is shown below.



The below figure shows the effect of uncertainty in the parameters of the beta-Poisson dose-response function reported by FAO/WHO.



The effect of this uncertainty is about 100,000 illnesses or about 29% above or below the baseline estimate.

Comment #60: p192-193. The method applied is reasonable, but just one of several options. The resulting range is so broad, that it would be a great surprise if the model was not validated. But the results of both approaches are not conflicting, and that is good news.

Reply: No response needed.

Comment #61: Clearly, some general comments above (e.g. about referring to annexes) are valid for this section too.

Reply: See response to above comments.

Comment #62: p 195-198. Anchoring. This is rather difficult to follow. Exposure assessment results are modified because the end result was unlikely (could not be validated). Modifications are based on FSIS testing data. I would like to see equations instead of tables (5-19 to 5-21). By now I don't know how to reproduce these tables, and I cannot assess the validity. The choice to only modify the baseline result for egg white in the end is somewhat arbitrary. I would like a discussion on how it can be that the baseline results for egg white are obviously wrong. Isn't the same argument valid for other products?

Reply: Reply: The other products are not in conflict with FSIS testing data. This section has been rewritten to provide a better explanation.

Comment #63: p. 200. Refer to table number (3.31) if you refer to a table.

Reply: This has been fixed.

Comment #64: Table 5-26. A pie chart helps.

Reply: A pie chart shown below has been included in the revised version.

Comment #65: Table 5-28 and 5-27. The difference between the predicted illnesses is very large. I hope this is not because (as you say) the number of iterations differs (I guess), but because you do two independent runs of the model. It implies your end result is quite uncertain. This should be explained better.

Reply: It is because the runs have different numbers of iterations. The first 100,000 iterations should be the same in the baseline model. Unfortunately, the model takes a long time to reach stability; 500,000 iterations seem to be enough to get a reasonable standard error of the mean. Nevertheless, even though 100,000 iterations do not generate a stable baseline value, this many iterations does show the relative differences between mitigations. If line by line outputs of each iteration are not needed the model can be run for more iterations depending on memory availability.

Comment #66: Fig 5-19 is it 3 (text) or 5 (figure) log reduction? (Five probably)

Reply: Fig 5-19 represents a five log reduction. The figure and accompanying text have been revised to reflect this.

Comment #67: p 205. Stability: see remark for table 5-28. So the standard error of the mean for probability of illness is 6% for each of the products?

Reply: Yes, about 6%.

Comment #68: Correlation sensitivity analysis: Please explain what you do, and how to interpret the tables.

Reply: "Rather, standard correlation coefficients were calculated for different inputs and intermediate outputs" have been replaced with "Rather, standard correlation coefficients using the Excel function Correl(input array, output array) were calculated for different inputs and intermediate outputs,"

Also, an explanation of how we interpret the table is now provided: "The table shows that the probability of a negative serving is negatively correlated with the concentration of bacteria in the raw product. In other words, lower concentrations in raw product are more likely to be associated with negative servings. At first glance, it might be expected that the correlation should be more pronounced. Because most raw product has generally

low concentrations of bacteria and serving sizes are relatively small, a less pronounced correlation makes sense."

Comment #69: Fig 5-26 and 5-27 cannot be read (black x-axis labels).

Reply: These figures have been clarified.

Comment #70: Validation: First, validation of a model which is modified because earlier validation failed is doubtful. Second, the line of reasoning with reference to outbreaks is not very clear. I think you mean to say that for each 15000 cases you would expect an outbreak, and therefore with 50.000 cases you should see about three outbreaks a year. Because you don't see any, there is something going on... It is interesting, but not convincing.

Reply: First, yes. Second, this was done to begin to estimate how many salmonellosis cases are due to egg products. On the one hand, it is not convincing because there is not evidence that shows illnesses due to egg products. On the other hand, however, it is difficult to make an argument that egg products are not a problem simply because a problem has not been observed. Validation will remain problematic for the egg products portion of the risk assessment.

Annex A

Comment #71: This is informative, not a general introduction to the annexes, but to the report. Why not put this in the report itself?

Reply: We deem it best if this introductory portion remains part of the annexes rather than the main report.

Comment #72: Uncertainty: Good discussion. You have to read this to understand what is meant by 'state-of -knowledge' in the report, which is essential information. What is the difference between 'state-of-knowledge' and 'guesstimate'?

Reply: A state-of-knowledge estimate is one based on careful consideration of extant data by persons familiar with the subject; "guesstimate" and similar terms imply a casual or indeed lazy approach to analysis, which was not the case here.

Comment #73: Throughout the report uncertainty analysis and sensitivity analysis are mixed up (e.g. p. 5. of the report). It should be clarified somewhere which is what and why you do it.

Reply: Yes. Mostly what is done is sensitivity analysis. The effect of uncertainty, especially the global effect, is generally not characterized. This has been clarified in the report.

Shell egg model.xls

Comment #74: The spreadsheet is not well documented. The color code of cells is not clear and seems inconsistent. I am not an expert in Visual Basic and cannot really check whether the model does what it promises to do. The link between parameters in main text, annex and spreadsheet model is not evident: It is difficult to find parameters from the report (as reported in the tables) in the spreadsheet. The names of parameters and variables differ in the spreadsheet and report; this complicates reading the spreadsheet model.

Reply: All of the model inputs have been placed in user forms to make auditing the model easier.

Comment #75: For example: the probability of illness in the results sheet is not the probability of illness (it is not a probability) but the illness rate ("number of illnesses", as you call it). Adding here that it is I_E would help a lot.

Reply: It is not a rate because it doesn't involve a time component. As discussed previously we have changed the label of this value to frequency.

Comment #76: I cannot reproduce the figures in the report with this spreadsheet model at hand. If I run the model send to me on CD I first could not reproduce the baseline results given in the report. The 'baseline' model in the spreadsheet (the one I got on CD) is not the baseline as in the report, where baseline = no pasteurization. What is the use of the pasteurization model for shell eggs if you only use no pasteurization, 3 logs or 5 logs? Apparently, the 'baseline' in the spreadsheet is 1 log reduction (on average).

Reply: The reviewer must have inadvertently been sent a model that had the pasteurization input set to 1 rather than 0.

Comment #77: From the spreadsheet results I read that numbers of bacteria are not integers (except when samples from the Poisson distribution). This is not a good modeling approach when the numbers of bacteria per egg are low. (1.5 bacterium should be 1 or 2).

Reply: The growth equations that are used require fractions of bacteria to be modeled at each interval. The biological process that allows one bacterium to become two bacteria is modeled by generating an increasing decimal value until two bacteria are achieved. The results show intermediate points of a continuous process. For instance, there may be 3.4 bacteria after on-farm storage because 3 bacteria are on the way to becoming more. If this were to be modeled as 3 then the amount of progress toward reproduction that has taken place is dismissed. Consequently, modeling low numbers of bacteria as fractions is essential in ensuring that growth is properly modeled. The modeled stages are arbitrary divisions of a continuous process. If the numbers of bacteria were rounded at the end of each stage, then the numbers of bacteria would depend on how many roundings took place.

There are, however, points at which numbers of bacteria must be represented by integers. After pasteurization bacteria are either present or they are not. If bacteria are present, new growth can modeled; if bacteria are not present, no amount of time and temperature abuse can generate the contamination which has disappeared. The Poisson draw returns integer values for the number of bacteria after pasteurization. On the other hand, bacteria after cooking are represented as fractions because these values are used in the dose-response function.

Comment #78: Likewise, the exposure (column AS in the results) holds non integer values of number of bacteria in an egg. The vast majority is a number between 0 and 1. This cannot be, 0.01 bacterium does not exist. Either my interpretation of the spreadsheet is wrong, or there is a serious mistake in the modeling concept here.

Reply: The growth equations that are used require fractions of bacteria to be modeled at each interval. The biological process that allows one bacterium to become two bacteria is modeled by generating an increasing decimal value until two bacteria are achieved. The results show intermediate points of a continuous process. For instance, there may be 3.4 bacteria after on-farm storage because 3 bacteria are on the way to becoming more. If this were to be modeled as 3 then the amount of progress toward reproduction that has taken place is dismissed. Consequently, modeling low numbers of bacteria as fractions is essential in ensuring that growth is properly modeled. The modeled stages are arbitrary divisions of a continuous process. If the numbers of bacteria were rounded at the end of each stage, then the numbers of bacteria would depend on how many roundings took place.

There are, however, points at which numbers of bacteria must be represented by integers. After pasteurization bacteria are either present or they are not. If bacteria are present, new growth can modeled; if bacteria are not present, no amount of time and temperature abuse can generate the contamination which has disappeared. The Poisson draw returns integer values for the number of bacteria after pasteurization. On the other hand, bacteria after cooking are represented as fractions because these values are used in the dose-response function.

Comment #79: The model can be simplified by skipping all calculations where cooking has a 12 log decrease effect. This is simply because an egg cannot contain more than 10^10.59 = 38.904.514.499 cells. With twelve log reduction there will be none left whatsoever. More then 50% of the model runs will therefore yield an expected exposure of < 0.1 cfu SE, which can be simplified to no exposure et al. Calculations do not make sense; it saves half of the computing time.

Reply: An expected exposure of less than 0.1 cfu can not be simplified to no exposure. At an expected value of 0.1 cfu, one in ten servings would be expected to have 1 organism. When there are many servings even very small probabilities can result in illness.

Comment #80: Cell D18 = j does not have the value as given in the report (Table 3-1).

Reply: Cell D18 represents an adjustment that considers a flock should be modeled as "molted" only in the immediate post-molt period. As the text notes:

> At any given time of year, the fraction of all flocks that are molted is estimated to be about 22%; only those flocks that are molted and in their first 20 weeks of production post-molt are of interest for this part of the exposure assessment. A non-molted flock will produce eggs for 52 weeks. Therefore, over 2 years there are 104 weeks of production. If the flock molts, the period in molt is about 10 weeks, and there are 94 weeks of production available. As such, the pre-molt and post-molt production periods constitute about 47 weeks each. The first 20 weeks of one of these production periods is about 42% of the production year. Consequently, 9.4% (22% x 42%) of flocks are molted and in their first 20 weeks of post-molt production. This fraction of infected flocks represents the flocks producing contaminated eggs at higher frequencies than the remainder of infected flocks.

The table has been adjusted to reflect the value used and relabeled "Fraction of flocks in immediate post-molt period."

Comment #81: I get another mean for M(t) (3.1 not 2.86)... Or is it the ML value? (See table 5-7).

Reply: This is correct. The mean considering weeks 0 through 20 is 3.1. The model considered eggs produced from 1 through 20 weeks rather than from weeks 0 through 20. Using either value will not make a substantial difference in the result, although using the value for weeks 1 through 20 may help account for the lower initial egg production hens experience after the return to molt.

REVIEWER #2

Comment #1: *Evaluate whether the risk assessment answered the specific FSIS risk management question.* "It is never clearly stated in this document. The authors infer that refrigeration-storage temperatures, and pasteurization are important critical control points in management of SE in table eggs and they never state Pre-harvest, on-farm management practices that impact on SE prevalence among layer flocks. One assumes that biosecurity, depopulation of SE-positive flocks, vaccinations, etc are important factors in control of SE. It would have been informative to all to address the purpose of this risk assessment, how it's different and/or builds on past SE risk assessments AND clearly identify management practices important to building this risk assessment."

Reply: The purpose of these risk assessments was to address the risk management questions specifically. Itemized responses to each of the questions may be found in both the Executive Summary and the Rick Characterization sections of the report.

Comment #2: *Evaluate whether all key studies and data been identified and critically evaluated. Have all key studies and data been identified?* YES.

Reply: No response needed.

Comment #3: *Have the data been correctly interpreted and emphasized?* "It is questionable whether it was appropriate to average SE layer flock prevalence for US. Rather it should have used a range of values representative of extremes in flock prevalence, including size of layer industry in that region and total egg production per region in the risk assessment. This would be interesting in terms of *Salmonella* incidence comparisons generated from CDC FOODNET with projected incidence of SE in table eggs produced for that region."

Reply: Because the risk assessment was designed to answer questions about the per annum risk for the nation, it was appropriate to average SE layer flock prevalence in the US.

Comment #4: *Were input data valid and appropriate for use in this risk assessment?* "Listed, are several values inputted into the model where their derivation is unclear or elimination from modeling adequately justified."

Reply: See below

Comment #5: False-negative rate: Emphasis is placed on single reference on false-negative rate, 50% (Waltman et al. 1992), and yet authors choose 15% to model into their calculations, why?

Reply: As explained in the text, the basis behind using a 15% false-negative rate was as follows:

> While a 50% false negative rate may be high, such a rate cannot be dismissed, particularly for low level SE-infected flocks. It is possible that the false negative rate would be a function of the percentage of positive test – a higher percentage would, or might imply higher levels of SE, generally, which would imply a lower false negative rate. No information on this is available, and thus, for simplicity, a moderate false negative rate of 15% was assumed in the above analysis.

Comment #6: The authors illustrate problems with current culture methodologies for detecting *Salmonella* that are influenced by sample type or *Salmonella* serotype. Also with incorporation of PCR into *Salmonella* surveys and *Salmonella* sero-prevalence data, this reviewer believes a "sound" false-positive rate can be made, rather than an arbitrary value of 15-percent. In addition, this value is not used consistently throughout Annex B, see B-42 where authors state false-negative rate of 10% in the risk assessment.

Reply: The polymerase chain reaction (PCR) was not used in the *Salmonella* surveys cited in the report. The discrepancy between 10 and 15% has been corrected.

Comment #7: Egg yolk contamination (B-17): authors minimize potential for egg yolk contamination and yet, although low, paper(s) they cite state that this does occur, experimentally.

Reply: Egg yolk contamination has been demonstrated experimentally. However, as discussed, we believe the potential for it to occur under natural conditions is low.

Comment #8: Reliance on experimental data, although necessary, may generate erroneous conclusions concerning *Salmonella* prevalence, persistence, and cell density within flock, individual bird and eggs. What is especially troubling is reliance on data generated from oral challenges with high inoculums that may not reflect their true levels in nature.

Reply: We agree that one of the drawbacks to using experimental data is that they may not reflect precisely what occurs in nature. However, in absence of data from natural environments it is appropriate to use data from laboratory experiments.

Comment #9: Percentage of flocks estimated to be molted is 22%, and yet for model, 10% was chosen. Is this an arbitrary value and if so why was it chosen?

Reply: At any given time of year, the fraction of all flocks that are molted is estimated to be about 22%; only those flocks that are molted and in their first 20 weeks of production post-molt are of interest for this part of the exposure assessment. A non-molted flock will produce eggs for 52 weeks. Therefore, over 2 years there are 104 weeks of production. If the flock molts, the period in molt is about 10 weeks, and there are 94 weeks of production available. As such, the pre-molt and post-molt production periods constitute about 47 weeks each. The first 20 weeks of one of these production periods is about 42% of the production year. Consequently, 9.4% (22% x 42%) of flocks are molted and in their first 20 weeks of post-molt production. This fraction of infected flocks represents

the flocks producing contaminated eggs at higher frequencies than the remainder of infected flocks.

Comment #10: Authors discuss yolk membrane breakdown, an important event for modeling SE growth in eggs. It is unclear as presented why YMB is documented when SE reaches 5.3 log10 CFU/ml, and yet stated later, E-15, YMB appears to be a function of time and temperature. Are the authors inferring that this level of SE in the egg is responsible for YMB or an indicator of YMB? Which of these three variables are important in modeling YMB and bacterial growth in the egg? Obviously, as stated earlier, we need a reliable measure for when this event occurs.

Reply: The value of 5.3 $\log_{10}$ CFU/ml *Salmonella* was taken as an indicator of YMB, not as a cause.

Comment #11: Lag phase, particularly its length, is an unknown and important variable that this risk assessment identifies as data gap. It impacts on estimation of growth rate, especially if sampling time points are few. Also important is determining when non-exponential growth (late-exponential and stationary phase) commences. Thus, the sampling window for estimating growth-rate is during exponential growth and this is critical in accurately estimating μ. The authors discuss this later, but it is imperative to place it early in discussion of the model and remove extraneous information, that is OFTEN distracting.

Reply: The discussion of lag phase length et al. has been shifted to an earlier point in the discussion. Efforts have been made to remove unnecessary information from the document.

Comment #12: In attempt at following discussion in text with data presented in tables, this reviewer comes across the following contradictions. Table E-9, subscript b, the reported inoculums are 0.079 log10/ml and 1.079 log10/ml but different values are given on E-25 of the text.

Reply: The text has been corrected.

Comment #13: Annex E, as written, is difficult to determine whether the authors believe albumen growth is important and relevant to modeling bacterial growth, especially as YMB appears to be important in this discussion. From this discussion, it's apparent that inoculums administered to egg albumen impacts on growth results, but not for the reasons presented, (probability vs. physiology), and therefore, data from low inoculums should be excluded from any calculations and discussion. Other relevant factors that need to be assessed, or at least discussed, are geographic and seasonal differences with regards to SE prevalence in layer flocks. Also with regards to consumers' behaviors/habits, seasonality should also be considered (ex. eggnog).

Reply: Data from low inoculums should not be excluded as these types of inoculums likely represent contamination in the natural environment. Indeed, the researcher in

whose laboratory the work was performed states that in his view "an inoculum of <10 cells per egg produces the most reliable data."

The risk assessment sought to answer questions about per annum risk; analysis of geographic and seasonal differences was outside of its scope.

Comment #14: *Comment on the overreaching logical structure of the risk assessment. Comment on the biological plausibility of the assumptions made in the risk assessment. Are the mechanics of the model consistent with known biology?* Comments on points 3, 4 & 5: Overall, the risk assessment is excellent in presenting the continuum of events necessary to causing the end result associated with consumption of egg, egg-product: illness. Many of the explanations within the appendix range from excellent to poor. There several factual errors and superfluous sections that makes reading and understanding of this assessment difficult. This reviewer found them especially distracting and wonder if authors truly understand microbial physiology important to modeling bacterial growth rate and other physiological parameter important for understanding SE behavior in eggs. The following are specific examples.

Reply: See below

Comment #15: A-8:How does quorum-sensing and swarming impact on growth rate and physiological adaptation to the environment? Authors do not explain physiological basis for statements made in this section.

Reply: The section on quorum-sensing and swarming has been removed. It was originally offered simply as an explanation for the biology underlying growth of SE in albumen; however, none of the information presented in the section was directly related to the risk assessment.

Comment #16: Also erroneous statements are made. For example, investigators have recently determined that the quorum sensing molecule is not acyl homoserine lactones.

Reply: This section has been removed.

Comment #17: B-44: How is discussion of flagella phase-variation relevant for MONO-phasic salmonellae like SE?

Reply: Discussion of flagella phase-variation has been removed.

Comment #18: B-45 to 49: Is way too long, convoluted with contradictory statements. This section needs to be reduced to one page, tops. The only relevant information should be from those that examine defined mutation(s) in fimbrial genes and comparisons to parental strain, as the authors recommend.

Reply: The section has been removed.

Comment #19: E-39: Bacteria do not restrict free iron, their animal hosts due by producing proteins with high affinity for iron (transferring, lactoferrin, and ovotransferrin).

Reply: This section has been removed.

Comment #20: E-39: Siderophores ARE NOT proteins, but rather low molecular weight, phenolate derivatives.

Reply: This section has been removed.

Comment #21: E-40: Authors state that enterobactin is capable of competing with ovotransferrin for iron, yet on previous page supplementing albumen with iron allowed SE to grow and authors reference Humphrey study that SE reaches cell density of 10^5-10^6 cells. There is apparently something, essential for growth that limits SE cell density in albumen. This circuitous discussion is one example of many places in this document where this type of discussion is distracting due to its contradictory statements.

Reply: This section has been removed.

Comment #22: E-40: Here, as well as in several other sections, bring in quorum-sensing and biofilms to explain SE behavior where in many cases it is not only possible explanation, but does illustrate a more important point to consider, SE may exist as a heterogenous population, at least with regards to its physiological state. This has important implications with regards to interpreting results from low vs. high dose inoculums, culturing and enumerating *Salmonella*. Since authors state that several of these studies used cultures in stationary phase, chances are this represents heterogeneous population of cells: 1) stationary vs. exponential; 2) planktonic vs. sessile (biofilm).

Reply: Information about quorum sensing, etc. was introduced solely for the purpose of offering an explanation of how growth of SE may occur in albumen; this information is not directly related to the risk assessment model and has thus been removed.

Comment #23: The other point that is problematic with discussion of low dose data is "how confident is the investigator that 2 cells were administered per egg". It would appear that study design needs the sample size and analysis associated with Poisson distribution for rare events.

Reply: We agree with the comment's implication that design effects, particularly at this inoculum level, could have had an effect on the results. This concern is manifested in the inconsistency of the results that are associated with this inoculum level, when compared to the other results. On Table E4 of Annex E, it can be seen that at 30ºC the percentage of eggs showing large relative growth is greater, by a small amount, for the eggs inoculated with 2 cells versus those inoculated with 25 cells (30% versus 23%); on the other hand, at 20ºC, the relationship was reversed by a substantial amount (7% versus 30%). Note also that for the eggs inoculated with 25 cells, the percentage showing large growth was

greater at 20ºC (30% versus 23% at 30ºC). Accounting for the eggs that actually had no cells being inoculated would increase the actual percentages by small amounts which would accentuate the inconsistencies even more. We could explain these data (and the inconsistency) by hypothesizing that a temperature effect only occurs for low initial levels and that otherwise, there is no temperature effect. And to fit the data well, we could have constructed a model that had this property – that the likelihood of large relative growth was temperature and level dependent when the temperature was below 30ºC (how much below would be anyone's guess), and when the levels were below 25 cells (again how much below is not known. But our concern was that the results at 20ºC, with such a low level of inoculation could be incorrect for reasons connected with the actual experiment, and consequently it was decided not to use these data for the inoculm of 2 cells. Even if the results represented the situation accurately the bias introduced by deleting these data would not be large: it is only the one cell – at 20ºC – that had significantly lower proportion of egg showing growth.

Comment #24: Finally, to determine growth rates within the egg and its various compartments, one would use exponentially grown cultures rather than stationary phase cells. This is the general approach used in nutrient down-shift experiments to study growth rate, growth kinetics as cells go from nutrient-rich to nutrient poor conditions.

Reply: No response needed.

Comment #25: *Review and analysis of model:* This risk assessment clearly illustrated, at least to this reviewer, inadequacies of current methodology for assessing SE growth-rate and enumeration in vivo and within the environment.

Reply: No response needed.

Comment #26: *Have the risks been appropriately characterized.* YES.

Reply: No response needed.

Comment #27: *[Did the risk assessments identify] Key sources of variability and uncertainty?* YES

Reply: No response needed.

Comment #28: *[Did the risk assessments identify] Critical assumptions.* YES

Reply: No response needed.

Comment #29: *[Did the risk assessments identify] Important data gaps.* YES

Reply: No response needed.

Comment #30: *Does the risk assessment identify and characterize the following:*

User friendliness of the model. Clarity of risk assessment report. On whole, the authors did an excellent job identifying all factors and variables within the continuum, from farm to fork, important to a risk assessment for SE. However, this report, as written, is extremely difficult to follow. It gets "bogged-down" mostly due to minutia, where at least half the information is irrelevant to discussion of the model and therefore should be eliminated from discussion. This report also needs to include discussion of previous risk assessments, and hi-light departure that this new assessment took in evaluating risk associated with consumption of table eggs.

Reply: Much text has been removed in an effort to streamline the document. This includes discussion of possible mechanisms (iron sequestration, etc.) for bacterial growth in albumen.

REVIEWER #3

Comment #1: *Response to the evaluation criteria: Evaluate whether the risk assessment answered the specific FSIS risk management questions.* The risk assessment clearly and explicitly answers the risk management questions as summarized on p215-216. The risk assessment is properly aimed at answering those questions.

Reply: No response needed.

Comment #2: *Remarks on some of the specific questions: The number of SE in shell eggs before and after pasteurization:* I could not reproduce the quantitative results, but can subscribe the conclusion that 3 and 5 log reduction are not reflected at consumption. If this management question would be asked to me, I would ask for clarification: this "number of SE" is variable and can be described by a distribution, but why should that be of interest to a manager?

Reply: No response needed.

Comment #3: I have strong doubts whether the mean is the proper measure to reflect "the number of SE in shell eggs".

Reply: The revised risk assessment report presents the potential for illness at each point in the shell egg production continuum, rather than the mean number of SE cells.

Comment #4: *The number of illnesses per serving and annual number of illnesses:* Some information on the uncertainty about the differences between the three scenarios would be welcome here. This may be derived from different sets of model runs, which will not yield 'total uncertainty', but indicate the uncertainty associated with the risk assessment model used.

Reply: The only difference in the model runs is the amount of log reduction applied to shell eggs. Log reduction is a constant for a particular simulation. Because the same seed value is used for each of the model runs, all draws from the distributions are identical, and the results represent only the effect of a particular log reduction. When different seed values are used, the shell egg model has an approximately 6% standard error on runs of 50,000 iterations.

Comment #5: *Evaluate whether all data were identified and critically evaluated. Have all key studies and data been identified?Have the data been correctly interpreted and emphasized?Were input data valid and appropriate for use in this risk assessment?* I cannot judge this.

Reply: No response needed.

Comment #6: *Comment on the overreaching logical structure of the risk assessment.*

The structure of the risk assessment is according to international standards. Models are linked in a logic way, probability distributions are properly linked and interpreted.
I do have some concern about the use non-integer values representing (low) numbers of bacteria. This is not a good modeling practice in quantitative microbiological risk assessment. It is not clear from the report and the spreadsheet whether this issue is properly addressed, I get the impression it is not.

Reply: The growth equations that are used require fractions of bacteria to be modeled at each interval. The biological process that allows one bacterium to become two bacteria is modeled by generating an increasing decimal value until two bacteria are achieved. The results show intermediate points of a continuous process. For instance, there may be 3.4 bacteria after on-farm storage because 3 bacteria are on the way to becoming more. If this were to be modeled as 3 then the amount of progress toward reproduction that has taken place is dismissed. Consequently, modeling low numbers of bacteria as fractions is essential in ensuring that growth is properly modeled. The modeled stages are arbitrary divisions of a continuous process. If the numbers of bacteria were rounded at the end of each stage, then the numbers of bacteria would depend on how many roundings took place.

There are, however, points at which numbers of bacteria must be represented by integers. After pasteurization bacteria are either present or they are not. If bacteria are present, new growth can modeled; if bacteria are not present, no amount of time and temperature abuse can generate the contamination which has disappeared. The Poisson draw returns integer values for the number of bacteria after pasteurization. On the other hand, bacteria after cooking are represented as fractions because these values are used in the dose-response function.

Comment #7: *Comment on the biological plausibility of the assumptions made in the risk assessment.* Not my expertise.

Reply: No response needed.

Comment #8: *Are the mechanics of the model consistent with known biology?* As far as I know biology, they are, except for the use of non- integer values for numbers of bacteria. Half a bacterium is not a bacterium.

Reply: Removing use of non-integer values implies setting a threshold for pasteurization. In other words, a 3 $\log_{10}$ reduction applied to 2 $\log_{10}$ of bacteria would always result in 0 bacteria, rather than an expected value of 10^{-1}. Similarly this implies that if a serving is contaminated with 95 bacteria (1.98 $\log_{10}$) then a 2 $\log_{10}$ reduction will always be sufficient because the result would be 0.95 bacteria which is only a fraction of a microbe.

The approach in the risk assessment assumes that log reductions are probabilistic events. Thus, if 0.95 bacteria are expected after a log reduction, then 61% of the time (assuming a Poisson distribution) there will be at least one surviving organism.

Similarly, the beta-Poisson dose response curve considers average doses for populations rather than a single dose for an individual. Doses of 10^{-4} cells are representative of a population of individuals receiving this as an average dose, not one person consuming 0.0001 microbes.

Comment #9: *Review and analysis of model:* It is difficult to check the spreadsheet model, because the links between the report and the model are missing or at least not obvious. The documentation accompanying the model (2 pages) is far too little to examine the model thoroughly in a few days time. The color codes in the spreadsheet are not clear, and apparently neither consistent. There is (for example) no explanation about how to interpret the results sheet, and this cannot be checked without unraveling the code in Visual Basic. (Tools available in Excel cannot be used.) Likewise, there is no explanation what the different macro's are doing, or how to interpret them. It is not clear how to get many of the results presented in the report when you have the spreadsheet model available.

Reply: Documentation has been revised throughout in an effort to increase clarity. The liquid eggs product model has been rewritten to present a more user-friendly interface. Inputs are now presented in a series of user forms. Additionally, all inputs and summary results for each simulation are automatically archived in a separate worksheet. This has allowed removal of most of the macros written to run specific simulations.

Comment #10: *Comment on the appropriateness of modeling techniques (model mathematics and equations).* Details are predominantly hidden in the annexes, which are not written very 'user-friendly'.

Reply: Efforts have been made to streamline text and add clarity.

Comment #11: In general modeling techniques are of high quality, at or ahead of the current international standards. Some discussion on the appropriateness may be added, it is interesting to find out what is gained by applying more advanced modeling techniques.

Reply: In the introductory portion of the report we discuss the fact that advanced modeling techniques was one of the driving forces behind the decision to undetake new risk assessments for salmonellae in eggs and egg products.

Comment #12: *Examine the methodologies used in the risk assessment for estimating parameters from the data:* I am not an expert in this kind of statistics. In general, representativeness of data is more important than the precise fitting technique (which usually gets most attention). This should be discussed.

Reply: Discussion of data representativeness has been added at the end of annex A. Also, the sensitivity analyses performed as part of the risk assessments identified those parameters which most affected model output.

Comment #13: *Examine/check the data analyses (spreadsheets) for compliance with the methods and overall accuracy:* This is difficult to check throughout. However, I had some problems. Looking for example at the molting multiplier (cell d19 in sheet Inputs of the shell egg model) the 2.86 does not follow from equation M(t) on page 45. To calculate E (fraction infected eggs) you need only the mean of the given distribution, which I calculate as 3.1. At page 179 2.86 is the ML value, not the mean. They may of course be identical, but this is not clear.

Reply: This is correct. The model considered eggs produced from 1 through 20 weeks rather than from weeks 0 through 20. Using either value will not make a substantial difference in the result, although using the value for weeks 1 through 20 may help account for the lower initial egg production hens experience after the return to molt.

Comment #14: Also, I could not reproduce table 5-2 with pasteurization.

Reply: This table was based on data that is obsolete. The table has been removed.

Comment #15: *Examine/check the source code for overall accuracy.* The model is built in an Excel environment, but all essential modeling details are in Visual Basic. My expertise in Visual Basic is too much limited to check the source code. Without good background documentation this is a very time consuming job anyway.

Reply: No response needed.

Comment #16: *Have the risks been appropriately characterized?*Roughly they are. I see no reason why risk managers should not believe the final conclusions. I do have comments on details, as outlined above.

Reply: No response needed.

Comment #17: *Does the risk assessment identify and characterize the following: Key sources of variability and uncertainty?* I am very pleased with the way the uncertainty / variability issues are handled. It appears to be done in a consistent, logical way, fit to purpose. I cannot judge whether key sources are addressed: The only reference I have for identifying key sources is this report itself.

Reply: No response needed.

Comment #18: *Critical assumptions:* The sensitivity analysis is aimed at identifying critical assumptions. Using different types of analysis is a good approach.

Reply: No response needed.

Comment #19: *[Did the risk assessments identify] Important data gaps?* Yes.

Reply: No response needed.

Comment #20: *User friendless of the model: Is the model documentation adequate to allow individuals to conduct "what-if" calculations and alter sensitivity parameters?* The model is by no means user friendly. Model documentation is inadequate. It is not really clear what you are doing with any calculation.

Reply: The model documentation has been revised throughout in an effort to increase clarity. The liquid egg product model has been rewritten to provide a more user friendly interface. Furthermore, the model has been modified to provide archiving of summary results of past model runs and to allow multiple serial unattended simulations as an advanced option. Although increased use is made of tips and text to explain options, it is easier to run the liquid egg products model after about 30 minutes of training.

Comment #21: *Clarity of risk assessment report: Has the report been written and presented in such a way to clearly communicate the results of the assessment?* The report is fairly well written. I am not sure whether it is all suitable for the primary audience (FSIS risk managers), who will probably be mainly interested in the executive summary. The secondary audience may pick up those parts it is interested in. In general, it is a huge task for anyone to read the whole report and understand all the details. For a risk assessor, it is a pity that the interesting modeling details, which are essential to evaluate the modeling concepts applied and their validity, are put away in annexes. I would prefer to have the (essential) models in the report, and tables of data (which have a look-up function) in the annexes. I know model equations may scare off readers, but they are the backbone of risk assessment, and required to guarantee the reproducibility of the results. One should ideally be able to make a computer program of the whole model without having the spreadsheet model available.

Reply: The overall theme raised by the reviewer is a good one – one we struggle with continuously. On the one hand, the risk assessment report must give enough technical detail to enable scientists and other experts to offer a substantive critique, understand the model, and attempt to reproduce the results. On the other hand, the report must be clear and reader friendly to risk managers and others who may not have technical expertise in the varied facets of risk assessment modeling (and it must be done so in a fashion that is not derogatory or presumptuous).

While we understand the reviewer's desire for incorporating more of the equations into the body of the text, we respectfully maintain that these are best placed within the annexes. We have in the past made attempts to place more of the mathematics of the assessment into the main body of the report; doing so, however, only serves to make the report (more) cumbersome and convoluted. This it is we chose to present the equations in annexes – we feel they tend to streamline the main body of the report while at the same time ensuring the reader is fully informed of the data and modeling techniques employed in the assessment.

Comment #22: Referencing to other parts of the report is insufficient: the reader has to find details that are referred to himself, which is not helpful in understanding the logic.

Reply: Improved cross-referencing has been included in the revised report.

Comment #23: When referring to Annexes, the precise page or chapter of the annex should be given. Also, links between chapters should be indicated better. Naming of model parameters and variables should be consistent throughout the model; a table with all of them would be of help.

Reply: Please see reply to comment #22.

Comment #24: *Final comment*. I miss a discussion on the added value of this risk assessment compared to the previous one (1998) and the JEMRA one. What makes the difference?

Reply: The current risk assessment reflects important information from various technical publications and baseline studies, as well as new and improved modeling techniques unavailable at the time of the 1998 risk assessment. The Executive Summary states:

> Since 1998, however, data have become available to develop robust risk assessments for *S.* Enteritidis in eggs and *Salmonella* spp. in products. First, FSIS has conducted a national baseline survey to measure *Salmonella* levels in liquid egg products produced in the U.S. (FSIS, 2001). Second, recent experimental studies have clarified some of the scientific issues associated with *S.* Enteritidis contamination in egg yolk (Gast and Holt, 2000a, 2000b, and 2001). Third, the United Egg Board sponsored studies to produce valuable data on the lethality kinetics of *Salmonella* spp. in a wide variety of liquid egg products (United Egg Producers, 2001). Fourth, Codex developed an improved dose-response model for *Salmonella* spp. (WHO/FAO, 2001), one which is favored by FSIS risk managers because it is considered more protective of public health.
>
> As a result of these newly available data, two new risk assessments were undertaken. One estimates the risk of illness associated with *S.* Enteritidis in shell eggs. The other estimates the risk of illness associated with all *Salmonella* spp. in pasteurized egg products. The purpose of these risk assessments is to assist FSIS risk managers in developing performance standards to mitigate the likelihood of *S.* Enteritidis contamination in shell eggs and *Salmonella* spp. in egg products.

The risk assessment conducted by the Joint Experts in Microbiological Risk Assessment (FAO/WHO) to which the reviewer refers was for *S.* Enteritidis in shell eggs and multiple *Salmonella* serotypes in broilers. A substantive difference between the FSIS risk assessment and JEMRA risk assessment is that the former estimates risk for *Salmonella* spp. from liquid egg products whereas the JEMRA did not.

REVIEWER #4

Comment #1: *Overall comments.* This risk assessment is certainly an impressive work and it obviously represents a herculean effort by FSIS staff. I am not yet completely sure about the reasonableness of the model or its implementation, but it's surely a great beginning. Even though I have spent more than the four days allotted to me to review the assessment, I still feel as though I have only scratched the surface of this extremely complex product.

Reply: No response needed.

Comment #2: I believe that there are some issues that should be reconsidered, or maybe just re-argued, in a revision of the assessment. I firmly believe that considerable further checking is required, including some automated checking by computer, before one could reasonably have a sense of general confidence in the model as a whole and the results of this assessment.

Reply: Individual segments were built in Excel spreadsheets. The calculations were reproduced in VBA code. This code was run and checked to make sure the same answers were generated.

Comment #3: I would be interested in hearing feedback from the developers at FSIS regarding my comments, and in seeing the comments from other reviewers. My criticisms below, which may feel alternately harsh or pedantic, are offered in a genuinely collaborative spirit with much respect for the effort and accomplishment this assessment represents.

Reply: We plan to share the reviews together with our replies with each individual reviewer.

Comment #4: *Modeling issues.* The style of this assessment, and some of the thinking it entailed, is familiar to me from FSIS' recent *E. coli* assessment, although the *Salmonella* effort seems better in many respects, especially in the implementation and presentation of the modeling. For instance, I could get this simulation to run to completion and actually produce results (this is very good and was not necessarily guaranteed given the size and complexity of the model). On the other hand, an unfortunate similarity with the *E. coli* assessment is a profound lack of transparency in the documentation and the software. No doubt this is due in large part to the inherent and inescapable complexity of the underlying scenarios considered. But however bad this complexity may be, it does not erase the requirement that the assessment be intelligible and transparent enough to be reviewable and reproducible.

Reply: Based on this and similar feedback, we have worked to increase the model documentation's clarity.

Comment #5: My most serious concerns about the assessment from a perspective of modeling and risk analysis are (i) major sources of uncertainty seem to be ignored or relegated to an incomplete analysis, and (ii) some sources of variability seem to have been substantially underestimated.

Reply: These are addressed below where specific instances are noted.

Comment #6: In the introductory discussion of the modeling plan, on page 42, the nature of the theory to be used for handling and distinguishing uncertainty and variability is unclear. It would be helpful to cite the literature appropriately so risk analysts will be able to understand what you're thinking. The approaches used in the assessment, insofar as I understand them, seem to be neither state-of-the-art nor entirely sufficient for the task of risk assessment.

Reply: A more complete explanation of how we conducted a first order model and conducted a sensitivity analysis of the inputs is given in the revised report.

Comment #7: *Averaging and regressing away variability.* In a few places throughout the assessment, the developers have elected to use averages of random variables rather than model the intrinsic variability of the random variables. For instance, on page 63, the discussion considers exponential cooling coefficients. Instead of discussing the individual cooling rates that were observed, we see only the means. In principle, this results in an underestimate of the variability present in this parameter. Apparently, the modelers feel that stochastic variability (as opposed to trends induced by packing geometry inside egg pallets and cases) is not terribly important in this parameter, because they later abandon modeling it at all except in a crude discrete distribution with three points of support. Indeed, a grand average of the means displayed in the Table 3-10 is taken (and arbitrarily simplified). This averaging reduces even the apparent range of 0.0063 to 0.615—which corresponds to a range of thermal equilibration periods from 20 to 2 days—to one or a few points with a much smaller variance. It's not entirely clear from the discussion why this is a reasonable decision. How could it be justified by subsequent sensitivity analyses if the original variation is erased before the simulation begins? The argument to simplicity on page 64 is by no means compelling. You may not be able to disaggregate this variation, but you can still model it *statistically*, that is, phenomenologically.

Reply: The *k* values represent only the central egg. We did decide that where an egg is within a pallet was probably the most important factor for cooling. The equation for adjusting the cooling factor by accounting for the location of the egg is at best an approximation as the cooling of all eggs also depends on convection. This drove the early decision to simply represent cooling constants by one of three possible values. The sensitivity analysis confirms that it does not matter substantively what cooling constant is assigned to the central egg of a pallet or a case. This is because many of the other eggs will equilibrate much more rapidly.

Comment #8: A similar problem, with the same consequence of underestimating the variability in the system, arises when regression models are employed to model inter-variable relationships. The standard errors for the regression parameters are given in the documentation and on the Inputs worksheet. Although I was unable to track where or even whether these standard errors were used in any of the calculations, I presume they were used somewhere (perhaps only in sensitivity studies?). If they are not used, then the variability and uncertainty from the regression has been totally ignored. Even if the standard errors are used, they are underestimating the true variability present. This is because standard errors of the regression constants are not sufficient measures of the reliability of the regression. To *reconstruct the scatter* of the regression (which is what a risk analyst has to do to faithfully propagate the variability), one needs the σ from the regression analysis. These values do not appear to be mentioned anywhere, nor is there any evidence that they are used in calculations to reconstruct the variability in the original regressions.

Reply: This refers to the parameter estimates for the growth equations. Because they were used to represent uncertainty for the estimates, the standard errors are used in the sensitivity studies only.

Comment #9: *Uncertainty analysis.* Apparently, nominal range sensitivity analyses and "correlation analyses" (a terrible misnomer) were the extent of the uncertainty analyses in this assessment. I agree with two of the three arguments given on page 174 for why a second-order Monte Carlo simulation could not be conducted. It is clear that the sometimes gross uncertainties cannot themselves be probabilistically characterized. It is also clear that a two-dimensional simulation would be computationally impractical anyway. (The first reason mentioned on page 174, however, doesn't make sense as a reason not to do a second-order assessment.) Nevertheless, it not entirely reasonable to hold that a comprehensive uncertainty analysis is not still needed.

Reply: Uncertainty was generally not evaluated in this assessment because the global uncertainty in the model overwhelmed the uncertainty present in the epidemiologic evidence. This is because many of the inputs were based on limited data. Thus, the effect of individual inputs was evaluated with a sensitivity analysis.

Comment #10: And the sensitivity analyses that were conducted are really only a pale shadow of what a full uncertainty analysis would provide. I believe it makes sense in some cases—and perhaps this is one of those cases—to adopt a bounding approach to handle the uncertainty about probability distributions. Analysts often find it much easier to circumscribe interval bounds on probability distributions even though prescribing distributions for their parameters (second-order distributions) would be burdensome.

Reply: The uncertainty present in just the production segment of the model (proportion of contaminated eggs) is larger than the uncertainty present in the epidemiologic evidence. Thus, nominal range sensitivity analysis was used to show effect of individual inputs.

Comment #11: The methods of probability bounds analysis can be used to project this uncertainty through calculations in a way that is computationally convenient and yet also mathematically comprehensive. As it stands now, one must conclude that the assessment has not taken the question of uncertainty about the probabilistic inputs seriously. Further work remains to be done.

Reply: The text states:

> The uncertainty and variability about the likelihood of human illness from SE in shell eggs are characterized in Chapter 4 of this report. The characterization is based on epidemiologic evidence regarding the occurrence of human illness.

We would agree that a second order model that was able to narrow the uncertainty of this likelihood would be useful. If all of the uncertain inputs in this model, however, were propagated throughout the model, the resulting uncertainty would be greater than that derived from the epidemiologic evidence. Such a characterization would be unhelpful for decision makers. It is the goal of this risk assessment that the most likely scenario fall within this range of uncertainty given by the public health estimates. We have good estimates of the risk, primarily gleaned from data generated through the Foodborne Disease Active Surveillance Network (FoodNet) and other CDC surveillance data, even if we are very uncertain about many of the model parameters. See Schroeder et al. *Emerging Infectious Diseases* **11:**113-115; 2005, for example.

Comment #12: *Correlation and dependence.* Some correlation matrices appear in the document and in the Inputs worksheet. The text seemed to suggest that the correlations among the variables involved in the growth submodel would be represented in the simulation. If this were so, the modelers should be commended for addressing the correlations among parameters where they could. Correlations and dependencies are often overlooked but can be very important in risk analyses. However, the only correlations considered are between quantities taken to be constants in the model, so, apparently, correlations are not used at all in this assessment, at least not in any way that I can understand. My initial enthusiasm was dashed.

Reply: The correlations are available for use in a second-order model. They were used in the sensitivity analysis to determine whether to move bounds together or separately.

Comment #13: Is it reasonable to assume that all the stochastic inputs are independent of one another? This seems unlikely to me. The entire process of modeling is, in a sense, a process of teasing out these functional and statistical dependencies among variables. The modelers might therefore argue that they *have* included the important dependencies that they could by explicitly modeling the functional relationships among the variables. But there is another question about residual statistical dependence in the variation represented by probability distributions in the model. Is it reasonable that all these distributions are stochastically independent? Would it not be expected, for instance, that the random fluctuations in temperature in the laying house might be correlated with the temperature "on farm"? The argument at the top of page 55 that eggs laid in the same house, packaged, transported and processed together should nevertheless be modeled as

independent seems completely specious, to put it politely. When I pool eggs from the same carton out of my refrigerator to make a cake or an omelet, the risks associated with those eggs are unlikely to be stochastically independent.

Reply: Our observations of layer houses lead us to conclude that the fluctuations of temperature within a layer house are likely mostly independent of "on-farm" temperatures. The way in which the temperature is maintained in the house will have little effect on whether the producer refrigerates eggs after collection.

Time and temperature, however, may not be as independent as we have modeled. Producers storing eggs for long times may store eggs at different temperatures than producers storing eggs for short times. However, we have no data or observations to inform us on this point.

The model does make the assumption that one or more individuals will be exposed to only one contaminated egg at a time. We did this because a flock shedding contaminated eggs at a prevalence of 1 per 1,000 would likely be considered a very high shedding flock.

Comment #14: *Shapes of probability distributions.* The justification offered on page 56 for the use of a lognormal distribution is not serious. It is incorrect that a lognormal is appropriate for any variable that can be decomposed as a product of factors. This is an absurd oversimplification of the Central Limit Theorem. The resulting argument on this page is specious and should be reconsidered. Lognormal distributions might nevertheless be entirely reasonable as models for these variables if the fit to empirical data is reasonably good, preferably both on arithmetic and probability scales that emphasis tail behavior. They might also be justified by well-reasoned mechanistic arguments about the origins of variability in the quantities. In other instances, it seemed that the distribution selections were generally more reasonable.

Reply: The reasoning on this point was taken from Vose (1997).

> The Lognormal distribution is useful for modeling naturally occurring variables that are the product of a number of other naturally occurring variables. The Central Limit Theorem (see Section 3.4.2) shows that the product of a large number of probability distributions is Lognormally distributed. For example, the volume of gas in a petroleum reserve is often Lognormally distributed because it is the product of the area of the formation, its thickness, formation pressure, porosity and the gas:liquid ratio.

Thus, it seemed reasonable to use the lognormal as the default distribution. Moreover, the lognormal appears to fit the data that are available.

Comment #15: I was interested and amused to find in this assessment what may be one of the few appropriate invocations of a uniform distribution anywhere in risk analysis: for the location of a random egg from within a rectangular volume created by the pallet or case. Finally someone using the uniform legitimately!

Reply: No response needed.

Comment #16: It is appropriate to employ finite upper bounds on the distributions that might mathematically extend to infinity. The developers are right to ignore the advice to the contrary by some prominent risk analysts.

Reply: No response needed.

Comment #17: It might, however, be advantageous to identify mechanistic or policy-driven upper bounds, rather than simply truncating the distributions at uniform but arbitrary percentiles. Are the observed truncation limits plausible? We saw 69 days as the largest period of preprocessing storage (of 50,000 replications). Could it really be so long? Maybe it could. The largest temperature was 45ºC. Maybe that's not hot enough to fry the egg, but it seems pretty dang hot, even for an unregulated chicken coup in the Deep South.

Reply: These truncation limits seem plausible. For instance, it is possible that the temperature in certain buildings or vehicles may reach 45°C (113°F). Also, as noted in the report, NAHMS reported some eggs held on farm for longer than 10 days, and RTI reported some eggs held in preprocessing for more than 20.

Comment #18: The number of bacteria per egg seems to be limited to 38.9 trillion. It's not clear whether that's a modeling decision or an artifact of Excel. I think this value should be justified.

Reply: The limit is 38.9 billion or 10.59 $\log_{10}$. This is a modeling decision reflecting a maximum population density in an egg.

Comment #19: Mightn't it be reasonable to use a *discrete* distribution for the number of days between egg pickups?

Reply: Yes, though the same could be said for other time variables within the model. This really depends on the smallest unit of interest. Does it make a difference in growth of SE if the truck picks up eggs in the morning or the afternoon? Collections at a farm might be separated by units approximating days, but collections of eggs at different farms probably would not.

Comment #20: I have grave reservations about the use of "Pert" distributions. They are certainly convenient to employ. But, as Bertrand Russell used to say, theft is more convenient than honest work too. I recognize that advisors will disagree on this issue, but it seems to me that there cannot be any serious justification for their use in an assessment such as this. They are only gross cartoons of reality. For this reason, it is all the more important to explore the second-order uncertainty in a comprehensive way. This has apparently not been attempted.

Reply: This comment refers to the expert opinion generated by Research Triangle Institute for the egg products model. These were presented as variability distributions but look more like uncertainty distributions. They are used as variability within the model. This is an issue we need to discuss more as we look for expert opinion in other assessments. Regardless, the uncertainty associated with these distributions was modeled by setting the midpoints of the pert distributions to the upper and lower bounds specified by the expert committee. The effect of these changes is shown in the risk characterization chapter.

Comment #21: The distribution shapes (uniform, lognormal, discrete) are specified on the Inputs worksheet as though they were subject to change by the user. However, the distribution choices are actually hardcoded in the macro. Consequently, there is currently no way to vary the distribution shapes as a part of a sensitivity analysis about model uncertainty. Perhaps these cells could be locked to indicate that they are not really changeable.

Reply: These were meant only to be informational. We have deemed it better to remove the descriptions from the revision rather than locking them as there are other distributions not described in the sheet.

Comment #22: *Inappropriate mixtures.* At the bottom of page 65, the construction of an average over treatments is discussed. The addends of this average should be weighted by the relative *number of eggs* experiencing these different treatments. Otherwise, the average is meaningless. I could have further subdivided the treatments into as many categories as I want: foam, wood, fiber, plastic, wire, screening, wicker, packed on Tuesday, etc. Averaging the *k* values for these treatments without weighting ignores the fact that very few or even zero eggs are stored on wicker. If I don't weight by the number of eggs per treatment, then I can get a nonsense result that reflects how I made up the categories rather than anything about egg storage.

Reply: We agree. The averaging over treatments has been removed.

Comment #23: *Population growth modeling.* I did not have a chance to study the mathematical model used to represent growth of the bacterial populations. However, I recommend a seminal paper (Ginzburg, L.R., Slobodkin, L.B., Johnson, K. and Bindman, A.G. 1982. Quasiextinction probabilities as a measure of impact on population growth. *Risk Analysis* 2:171-181) that gives analytical formulations for the first passage time—which I believe is what you'd be wanting—for stochastic population growth. They were thinking of population extinction, but the same formula applies to population explosions too. This work generalizes and applies mathematical methods due to Capocelli and Riccardi for computing first passage times. See also a later paper on the same subject (Lande, R. and H. Orzack. 1988. Extinction dynamics of age-structured populations in a fluctuating environment. *Proceedings of the National Academy of Sciences USA* 85:7418-7421). The equation you want is actually used as cover art on a new book *Quantitative Conservation Biology: Theory and Practice of Population Viability Analysis* by Morris and Doak (Sinauer, 2002). I'm not sure, but it may actually be possible to simply

compute the distributions you're currently simulating with expensive Monte Carlo methods.

Reply: We thank the reviewer for the references, and the background history of the development of first passage times for stochastic processes. The problem being discussed is the modeling of growth from a possibly small number of cells. We conceptualized that each cell would be initially in a lag phase, and that, after some period, which was assumed to be a random variable that is distributed exponentially, the cell would enter the exponential phase of growth. The number of off-spring cells, from a given cell once in the exponential phase, was assumed to be distributed as a geometric distribution, following the standard simplifying assumptions associated with the growth phenomena for bacteria. Thus the modeling problem needed to address two components: i) the times that cells change from a lag phase state to an exponential state of growth; and ii) the number of offspring from each of the cells once in a exponential phase.

We understand the reviewer's comment as addressing the first of these. If so, the comment seems to suggest that it would be sufficient to use the equation for first passage time developed from an assumed Weiner process for approximating the earliest time that one of the cells would enter the exponential phase and begin to grow. The equation that the reviewer referred to can be described as a weighted sum of cumulative normal distributions. Specifying this equation involves identifying values of three parameters. The estimated values would depend upon the known parameter value characterizing the assumed exponential distribution of the cell-specific lag phase duration. However, given the assumptions described above, a more direct equation for the first passage time is obtained, by considering the minimal value from independent realizations of n random variables, each distributed exponentially with parameter, λ, namely: $(1-\exp(-\lambda t))^n$, where n is the number of cells at time $t = 0$, and λ is a parameter. Once this minimal time (the first passage time from lag to an exponential phase among n cells) is obtained then we presume the reviewer would suggest that the model proceeds and generate a value from the appropriate geometric distribution.

However, we would be concerned that, typically, the approximation described above would be negatively biased, because the growth component of the model would then be based on the number of off-spring from one cell - the first one that changed from the lag phase to the exponential phase state of growth. This approach would be ignoring the number of off-spring cells that might have grown from the other cells, thus resulting in a biased estimate of the total number of cells in the population.

Our approach was based on calculating the conditional means and variances of growth given that some growth took place. The conditional means and variance are directly calculated given the values of certain parameters. The question facing us was what simple distribution to use to approximate the number of cells in the final population. Simulations were performed and by comparing the results of the simulations with various distributions: gamma, lognormal, and Weibull, the latter distribution was determined to provide the best fit.

A paper describing this approach was presented at the 2003 Fourth International Conference on Predictive Modeling in Foods, and published in the International Journal of Food Microbiology 100:275-287.

Comment #23: *Yolk membrane breakdown.* The simulation strategy described at the top of page 71 doesn't make any sense to me. The probability of YMB increases with time, so the occurrence of YMB depends on time. And, once it has occurred at some time t_{YMB}, it's broken for all subsequent times. That means that you need to know a time horizon to make the calculation. It doesn't make sense to make this calculation for each of many time steps unless you somehow account for the fact that past and future time steps are not independent of one another. I could not find the definition of $PYMB_t$ anywhere, but I presume it is a discrete analog of the instantaneous failure rate. This is an awfully cumbersome way to skin this cat. By sampling from a Bernoulli distribution (binomial with $n = 1$), you've simulated *whether* YMB occurs. I don't think you want to simulate whether YMB occurs, but rather *when* it does (if the time is infinite, it didn't occur). To do this, you can use elementary methods of reliability theory that depend on $P(M_t)$

Reply: YMB is dependent on the time and temperature as well as the number of bacteria. As temperature and bacteria increase the amount of time for YMB to occur decreases. The text stated:

> Given the probability of YMB calculated in this way, the occurrence of breakdown is estimated using a binomial distribution with $n = 1$ and $p = PYMBt$.

This was a misstatement. p is a random value from 0 to 1 drawn at the beginning of the iteration. As subsequent increments are modeled the value for *PYMBt* is updated and compared to p. When *PYMBt* exceeds p then YMB has occurred and *PYMBt* is no longer estimated. The text has been reworded to explain this.

Comment #24: *Other miscellaneous issues.* What does it mean when the results suggest that an egg has a "1000% probability" of causing illness? It's not entirely clear, but it seems as though the modelers are interpreting such a result as saying there would be 10 disease cases attributable to that egg. If this is so, there should be a full explanation and justification of the underlying idea, 'cause, right now, the result looks more like a bug. The text around page 60 seems to suggest that values below unity are to be understood as frequencies and values above unity are to be understood as counts. This would strike most readers as an odd idea, and I don't believe it is tenable. The algebras for frequencies and counts are quite different.

Reply: All of the values are meant to be interpreted as illnesses per egg. The text has been reworded. "Probability" has been changed to "frequency" in referring to illness per egg.

Comment #25: I had similar though somewhat lesser consternation about your use of the word "expected" to describe the point values used in the gray boxes on pages 40ff. S_1 would be the expected number of SE only if you used expected values for S_0, G and P

and they were all mutually independent. In the top box on page 41, the quotient S_2 will not be an expected value in any case (the expectation of a quotient is not the quotient of expectations). Why not just use the word "estimated" rather than "expected"?

Reply: "Expected" has been changed to "estimated" at these portions of the text.

Comment #26: The Excel interface is obviously a considered decision, but it may not be the best one. With its concurrent calculation, Excel is certainly an odd choice of a programming environment for conducting temporally explicit modeling. (This must lead to some ugly hacks here and there.) Running macros under Excel is surely vastly slower than can be achieved in a stand-alone program. References to the inputs worksheet are rather awkward and error prone (e.g., "worksheets(sInputs).Cells(28,4)"). It is not altogether clear that the Excel interface has advantages to programmers or users beyond those that would be enjoyed by a much simpler disk file interface. I presume that the programmers like this environment because it allows them to develop fairly complicated programs (macros) that can be modified by others without requiring a separate compiler a user would have to buy. I agree that this is a distinct and important advantage. Nevertheless, I would hope that the developers will consider opportunities to migrate the Basic code out of Excel and into a more flexible stand-alone program.

Reply: The use of Excel with VBA was meant to allow use and review of the model by anyone with Microsoft Excel. The Excel worksheets are not used to run the model but rather to hold inputs and to summarize outputs. Standalone programs require a way to summarize the output. This may be done by using third party graphical libraries. However, the use of third party libraries in addition to the compiler means additional resources are needed for anyone wishing to make modifications to the code.

Comment #27: *Matters of form, format and cross referencing.* These issues of form, format and cross referencing are chiefly related to the lack of transparency in the assessment. I appreciate the extensive effort that went into constructing the tables of variable names, descriptions and comments on their estimation that have been integrated into the text. These tables really must include the units for each variable. It is not merely a pedantic instinct to want these units. They are essential to prevent ambiguity, such as that which arises when both units of days and hours have been mentioned in the text for temporal variables. In cases such as that of the variable "internal egg temperature at time = t", units for both the temperature and the time should be given.

Reply: Cross referencing has been improved throughout the document.

Comment #28: A very simple check on the integrity of the equations used in the assessment is to confirm that the units balance. The omission of the units from the tables hampered and often prevented my conducting this check. I did notice a few units problems, although they may be merely typographical mistakes and not modeling errors. For instance, V in the upper gray box on page 41 needs units of "servings *per egg*". In the box on page 63, the denominator in the formula for T_{i3} should not have the unit "hours". The variable k already embodies that unit. I suspect that there are more unit

errors, and perhaps some that influence the calculations. Having units easily accessible in the tables would enable me to check this quite easily.

Reply: Please see above comment.

Comment #29: I could not locate the software code promised as an appendix to chapter 3. I feel this was a serious omission.

Reply: The software code is now available within the model.

Comment #30: I could, however, access the code by using the macro editor within Microsoft Excel. Although I love the Basic programming language, I haven't worked in it for over twenty years, and there are about 40 pages of Basic code in the ModelEgg macro alone. My superficial review of the code did not encounter the most scrupulous standard of programming practices with regard to structured style and commenting. The variable names were mnemonic with a fairly obvious (but unexplained) structure to designate doubles, arrays, constants, etc. It was certainly better than my own programming style, but really not adequate as a medium to express the model used by FSIS in conducting this assessment. That would have required a lot more modularity and other structuring. In the absence of self-documenting software, the documentation becomes the only viable means to express the model, so it must be complete and sufficiently detailed to allow someone to reproduce the model. I did not find the document to meet this high goal.

Reply: We converted procedures that had been developed in Excel spreadsheets into Visual Basic to ensure equivalent answers were obtained.

Comment #31: The continual and pervasive cross referencing is brutal on a reader. Sometimes the references were not all that useful. For instance, Table 3-15 on page 71 refers to Annex E for the values of the constants *B*, *E*, *FY*, and *W*, but I could not easily locate these in Annex E, which is itself a 54-page document. Such references should include a page, table, or at least a section reference.

Reply: Cross references have been updated.

Comment #32: It would be desirable to include the numerical values of the constants in the original calling table in the first place. We should have to go to an annex for the justification of the number, but not for the number itself. Table 3-14 on the previous page merely characterizes the values of *D, G, G* (sic), *K* as constants, without bothering to say either what the values are or where we can find the regressions from which they were derived. This amounts to a Potemkin village description of the model and is not acceptable.

Reply: The revised document includes improved cross-referencing to deal with these and similar issues.

Comment #33: I did find mention of variables *e, f, b* and *w*, and mention of *d, f, g, k* in Table E-1 of Annex E. Presumably these are what are used in the model. (If we can ignore italics, I guess we can ignore capitalization too. Perhaps the Y and the second G are typos.)

Reply: The information in the table has been corrected.

Comment #34: The column labeled "Values" in Table E-1 refers to what appears to be the data on which the regression was based, but I'm not sure of this at all. The values of the constants, together with their standard errors and correlation matrix, are apparently first given in tables mentioned in the column labeled "Uncertainty". But we still don't know even the units of the constants or much about the regression from which they came. What was the sample size? What does a plot of the residuals look like? Critically, what is σ for the regression? To estimate four or six independent parameters from any regression one would need pretty large sample sizes. I find it hard to believe that such abundant data are available. Are these regression constants reliable at all? It seems dubious.

Reply: Table E-1 refers to the values which are used in the growth model. It is an index to other tables in the annex. The data on which the model is based is referenced in other parts of the annex. The uncertainty associated with the parameters is referred to in table E-1 and further explained throughout the annex. We recognize the growth model may be overly complex and there may be simpler approaches that would also work. Nevertheless, after careful consideration we felt this was the most biologically realistic growth model and it does fit the present data.

Comment #35: In the case of the first batch of constants, there are six different papers cited from the literature. Are we supposed to read them to find these critical details? I was hoping to get a quick idea of these essential issues by scanning the tables and graphs. This is not possible. Perhaps they can be gleaned by careful reading of the text, but I'm not sure. I guess what I'm saying is that it seems that too little effort has been invested in making the synopses easy to follow and the arguments about modeling choices compelling. Therefore, I worry that the documentation does meet the high goal mentioned above.

Reply: Efforts have been made throughout the document to present a clear and concise summary of research drawn upon to create the risk assessments. As with any report, there are some instance in which references are given in support of statements, without a detailed description of the work referenced.

Comment #36: I very much appreciated the gray boxes that occasionally appeared in the text to explain important issues. Sometimes, however, they seemed to increase my confusion rather than reduce it. On page 11, the discussion of SE-infected shell eggs confused me. Is it one egg per 10,000 (as the quantity 0.01% suggests), or is it one in 20,000 (as the quotient 2.3e6/47e7 suggests)?

Reply: The 1 per 10,000 reference is more of an historical perspective and in hindsight was not required. It has since been removed.

Comment #37: In the box on page 13, the sentence beginning "Lethality performance standards…" is a doozy.

Reply: The entire paragraph has been replaced:

> Lethality performance standards are expressed as the number of decimal reductions (x $\log_{10}$) of the target pathogen(s). This can also be expressed probabilistically. A performance standard for a 3 $\log_{10}$ reduction, for example, means that 99.9% of bacteria would be killed. If there was one bacterium, the probability of it being killed would be 99.9%. Egg-handling performance standards establish the maximum relative growth of *Salmonella* allowable in eggs during handling and storage.

Comment #38: Most of the main chapters of the document include a final summary section. I found these summaries rather helpful to orient one's reading. The cruel exception is the 100-page chapter 3. Where's its summary?

Reply: Chapter 3 now has its own summary.

Comment #39: You need to specify, perhaps in a central table somewhere, the various parameterizations for probability distributions you're using. For instance, there are different ways to parameterize the Weibull distribution mentioned in Table 3-1 on page 45. The book by Evans et al. (which should probably be considered the standard here) suggests the first parameter is the scale or characteristic life, and the second is the shape parameter, such that the median of the Weibull(b, c) is $b\ln(2)^{1/c}$. This does not seem to be the parameterization you used since the values you mention would imply an immense median. But how can we tell what you intend?

Reply: Table 3.1 has been revised to describe the Weibull distributions used in equations 3.22 and 3.23.

Comment #40: Likewise, you need to say how you simulated Poisson deviates without zeros. What it $P + 1$, or perhaps max(P, 1)? It's not obvious and it can make a difference.

Reply: Simulating Poisson deviates without zeros: A random draw, $p1$ is generated from a Uniform(0, 1, rand()); the Poisson probability of zero is calculated ($P(0)$); the $p2$ for generating the deviate is taken from a new Uniform(P(0), 1, $p1$) distribution; this is used to generate the deviate from a Poisson(mean, p). This is now explained in a textbox.

Comment #41: It took me a surprisingly long time to be sure that the 50,000 iterations in a simulation represented 50,000 eggs, rather than, say egg meals or some other unit. Shouldn't this be clear in the documentation, the Excel interface, and the macros, and shouldn't it be obvious from the very beginning?

Reply: Yes. A brief summary that states the intent of the model has been added to the report.

Comment #42: *Typographical, grammatical and style issues:* The text should be copyedited before release. There are several mistakes and odd turns of phrase that, in aggregate, could be embarrassing.

Reply: The revised text has been copyedited.

Comment #43: I presume from your parallel usage of "*Salmonella* Enteritidis" and "*Salmonella* spp." that *enteritidis* is the name of a species from the genus *Salmonella*. But, if that is the case, shouldn't the word be italicized and the E be lower case? The Centers for Disease Control (http://www.cdc.gov/ncidod/dbmd/diseaseinfo/salment_g.htm) seems to treat the word like a species name. I would think you guys should know how the name should be written, but if this is an oversight, it appears throughout the document and in the title.

Reply: *Salmonella enterica* serovar Enteritidis. Here, "*Salmonella*" refers to the genus, "*enterica*" refers to the species, and "Enteritidis" refers to the serovar (a subtype of the microbial species). The generally followed convention for abbreviation of the organism is "*S.* Enteritidis."

Comment #44: In the middle of page 66, you assert that 50% of eggs are within 4 inches of the perimeter (I assume you mean the *faces*, that is, the four sides and top and bottom) of the egg storage unit with dimensions 3ft × 4 ft × 6ft. This looks like a computational error. It's closer to one-third, isn't it? The calculations should be checked!

Reply: The volume of a pallet with dimensions 36in x 48in x 72in is 124,416 cubic inches. The volume of a unit with dimensions 28in x 40in x 64in is 71,680 cubic inches or 57.6% of the original. Thus 42.4% of the volume of the pallet is within 4 inches of a face. Part of some eggs, however, will be within 4 inches of a face, even though the entire egg is not within 4 inches. Eggs that are touching the 4 inch face may have centers 5 inches from the face, so we get close to 50% of the original. Nonetheless, for the sake of clarity, the revised text simply states "approximately 40%."

Comment #45: This is perhaps a wee bit picky, but it would be nice if you italicized mathematical symbols. This is conventional in mathematics, and the text looks unprofessional otherwise. Actually, you sometimes use italics and sometimes not (on page 63, the symbol *k* appears twenty times, about ten of which are in italics), which is even worse than not using italics at all because it can be confusing.

Reply: Mathematical symbols are italicized in the revised text.

Comment #46: You sometimes use the same symbols to refer to different quantities. For instance, the symbol *e* is used as a regression parameter and the also the base of the natural logarithms. The symbol *k* is likewise overloaded for *three* quantities: a regression

parameter, the cooling exponents, and the fraction of infected hens in an infected flock. Actually, you variously use *k*, k, *K*, and K, but not in a way that the quantities are distinguished by the use of different symbol forms. This seems to happen with *f*, *g* and some other symbols too. This just seems to be sloppiness or lack of coordination. It'd help to have a grand table of all variables.

Reply: Throughout the revised report we have stated clearly what each symbol denotes and where appropriate, discuss the derivation of the symbol's value.

Comment #47: You seem to use both "log" and "ln" to refer to the same operation (e.g., in the figure on page B-13). It is not clear, however, whether this is the natural or common logarithm. My worst fear is that you are using both and not identifying which by any convention. This fear may be a reality: the example in the middle of page 58 is clearly a natural log, but elsewhere you talk about log reductions in population from cooking that seem to be in terms of common (base 10) logs. You have to tell the reader which you're using in each instance. It would be conventional to use "ln" to denote natural and "$\log_{10}$" to denote common logs.

Reply: This has been corrected.

Comment #48: Some text from figure captions seems to have invaded the table of contents (page iii) before the summary and conclusions section of chapter 2.

Reply: This has been corrected.

Comment #49: The authors of the assessment refer to themselves in the third person as "the authors" in several spots where the surrounding literature review of the work and opinions of other authors makes the text ambiguous. It would be appropriate and far more natural to use the first person ("we"), or at least a specific collective noun ("USDA" or "FSIS"). Even the archaic (and stupid) phrase "the present authors" would be preferable to the ambiguity.

Reply: Mentions of "the authors" have been rephrased.

Comment #50: The phrasing "eggs are provided reasonably unfettered access to the ambient air" is almost bizarre if it merely means that eggs are generally or often *exposed* to ambient air. Access? Provided? Reasonably? Unfettered?! The phrasing appears more than once.

Reply: The phrasing has been simplified.

Comment #51: The word lognormal should be a single word (not "log normal", not "log-normal").

Reply: This has been fixed throughout the document.

Comment #52: The use of the word "consistent" in the middle of page 62 may be misleading. The conformance in question is not consistent in any evidentiary sense, but rather only in the sense of mere redundancy in the absence of real evidence.

Reply: The wording of the text has been changed accordingly.

Comment #53: The even page numbers of both Annex E and Annex H are improperly formatted.

Reply: The formatting has been corrected.

Comment #54: There is no Section E1 preceding Section E2 in Annex E. Section E1 should not be confused with Attachments E1 and E2, which are also present in Annex E. Come on, guys!

Reply: This has been corrected.

Comment #55: Perhaps I'm confused, but it seems that the formula for $M(t)$ in Table 3-1 should refer to t instead of W. The words after the formula should include "and t is in units of weeks". And the formula for e_m should be $M(W) \times e_{nm}$.

Reply: $M(t)$ in Table 3-1 has been changed to $M(w)$. The formula for e_m has been changed to $M(W) \times e_{nm}$.

Comment #56: There's an extraneous parenthesis in the legend of Table E-2 on page 6 of Annex E.

Reply: The parenthesis has been removed.

Comment #57: There are missing symbols (at least in my printout) in the caption for Table B-4 after the first comma and in the definition of c.

Reply: The symbols (μ) have been added to the revised version of the report.

Comment #58: At the bottom of page 15, the phrase "the number of *Salmonella* spp." is confusing. You intend to refer to the number of *cells*, or perhaps colony forming units, but not the number of *species*. It would be grammatical if you omitted "spp.".

Reply: We agree this wording is ambiguous. It has been revised throughout the text.

Comment #59: There seem to be five missing equal signs in the gray boxes on pages 40 and 41.

Reply: The symbols were lost when converting the document to a PDF**.** The equal signs in question are included in the revised text.

Comment #60: The equation reference in the section "Calculating Probability…" on page 194 is garbled.

Reply: The equation has been ungarbled.

Comment #61: Is it really called a "layer house". I would have expected "laying house". The hens are the layers.

Reply: Yes, like "hen house." Hens are also referred to as "layers."

Comment #62: I think the phrases "close to" on list items 4 and 5 on page 53 should be "far from".

Reply: We agree. The suggested change has been made.

Comment #63: The first sentence of the new section on page 65 mentions six values, but it looks like seven to me. Am I confused?

Reply: No. The *k* value of 0.0524 for "pallet of plastic basket cases" was inadvertently not included in the text. The text has been revised to include this value.

Comment #64: Does "palletized" mean "stored on a pallet" Do people actually use this word? Even if they do, the term "palleted" would be preferable grammatically.

Reply: The unabridged Merriam-Webster's dictionary recognizes pallet and palleting as nouns only. Palleted is not recognized. Palletize is a verb defined as "to place on a pallet: transport or store by means of a pallet." As for vernacular, a Google search resulted in 1,980 hits for palleted – many of which refer to items that have been placed on a pallet. A search for palletized, however, resulted in 47,900 hits.

Comment #65: On page 71, the "Dummy variable" should be "Seropositivity indicator". There's nothing dummy about it.

Reply: The suggested change has been made.

REVIEWER #5

Comment #1: *Is the report clearly written?* In general the report is well written, although there are a number of grammatical and typographical errors, which will presumably be dealt with when the final version is proofread.

Reply: The revised version of the report has been copyedited in an effort to reduce the number of grammatical and typographical errors.

Comment #2: There is a relative lack of reference to data from outside the US, but this is addressed to a large extent by the annexes. There is also some repetition of introductory data.

Reply: Because the risk assessment was designed to evaluate risk scenarios for egg production in the U.S., most of the focus is placed on data from the U.S.

Comment #3: *Does it follow a logical structure and layout?* The structure is logical and the layout is generally good. There is a surfeit of graphs, which often look exactly the same, but presumably this is because the target audience for this report are people engaged in risk assessment. It might be a good idea if the review parts of the report and the annexes were put together as a separate publication as they provide a wealth of very valuable information. The authors are to be congratulated and hopefully they will be identified in due course.

Reply: We thank the reviewer for encouragement and kind words.

Comment #4: *Does the background information sufficiently and accurately capture the current state of knowledge regarding Salmonella and egg safety?* I recognise that the task given to the authors of the risk assessment was made more complicated by the relative lack of authoritative data on SE in naturally contaminated eggs. It is of some astonishment to this reviewer that so little work has been done on the numbers of SE in naturally contaminated eggs in the US, particularly given that the pandemic has been in progress for over 15 years. There has also been relatively little interaction between scientists in the US and Europe over SE in eggs, which has, in part, led to some rather entrenched positions being adopted. When all this settles down I think that we will find that the various phage types and sub-types of SE behave differently in the reproductive tract and egg contents. Principal amongst this may be the behaviour of SE in egg albumen and some data are included at the end of this letter to illustrate what I mean by this. PT 4 is adapted to survive well in the harsh conditions of the albumen and does so better than some other PTs. This could begin to explain why in the US egg yolk contents and membranes are more likely to be salmonella-positive compared to in Europe, where albumen is more frequently contaminated. Detailed comments on this and other matters follow, these general points.

Reply: No response needed.

Comment #5: *Have all of the assumptions used in developing the assessments been clearly stated?* The simple answer to this question is yes.

Reply: No response needed.

Comment #6: *If so, is the rationale for these assumptions valid?* In general, the assumptions made are valid and where I have concerns they are addressed in detail below.

Reply: See replies below.

Comment: Statement on conflicts of interest. In the discussion of the report and the annexes it is necessary for me to make reference to the work of my research group, both past and present. This is done in a spirit of openness and with a desire to be helpful. In one case it is my opinion that a misinterpretation has been made and a detailed discussion on this is included in my report.

Reply: See specific reply to the reviewer's comment about potential misinterpretation below.

Detailed comments on the report

Comment #7: *Text box on page 11:* Some rather bold statements are made in this text box, which may not be supported by the data available. For example, it is stated that only a small number of hens in an infected flock shed SE at any one time and that only 0.01% of eggs are contaminated. More data should be presented to support these contentions.

Reply: The text box has been revised.

Comment #8: As stated in one of the annexes there can be 'clustering' of positive eggs, which means that many more than 0.01% of the flock output will be contaminated. This is more likely to happen at times of physiological stress on the birds such as entering lay, after moulting and when birds reach the end of economic production. Recent examination of eggs imported into the UK, largely from Spain found contamination rates of around 5%, compared to 0.3% from UK-produced eggs from non-vaccinated flocks. When egg pools were examined as part of an investigation of outbreaks in south London, c50% was found to be SE-positive. These reports are available on the Health Protection Agency Website (Anon 2002 and 2004).

Reply: We thank the reviewer for bringing these reports to our attention. Because the effect of vaccination is not modeled in the assessment (due to a paucity of data) we deemed it appropriate not to try to incorporate data from the abovementioned reports into the revised risk assessment model.

Comment #9: My work on naturally infected birds, which were caged individually, showed that 1% of eggs laid by these birds had SE in their contents. As part of an

outbreak investigation in 1988, Paul and Batchelor found that 5 eggs out of the 10 examined had SE in their contents. It may be that the statement in the textbox refers to the national situation, which will include eggs from both infected and non-infected flocks. The survey in the UK in the mid 1990s, which is mentioned below found that 1:600 eggs at retail sale had contaminated shells and 1:6000 had salmonella-positive contents.

Reply: No response needed.

Comment #10: *Hazard identification, p29:* At the beginning of this section a statement is made that 'SE has the unique to colonize the ovaries of hens and contaminate the internal contents of eggs'. This is factually incorrect on two counts. Firstly, other serovars, which share LPS structures with SE such as *S.* Typhimurium and *S.* Infantis, have also been recovered from the contents of eggs. A US study by Keller *et al.* 1995 examined the behaviour of *S.* Typhimurium in chicken reproductive tracts and showed persistence, as did Williams *et al* (1998), Leach *et al* (1999) and Jorgensen *et al*. (2000) in the UK. A paper from Trinidad reported the isolation of Typhimurium from naturally contaminated eggs and it has been reported that Infantis has been found in eggs in Japan. *Salmonella* Heidelberg may also be able to contaminate egg contents. A better statement to make would be that SE has an enhanced ability. The error is repeated on page 31. In contrast, on page 37 it is stated that 'SE is one of the few Salmonella serotypes to colonize the reproductive tissues of hens'.

Reply: The statement that SE has the "unique" ability to colonize ovaries of hens and contaminate internal contents of eggs reflects laziness in choosing words on our part. This has been corrected.

Comment #11: *P27, data on the site of contamination sites in eggs:* The relative importance of the sites of contamination within an egg has been mentioned above and will be discussed in more detail later. I would exercise caution on the use of data from hens infected artificially, which are often given inappropriately high doses of salmonella, sometimes by unnatural routes such as intravenous injection. These might overwhelm the hen's systems of protection. Please also bear in mind possible differences between US and European strains of SE.

Reply: These are both good points. As with all experimental data from artificially inoculated animals, the results may not reflect precisely that which occurs naturally. However, in this particular instance, we were unable to identify data from studies using anything but artificial inoculation. As for the difference between US and European strains, because this risk assessment is concerned with risk of illness in the US, attention was focused on US strains.

Comment #12: *Bottom of p46, growth in egg albumen:* A statement is made that 'growth in albumen in some of these eggs will never occur regardless of how the eggs are stored'. This seems unnecessarily strong and should be modified to introduce and element of caution, even though it may well be true.

Reply: The statement has been revised accordingly.

Comment #13: *P47:* Evidence should be provided to support the statement in the first sentence, which says 'the most common form of contamination is the albumen-contaminated egg in which contamination is far from the yolk and no growth occurs in the albumen'.

Reply: This was a case of awkward wording on our part. We meant to express the sentiment that we *believe* the most common form of contamination is likely to be in the albumen. It was not intended as a statement of fact. The text has been revised accordingly.

Comment #14: *P142:* Some very good points are made about human volunteer studies. It is also possible that continued bacterial evolution since the volunteer studies and the more recent data from outbreaks would have had an effect.

Reply: This is an excellent point, mention of which has been added to the text.

Comment #15: *P143 and chapter 4:* This page, and the section as a whole, contains a very good review of data on the infectious dose of salmonella. It would be sensible of the authors to add a rider to the effect the Japanese protocol of storing retained foods at –20C would have reduced the numbers of salmonella cells present and made survivours more difficult to culture. Thus it is probable that the numbers found in the food samples may have been an underestimate.

Reply: The suggested rider has been added to the text.

Comment #16: This chapter, and others, makes frequent mention of the paper by Mead *et al.* (1999). Some of the calculations in this paper are disputed by HPA in England and Wales (Adak *et al*. 2000) and the authors of this risk assessment may also receive some criticism for their extensive use of the Mead assumptions. However, estimates of deaths and hospitalisations due to eggs outlined in this chapter seem reasonable. The tables at the end of Chapter 4 are particularly useful.

Reply: Many of the multipliers used by Mead et al. are controversial. Some of those used for *Salmonella* were proxies taken from studies with *Shigella*, for example. Also, the estimates of Mead and colleagues are surrounded by uncertainty. In an effort to offer an objective evaluation of the multipliers used by Mead et al, we have appended a summary of the multipliers to the hazard characterization section of the report.

Comment #17: *Page 167 and estimated illnesses per serving of non-pasteurized shell eggs:* It is my view that the estimates in this section may be flawed, as they do not seem to take account of the fact that the contamination rates of egg contents can vary. There is extensive discussion above on the prevalence of contaminated eggs and the statement that the figure of 0.01% was too low, if all available data are taken into account. The authors

have chosen to use an estimated prevalence 0.03% in this section. Presumably this is referring to the contamination of contents only? If this is the case this is closer to the figure from the retail survey in the UK, which was 0.016%. It might help readers of this report if they were reminded that estimates are for eggs with salmonella-positive contents.

Reply: Although a prevalence of 0.03% is used, this value has uncertainty associated with it. Prevalences of 0.005% to 0.1% are possible.

Comment #18: *Page 196 and Table 5.18:* The value of the analyses, which can be applied to data in the table, is limited because of the marked variation in the number of samples tested in the range of products.

Reply: There is variation in the number of samples tested. However, we are unaware of additional data for *Salmonella* in pasteurized egg products. A sentence has been added to state that because the sample sizes vary the data representativeness may be affected. Notwithstanding, the data were useful for comparative purposes between model predictions and post-pasteurization *Salmonella*-positive egg sample incidence.

Comment #19: *Post-pasteurisation growth of injured bacteria:* This is an interesting concept. It would have been valuable to have seen calculations, which took into account the possible lower infectivity of damaged salmonella, which are likely to be more acid-sensitive, for example.

Reply: We have reason to believe post-pasteurization growth of injured bacteria contributes minimally, if at all, to illnesses from *Salmonella*. Due to modeling limitations, we necessarily considered the growth rate of *all* sublethally injured *Salmonella* to be one-half that of wild-type for *all* subsequent generations. This was an arbitrary assumption necessitated from lack of data on this issue. However, it is reasonable to believe that slowing of growth due to sublethal injury is limited to *Salmonella* cells that underwent the heat treatment and not their progeny. In other words, slowed growth from injury is not a heritable trait. Thus, once the heat-injured cells divide, progeny cells would be expected to grow similar to that of wild-type cells. Given the time between pasteurization and consumption, the effect of sublethal injury becomes negligible.

Comment #20: *Chapter 6: Research needs.* In general, the suggested research areas are sensible and would provide much needed data. This chapter is indicative of the whole report, however, in that it largely ignores data from outside the US.

- Will the study to be undertaken by Dr Richard Gast use naturally infected hens? If not, how will the birds be infected and at what dose?
- I would strongly support the call for a study to determine the site of contamination in naturally contaminated eggs. It might be difficult, however, to separate inner and outer albumen. This study could also include numbers, if samples of homogenised egg were held at low temperature while the bulk of the sample was tested for salmonella.

- I would also support the call for a study on washed eggs. An additional risk is that washing may allow the ingress of organic matter, which might negate the inhibitory nature of egg albumen. Studies by Board and Lock in the 1980s showed that very small amounts of faecal matter allowed the growth of SE in egg albumen.
- I would also support the flock survey. The high percentage of eggs going for breaking in the US would make this study feasible compared to the UK.
- Growth in egg albumen has been studied in the UK and some data are supplied at the end of this report.
- In my view, what is also needed are studies on how SE differs from other salmonella and why it appears to be able to persist in chicken reproductive tissues and egg contents, better/longer. It is believed that there about five salmonella, which have the capacity to infect eggs, *in vivo*.

Reply: The most important needs, those that would potentially change risk manager decisions, were identified using sensitivity analyses. Because the risk assessment was conducted using data from the U.S., and because it was designed to predict scenarios for U.S. egg production, the research needs identified as part of the report necessarily focus on egg production in the U.S. That said, some of the research needs, such as studies of growth of SE in eggs, could be accomplished by laboratory experiments, not fieldwork. As such, these experiments trancend geographical boundaries. Though the research needs identified here were based solely on analysis of the risk assessment model inputs, we agree with the reviewer that the abovementioned suggested research areas are important.

Comment #21: *Annex A:* This is a well written and a potentially very valuable document, which makes many important points. A particularly valuable point is made about the lack of authoritative data on the numbers/growth of SE in naturally contaminated eggs. Two studies in the UK, on eggs from flocks associated with outbreaks, found that naturally contaminated eggs examined within two weeks of lay had low numbers present (Humphrey *et al*. 1989; Mawer *et al* 1989). A further study in the UK, also on eggs from known infected flocks, examined the contents of over 5700 eggs. Thirty-two (0.6%) were positive. Most eggs had low numbers (<20 cells per egg) but three, which had been stored for more than three weeks at 20C, contained many thousands of cells. Another study in France found that one egg, purported to be seven days old, had very high numbers. There is no doubt that SE can grow in eggs. A study in the UK on eggs purchased from retail outlets and stored at 21C for five weeks before testing found that 50% of those that had SE in their contents had levels $>10^4$ per gram of egg contents.

Reply: We thank the reviewer for pointing out these important studies.

Comment #22: *Sites of infection of the reproductive tract:* The authors use data from infection of the intestine to theorise that cells of salmonella are on the surface of reproductive tissue cells. I am not sure how valid it is to extrapolate from intestinal colonisation to infection of the reproductive tract. Although it is possible that SE can track up from the cloaca, the blood borne route of infection may be more likely. If the authors have good data on this then it should be included in the report.

Reply: The reviewer is correct. Our argument was speculative on this point. We are unaware of data to add to the report.

Comment #23: *Effects of site of contamination in egg contents:* The position of the SE cells relative to the yolk will clearly have an impact on the growth of the bacteria. The effect will be greater when some yolk membrane breakdown has occurred such as when eggs have been stored at ambient temperature as in the Humphrey and Whitehead study.

Reply: We agree.

Comment #24: How were the studies by Duboccage *et al*. performed?

Reply: Growth was investigated for 6 non-*S.* Enteritidis strains and eleven *S.* Enteritidis strains in fresh eggs and in eggs of 2 and 3 weeks old at 20° C. Growth was measured 6, 13 and 23 days post-inoculation. Experiments were also done to investigate salmonellae growth in minimal medium with an iron source together with the iron chelator conalbumin, and in a medium without iron and conalbumin, for 12 hours at 37° C. Growth was measured in minimal media (with iron) at pH 8, 8.5, 9 and 9.5.

Comment #25: SE is able to grow in the forming yolk, *in vivo*. There are many reports of diseased and retained ovules in infected hens. To my knowledge, there is no correlation between yolk appearance and the presence of SE in eggs contents, suggesting that internal contamination of the yolk is a rare event in natural contamination. My group has examined many hundreds of contaminated eggs and none of those examined when fresh, had obviously diseased yolks. It may well be that the overtly diseased ovum is not recognised by oviduct tissues.

Reply: This is indeed an interesting hypothesis. We agree that there is not necessarily a correlation between yolk appearance and presence of SE and that internal contamination of the yolk is a relatively rare event in the natural setting.

Comment #26: *Physiological state of salmonella cells when they enter egg contents:* This annex makes a good point about the physiological state of the bacterial cells as they enter egg contents and contrasts natural and artificial infection scenarios. If the authors of this annex have any reliable information on the growth patterns of salmonella cells in infected tissues they should include reference to them.

Reply: Similar to above (see comment #22 and reply thereto), our argument was speculative on this point.

Comment #27: It would be of interest to compare the behaviours of cells in log and stationary phase in egg contents. Stationary phase cells have been used in many studies because there is a belief that salmonella in tissues may be iron-starved. I feel that the authors should temper the statement that growth in eggs might be rapid and immediate.

There is always a growth check when bacterial cells are moved from one environment to another.

Reply: Mention of rapid and immediate growth of SE in eggs in mentioned only inasmuch as it is one of the possibilities resulting from egg contamination. In most instances rapid growth would not be expected to occur.

Comment #28: *Annex B.* This is another excellent document, which will be valued by researchers worldwide. I would support the view that past surveys have underestimated the prevalence of SE-positive laying flocks. In general, serological surveys would find more positive birds than culture, particularly where faeces are examined because SE can be extra-intestinal, and this is a point made in this annex. A good point is also made about the influence of culture techniques applied.

Reply: We thank the reviewer for kind words and encouragement.

Comment #29: In discussion of infection of reproductive tissue, on page 20 a statement is made, 'data suggest that both the ovary and oviduct can be heavily contaminated with SE'. Does this refer to frequency of isolation or numbers of bacterial cells present? The authors should quote data, which supports this statement.

Reply: The statement was meant to refer to the number of bacterial cells present. References in support of the statement are included in the revised text.

Comment #30: The section on the frequency of SE-positive eggs is also very interesting. Care should be used, however, in interpreting data from studies from hens infected artificially. The intravenous route may give unrealistically high doses to the reproductive tissues, as might the very high inoculation levels (10^{10}) used by Bichler *et al*. (1996).

Reply: The revised text includes mention of the fact that such care should indeed be exercised. That said, we are unsure of what if any mathematical adjustments can be made to the model to address this concern.

Comment #31: Without wishing to sound patronising could I congratulate those who wrote the annex? The piece on the effects of moulting is fascinating and I look forward to quoting these data when the report is published.

Reply: We again thank the reviewer.

Comment #32: The assessors might also wish to consider another scenario. It is likely that moulted hens might also be producing more catecholamines than normal. These will have a growth-stimulatory effect on salmonella cells as they assist bacteria in the uptake of iron.

Reply: This is an excellent point. Regardless of the mechanism however, the data inputted into the risk assessment indicate that molted birds shed increased numbers of *S.*

Enteritidis compared to their non-molted counterparts. We are not aware of data to the contrary.

Comment #33: On page 39 the authors discuss shell contamination and rightly point out that a delay between lay and examination is likely to reduce the number of salmonella on the shell. There are few authoritative data on the survival of salmonella on eggshells but these bacteria can show a high tolerance to desiccation. A study in the UK in 1991 found that 1 in 600 eggs sampled from shops was salmonella at any site while 1 in 6000 was contents positive.

Reply: We were unaware of this study and thank the reviewer for drawing it to our attention.

Comment #34: The authors have quoted only one study on shell penetration, namely Schoeni *et al.* (1995). There are many others.

Reply: There are other studies on shell penetration of SE, however, the risk assessment models shell penetration as one of the possible sources of SE in a contaminated egg. On the other hand, growth of SE from this type of contamination is not modeled differently than the growth from eggs contaminated in the albumen *in utero*. Thus, we decided to not incorporate other data into this input.

Comment #35: *Biological reasons for differences in infection rates:* As with other sections in this appendix, this section is well written and generally informative. There is no doubt that cell surface structures play a major role in the processes of infection of the reproductive tract. One area not discussed in any detail is the effect of mutations in key genes, although this is alluded to in one sentence. Salmonella serovars like SE can show high rates of mutation (LeClerc *et al*. 1996). In UK studies mutations in the gene *rpoS* was found to reduce persistence in reproductive tissues of commercial hens, infected artificially (Humphrey *et al*. 1996; Jorgensen *et al*. 2000). This may be because *rpoS* regulates the expression of SEF17. *rpoS* mutants also survive less well in egg albumen (Cogan personal communication). The work of Dr Jean Petter in Athens, Georgia has shown that LPS structure is also important in reproductive tissue persistence.

Reply: We thank the reviewer for summarizing the work performed in these studies. This portion of the report was written in a good-faith effort to offer possible explanations to biological phenomena observed previously. It does not, however, directly affect the risk assessment model.

Comment #36: *Annex C.* This appendix is a reasonable attempt to summarise what is known to date about contamination levels of SE in egg contents. The lack of US data on contamination levels in naturally infected eggs is presumably what caused the authors to concentrate on the one study on eggs from artificially infected hens. If my understanding of these data is correct even with the high infection doses used only a few eggs were definitely salmonella-positive in yolk contents. It would reasonable to assume that the

studies of Gast and Holt are broadly representative of the natural situation as almost all available data on numbers of salmonella naturally eggs indicate that levels are low.

Reply: No response needed.

Comment #37: *Annex D.* The content of this annex is really outside my area of expertise. It is vital, however, that good data are obtained for egg cooling rates on-farm as that could have a profound influence elsewhere in the production chain. Eggs immediately after lay may be vulnerable to rapid growth of salmonella in some instances because the pH of the albumen will be around neutral and there will also be unbound glucose available.

Reply: No response needed.

Comment #38: *Annex E.* This is a very important annex and one, which challenges previously held views. Doubts are cast on some of the data produced by my research group in the past and this is to be expected in science and it is welcomed. I reserve the right, however, to counter challenge some of the assumptions in this annex. It may well be that some strains of SE are capable of growth in the albumen of some fresh eggs. Whether this is due to the site of contamination, faults in an egg or the SE strain present is not known. The authors of this annex quite correctly point out that ICMSF stated in 1995 that salmonella could grow at pH 9.5. It may be misleading to equate this to conditions in the egg. It is likely that growth identified in the ICMSF document was not assessed under iron-limiting conditions or in the presence of lysozyme. That having been said, it is wise of the authors to err on the side of caution. However, if growth in the albumen were a common feature it would be expected to see many more fresh eggs with high levels of contamination. One of the problems that the scientific community, and the authors of this report face is that many different SE strains and contamination models have been used.

Reply: No response needed.

Comment #39: *Growth in albumen before yolk membrane breakdown:* The authors of this annex present a very detailed and valuable critique of the work done by my group in the recent and more distant past on the growth of SE in eggs. Some explanation is supplied below. It is my hope that the comments are not seen as being unduly defensive. As stated above, I welcome the scrutiny of the data. Early work on the effects of storage on the quality of egg contents identified that when eggs are held at ambient temperature the albumen becomes progressively thinner and the yolk membrane becomes progressively more fragile. It was recognised in the UK that these changes would also have a potential impact on the growth of SE in egg contents. Thus the earlier studies were largely done to examine the effect of shelf life on the ability of eggs to support the growth of SE and thus were an indirect measure of egg yolk membrane integrity, although, later knowledge suggests that other factors can also play a part. At the time of these studies there was quite intense debate in the UK over shelf life in retail outlets and whether eggs should be refrigerated. The study was not designed to study growth

parameters. Its sole purpose was to use SE to monitor changes in egg contents. An inoculum of 500 cells was chosen in the hope that it would increase the sensitivity of the assay and reduce egg-to-egg variation in levels of salmonella placed in the eggs. The data from these studies indicate that a major change in the ability of eggs to support the rapid growth of SE occurs between 3-4 weeks, which is consistent with other work on egg quality. With the benefit of hindsight it could be argued that the inoculum used was too high and because of this the time to significant levels of yolk membrane breakdown was underestimated. Please allow me to repeat my claim that this work was [not] done to model growth rates in egg contents.

Reply: We appreciate the reviewer's claim that this work was not done to model growth rates in egg contents. Indeed, this is a common feature of risk assessments, namely data from studies not necessarily designed with risk assessment in mind often are used to create models, especially when little else is available.

Comment #40: The later work by Cogan *et al*. also had two specific purposes. One was to try and reconcile the published data on the rapid growth of SE in artificially inoculated eggs with the UK belief that, in general, growth was slow and often delayed, supported by the reports, which showed that low numbers of cells were present in 'fresh' eggs, and which were mentioned earlier. There been a number of studies, largely from the US, although a German group has also addressed this, which indicated rapid growth of SE in artificially contaminated eggs. Some of these are open to criticism in that one used eggs of unknown age and most of the others used very high initial contamination levels.

Reply: No response needed.

Comment #41: The secondary purpose of the Cogan work was to develop a model so that the genetic basis for the better survival of SE in egg contents could be determined. As the authors of this annex state the size of the inoculum has a profound effect of SE growth rates. Dr Cogan believes that this may be due to localised reductions in albumen pH around the inoculum, which permit iron to be released from the iron binding proteins in the albumen. It is my view that for most naturally contaminated eggs only a few salmonella cells are deposited in the albumen and that an inoculum of <10 cells per egg produces the most reliable data.

Reply: No response needed.

Comment #42: It is also my view that by ignoring the data from the low inoculum the authors are discarding potentially valuable data. The Cogan paper states that even with a target inoculum of c2 cells of SE per egg only 9% of eggs would have received no bacteria. Given the very sophisticated statistics used in this report it should surely be possible to use these data, which may most closely represent the natural situation. By ignoring low inoculum levels it is possible that the analyses will be biased to showing growth to be more rapid that it really is in naturally contaminated eggs.

Reply: We thank the reviewer for the compliments and recognition that statistical adjustments could have been made if we had wanted to include the data with the inoculum of 2 cells. We were concerned in part for the reason given in comment #23 by reviewer #2: we were not confident, even accounting for a possible distribution of actual number of cells, that the inoculum was, on the average, 2 cells as designated. We agree with the comment's implication that design effects, particularly at this inoculum level, could have had an effect on the results. This concern is manifested in the inconsistency of the results that are associated with this inoculum level, when compared to the other results. On Table E4 of Annex E, it can be seen that at 30ºC the percentage of eggs showing large relative growth is greater, by a small amount, for the eggs inoculated with 2 cells versus those inoculated with 25 cells (30% versus 23%); on the other hand, at 20ºC, the relationship was reversed by a substantial amount (7% versus 30%). Note also that for the eggs inoculated with 25 cells, the percentage showing large growth was greater at 20ºC (30% versus 23% at 30ºC). Accounting for the eggs that actually had no cells being inoculated would increase the actual percentages by small amounts which would accentuate the inconsistencies even more. We could explain these data (and the inconsistency) by hypothesizing that a temperature effect only occurs for low initial levels and that otherwise, there is no temperature effect. And to fit the data well, we could have constructed a model that had this property – that the likelihood of large relative growth was temperature and level dependent when the temperature was below 30ºC (how much below would be anyone's guess), and when the levels were below 25 cells (again how much below is not known). But our concern was that the results at 20ºC, with such a low level of inoculation could be incorrect for reasons connected with the actual experiment, and consequently it was decided not to use these data for the inoculm of 2 cells. Even if the results represented the situation accurately the bias introduced by deleting these data would not be large: it is only the one cell – at 20ºC – that had significantly lower proportion of egg showing growth.

Comment #43: It is quite clear, however, that rapid growth is possible in a small percentage of fresh eggs, as some outbreak data indicate. In our experimental model, even at two cells per egg there are around 5-7% of eggs, which allow the more rapid growth of SE.

Reply: No response needed.

Comment #44: There is no doubt, however, that even with eggs from the same group of birds, inoculated with the same strain of SE from the same culture at the same time, there will be differences in growth/survival rates. We are currently comparing individual hens and data could be made available to FSIS. It is my view that the authors of this annex have made a very good attempt to sort out the tangled mess of data from studies of the growth of SE in artificially contaminated eggs. One of the problems that the authors face is the lack of good data on this highly important topic. This has forced the modellers to use data in a way for which it was not intended. I suppose that all the microbiologists referred to in this study could argue that if they knew at the time that risk analysis was going to be applied to the data they might have done things differently!

Reply: Yes, this is a common point of frustration. Rarely are experiments designed with the explicit intention of using the resultant data for risk assessment. We are aware of this and try to evaluate the data accordingly.

Comment #45: *Lysozyme activity:* The authors need to exercise caution in the statements about the effects of lysozyme in egg white. Studies conducted outside that environment may not match that within an egg where the pH and magnesium levels are high and iron levels are low. Thus while iron limitation may be the major reason for a relative lack of growth in egg albumen, lysozyme may also play a role in growth inhibition/survival (Cogan personal communication).

Reply: The mention of lysozyme activity has been removed.

Comment #46: *A putative mechanism for growth in albumen:* This section is authoritative and provides an excellent review of available data. The arguments put forward for growth in albumen seem reasonable.

Reply: We thank the reviewer for the comment.

Comment #47: *Annex F.* This is also a very interesting body of work, which presents some quite startling and highly valuable data, particularly in Table F1. The table gives the age of these eggs, where known. Are data available on how these eggs were stored, pre-breaking?

Reply: No, but good question. It should be understood that the age reported represents the minimum age among eggs that were used. So in fact there could have been some (unknown) proportion of older eggs used in the product even with a young age reported; it is for this reason, possibly, that the age effect was not as strong as it might have been otherwise. However, in addition, it is important to realize that these data are survey data – collected to be "representative" of product at the time of being pasteurized. Consequently causal type inferences – attempting to estimate the effect of age or time of storage - cannot so readily be made; there could be many unknown, or unaccounted for, factors that affect initial levels of salmonellae.

Comment #48: The data where time of year is examined could suggest that eggs for breaking are not held under refrigeration. The age of the eggs is addressed to an extent in table F-7, although I did find it quite difficult to follow. It would be very interesting if more information could be obtained about the eggs currently classified as >1 day old. Are there any data available on the age of eggs entering breaking plants? Maybe the industry has this?

Reply: Please see above reply.

Comment #49: It will also be very good to get information on the serotypes present. In my experience, these are almost always SE.

Reply: No response needed.

Comment #50: *Survival of SE on eggshells:* On page F-28, the authors discuss attempts to differentiate between sites of contamination and, in particular, to identify the contribution of internal contamination. They state; 'Therefore, if the eggs were kept in a dry environment soon after they were laid or contaminated, the distribution of salmonella levels due to contamination on the exterior of the shell would not likely to be affected by the age of the egg before breaking'. This is likely to be factually incorrect. Although salmonella can be isolated from eggshells long after lay and the UK study referred to earlier found positives after five weeks' storage, the numbers present will reduce.

Reply: This is an excellent point, one which we did not consider. The text has been revised accordingly.

Comment #51: *Annex G.* Table G-1 is interesting as they confirm that SE strains/PTs can show different heat sensitivities. It might improve the value of the data if additional information was given on standard errors and number of replicate experiments.

Reply: Shah et al. performed replicate experiments for one of the *S.* Enteritidis strains described in the report (strain C 398). The percent coefficient of variation from two determinations at 57.2°C and two at 60.0°C was 5.7% and 0%, respectively. This is now included in the text.

Comment #52: The paper [see above comment] is unlikely to have much other information on whether any of the isolates were *rpoS* mutants, for example, because the work was carried out before the significance and frequency of such mutations had been brought to public attention.

Reply: No response needed.

Comment #53: Given the low number of strains tested from any particular source that was examined, I would caution the authors of this annex about making too much of the effects of source. To my mind the data simply reflect the variation in heat resistance seen with SE, and other salmonella serotypes. In fairness, the authors do take this on board. The authors of the annex discuss the point that some serotypes of salmonella may show enhanced tolerance to environmental extremes than the average for salmonella. This is an area of much scientific debate. There is a view that SE and perhaps, *S.* Typhimurium may well have an overall greater tolerance. This may be a reflection of possible higher numbers of mutators in these serotypes. With the exception of *S.* Seftenberg 775W, it is my view no salmonella possess exceptional heat tolerance, although each serotype will contain variants with either greater or lower tolerance.

Reply: We thank the reviewer for providing this opinion. A statistically significant source effect (p-value = 0.04) was observed for the 17 *Salmonella* strains in question. However, it is not possible to say that the source variable implies a meaningful stratification of the population of *Salmonella* serotypes found in eggs. In the end, for our

model, the 17 *Salmonella* strains were considered to be a random sample of strains from the existing populations, and thus, the between-strain variance was computed, ignoring source and phage type.

Comment #54: *Survival curves in egg albumen at high temperature (page G-10):* It is surprising to see that the enrichment media for the survival in albumen studies contained sodium deoxycholate. Heat-damaged cells of salmonella are known to show increased sensitivity to this compound. What justification is there for its inclusion? I also see that the cultures were refrigerated before culture. Would this not have posed an additional stress on an already potentially damaged population?

Reply: Sodium deoxycholate selects for Gram-negative and enteric bacteria and inhibits most Gram-positive bacteria. It is not expected that refrigeration would have damaged the enrichment cultures. Presumably, these cultures consist of cells growing similar to those of wild type.

Comment #55: *Whole and egg yolk products (G-30):* While I accept that a lack of data on the survival of SE in egg contents components forces the authors of this annex to use alternative data, caution needs to exercised in the interpretation of the results of Blackburn *et al.* (1997). Firstly the choice of tryptone soya agar might have underestimated survival, because heat-damaged cells would have poor protection from oxidative stress. Secondly cells in naturally contaminated eggs may well have been exposed to a variety of different temperatures and there nutrient status could be very different to those in broth.

Reply: It is not clear how to treat the data from Blackburn et al. to account for these potential drawbacks. We have introduced a cautionary phrase regarding these two points in the text to alert readers to these two points.

Comment #56: *Pasteurisation of shell eggs:* On page G-39 the protocol of Schuman *et al*. (1997) is discussed. I note that the eggs were allowed to warm to room temperature, inoculated with SE into the yolk and then held for a further hour before heat challenge. Did Schuman *et al*. address the issue that the holding period may have increased the size of the inoculum and changed some of the cells from stationary to log phase? I am also concerned about the use of a mixed inoculum. Surely it would have been better to use an egg-associated strain with high tolerance. There were plenty available at the time this work was done.

Reply: A subtle distinction, Schuman et al. held eggs for ≤1 hour before heat challenge. However, we were unable to find precise values for egg holding before heat challenge. Also, we were unable to find discussion of the fact that the inoculums may have increased during this time. Similarly, though the authors describe their rational for using stationary cells in the inoculums (i.e., “because [stationary cells] are usually several-fold more heat-resistant that cells harvested in the log phase”), we were unable to find discussion of the fact that some of the inoculum cells may have entered log phase growth during egg holding prior to heat challenge. Lastly, it was unclear why the authors did not

use an egg-associated strain in the study. They did, however, use 1 strain from poultry manure, 2 strains from poultry belts, and 1 strain from live poultry. They also stated "a pooled, six-strain inoculum was used to compensate for strain-to-strain variations in thermal resistance."

Comment #57: *Annex H.* Although I read this annex in detail it does [not] seem necessary for me to comment on its content. A large range of food types is covered and the data on consumption matters could prove to be very useful for future risk assessments.

Reply: No response needed.

Comment #58: *General conclusions.* The risk assessment and the associated annexes are documents of value, which will inform risk assessors worldwide. An excellent attempt has been made to use existing data and, in general, the authors have also tried to use data from outside the US. There are, however, some potentially important omissions. I am also quite surprised that vaccination of laying hens was not addressed. Its introduction in the UK under the Lion Scheme is believed to have been the principal reason for the marked fall in human SE cases seen since 1997 (Anon 2001).

Reply: Vaccination does appear to be an effective mitigation for reducing infection of humans with SE from eggs. This point was also raised through public comments on the risk assessments.

With respect to the risk assessment for *Salmonella* spp. in liquid egg products, we used data from FSIS baseline studies of liquid egg products completed in 2003. Vaccination is a control measure introduced prior to egg laying; thus, in this sense, it is "upstream" of shell eggs or egg products sampled at processing or retail. As such, the effect of vaccination, as currently practiced, *is* included in the risk assessments and has not been overlooked.

With respect to the *S.* Enteritidis in eggs risk assessment, the effect of vaccination was not included in the shell egg risk assessment. Data were unavailable to assess the frequency of use or effect of use of current vaccines.

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Itemized Response to Public Comments to the Docket

for

Risk Assessments of *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Egg Products

United Egg Producers

Comment #1: FSIS employees announced at the public meeting on October 22, 2004, that the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) have had or will have an opportunity to review and comment on the risk assessments, but the comments from these agencies have not been incorporated into the drafts that were released. The egg industry feels that these comments from FDA and CDC should have been taken into consideration and incorporated into the drafts prior to public release of the documents. The drafts without feedback from FDA and CDC were prematurely released and of limited use to the public at this point. **We request that FSIS revise the current drafts incorporating any comments from other federal agencies, in addition to the peer reviewers, and re-release the drafts for public comment once those changes have been made.**

Reply: The revised risk assessment models and report have been updated based on comments from federal agencies, independent peer reviewers, and public comment.

Comment #2: *Vaccination of hens for* Salmonella. UEP and UEA [United Egg Association] are concerned about the absence of information on vaccine use in the risk assessments for both shell eggs and egg products. Vaccines are an effective tool for the industry to prevent *Salmonella* infections in hens. The shell egg industry and the egg processing industry both use vaccines to reduce the risk of *Salmonella* contamination. The industry has effectively used both killed vaccines and live attenuated vaccines to prevent *Salmonella* infection. In not addressing vaccine use, the current risk assessments have overlooked an important *Salmonella* control measure. We strongly suggest the addition of information on vaccines to the draft risk assessments.

Reply: In the liquid egg product risk assessment FSIS used data from baseline studies of liquid egg products. Vaccination is a control measure introduced prior to egg laying; thus, in this sense, it is “upstream” of egg products sampled at processing or retail. As such, the effect of vaccination, as currently practiced, is included in the risk assessments and has not been overlooked. The effect of vaccination was not included in the shell egg risk assessment. Data were unavailable to assess the frequency of use or effect of use of current vaccines.

Comment #3: *The number of annual illnesses attributed to egg products.* The risk assessment for egg products estimated that 50,000 – 200,000 illnesses per year are due to egg products. **This number is grossly overestimated by the draft risk assessment and needs to be corrected.** Since the Eggs Products Inspection Act (EPIA) went into effect, we are aware of no outbreak due to *Salmonella* in egg products. Arguably, egg products are more likely than other food products to cause an outbreak if contaminated, simply due to the quantities in each batch. Egg products have an exceptional food safety record. The industry works with FSIS and state inspectors in USDA inspected plants to produce safe egg products. If the agency responsible for inspection of egg products is saying that thousands of illnesses each year are due to egg products, something is wrong with either the inspection process or the risk assessment. We strongly suggest that the data and assumptions used to develop the illness estimates be reviewed.

Reply: The data and assumptions have been reviewed as a result of the feedback described throughout this document. The revised estimates are presented in the risk assessment report.

Comment #4: *Availability of data used in the risk assessments.* Several reports cited in the risk assessment are not publicly available. For example, the risk assessments refer to a survey of the industry, but this survey is not easily accessible to the public. In addition, a national baseline survey of egg products prior to pasteurization is mentioned in the risk assessment and the only reference is an abstract. A short report was posted to the risk assessment website after the public meeting; however, this report does not include adequate information for parties reviewing the risk assessment. In the PDF form of the report, the axes on the graphs are not labeled to allow the reader to understand the data presented in the graph. These important reports should be available in their entirety. **Releasing the draft risk assessments prior to the availability of all relevant data was premature.**

Reply: The draft risk assessments and related documents, including the results of the egg products baseline survey, are available at the FSIS website using through the following address http://www.fsis.usda.gov/Regulations_&_Policies/RD_04-034N/index.asp.

Some of the figures in the original report became unreadable when the document was put into PDF format. We have gone through the revised report to ensure that all figures, graphs, and tables are readable.

Comment #5: *Use of experimental research studies.* Because the infection rate of *Salmonella* Enteritidis (SE) in eggs is very low and it is almost impossible to use naturally contaminated eggs for research purposes, many research studies utilize inoculation techniques to experimentally infect hens and/or eggs with SE. Inoculation studies tell us a lot about the growth patterns of SE. However, caution should be used when extrapolating data from experimental studies to a natural environment. When data are available on naturally contaminated hens and/or eggs, those data should always be used instead of data from studies using inoculation of SE. Caution should be used when inoculation studies ate the only studies available on a subject. The data and methods

should be evaluated carefully. Not only will naturally occurring pathogen loads differ from the challenge doses used in the laboratory, but other factors such as the strains of birds will differ also.

Reply: We agree that caution should be used when extrapolating data from experimental studies to a natural environment. In these risk assessments we used data from naturally contaminated eggs or hens where available. Examples include the FSIS baseline survey of salmonellae in pre- and post-pasteurized liquid egg products and data from the Pennsylvania Pilot Project. However, as the commenter correctly points out, it is "almost impossible' to use naturally contaminated eggs for research purposes; therefore many of the investigations into growth of SE in hens and eggs has necessarily been performed using experimental inoculation.

Comment #6: The American Egg Board sponsored studies on lethality kinetics of *Salmonella* spp. in liquid egg products.

Reply: This has been corrected.

Comment #7: It is estimated that 80 percent of known-source SE infections are due to eggs. The reference cites data from 1988, 1993, and 1996. These data are 8 to 16 years old. The Centers for Disease Control (CDC) has several surveillance systems monitoring SE and the most recent data is available from 2002 and 2003. The most up-to-date information should be used when available.

Reply: Updated references have been included.

Comment #8: The background information about the regulatory requirements for shell eggs requires correction. The 1996 HACCP rule is referenced; however, egg products do not fall under this rule. The current wording implies that shell eggs and egg products are regulated under the 1996 HACCP rule.

Reply: The text provides an introductory overview of HACCP. It goes on to state that one of the objectives of these risk assessments is to assist in developing performance standards for eggs.

Comment #9: *Recent studies regarding SE contamination in egg yolk.* Methods used in the studies should be evaluated to make sure that when the yolk is cultured, contamination of contents with egg albumen or yolk membrane did not occur. It is well established that SE can be located in the egg white at the yolk membrane. Most studies indicate that contamination of the yolk only occurs after deterioration of the yolk membrane.

Reply: Methods used in all studies cited in the risk assessments are evaluated, to the extent possible, by risk assessors. We are unable to go into the laboratory in an effort to reproduce experimental results described by authors. Thus it is typically necessary to place a degree of trust in the study's authors. Particularly in the case of the peer-reviewed

literature, we make the assumption that published results are derived from scientifically valid and carefully controlled studies.

Comment #10: Under the section "*Egg product pasteurization scenario*" it states that "*Risk managers requested that these assessments consider egg product pasteurization scenarios in which the level of* Salmonella *in egg products is reduced by 7 to 12 log_{10}.*" Emphasis should be on control measures to prevent infection and growth of SE in eggs. A 7 to 12 log reduction is not practical for shell eggs or egg products when vaccines, on farm quality assurance programs, refrigeration, and proper handling are effective control measures.

Reply: The 7 to 12 log reduction included in the risk assessment refers to pasteurization of liquid egg products, not shell eggs. It should be noted that the baseline survey found consistent contamination of egg products with salmonellae, occassionally at high levels. Nonetheless, we believe the risk assessments effectively address risk management questions posed at the outset. The appropriateness of those questions is a matter for policy debate. The purpose of this document is to defend the technical merits of the assessments. That said:

i. We agree that emphasis should be placed on control measures to prevent infection and growth of SE in eggs. This is precisely what the FDA's proposed rule for Prevention of *Salmonella* Enteritidis in Shell Eggs During Production does (see http://www.cfsan.fda.gov/~lrd/fr04922b.html for details). By working in concert with FDA, FSIS strives to continue to improve egg safety.

ii. Based on data submitted by the commenter on current time-temperature practices used by industry for liquid egg pasteurization, a 7 to 12 log reduction is not only practical but commonplace.

iii. In light of the generally accepted estimate of between 100,000 and 150,000 illnesses per year in the U.S. from SE in shell eggs, whether the control measures currently in place are "effective" is a matter of opinion.

Comment #11: The *Salmonella* statistics on page 16 are not the same as the statistics on page 1 of the Executive Summary. Page 16 cites all *Salmonella* estimates while page 1 cites "foodborne" *Salmonella* illness estimates. *Salmonella* illness statistics are confusing and often misstated. It is important that the statistics be cited consistently and accurately. **We suggest FSIS choose a single set of statistics, clearly state what they represent, and use them consistently.**

Reply: The statistics are indeed different because, as the commenter goes on to state, those on page 1 refer to foodborne salmonellae whereas those on page 16 refer to all salmonellae. Infections from the former are estimated to constitute about 95% of the latter.

Comment #12: *"An individual consumes on average 230 eggs per year, not including eggs consumed as part of cake mixes, noodles, etc."* The reference for this statement is from 1998. The National Agricultural Statistics Service publishes up to date information each year and is available for 2003 (http://usda.mannlib.cornell.edu/reports/nassr/poultry/pec-bb/.) The American Egg Board also publishes an Egg Industry Fact Sheet each year with current information (http://www.aeb.org/eii/facts/industry-facts-2-2004.htm.) The risk assessment should include the most recent information available. It is also our understanding that consumption numbers include egg products consumed as ingredients in other foods.

Reply: The report from the National Agricultural Statisitcs Service and give data for egg production, not consumption. The report from the American Egg Board, which estimates consumption from production, suggest an individual consumed on average 254 eggs per year in 2003. This increase in egg consumption results in a slightly higher estimate of annual illness from SE in eggs; however, estimates from the revised risk assessment for SE in shell eggs have been anchored to CDC surveillance data.

Comment #13: *"Approximately 80% of vehicle-confirmed SE outbreaks have been associated with grade A shell eggs or egg containing foods."* The references are from 1988 and 1994 based on data from 1985-1991. The table referenced (Table 2-2) contains data from 1985 to 1987. More recent data is available from the CDC estimating the percentage of egg associated outbreaks. **Using data that are 13 to 19 years old is unacceptable when recent data is available.**

Reply: As cited in comment #13, the sentence in question was taken out of context, disregarding the two sentences that immediately followed.

> Between 1993 and 1997, an average of 80% of vehicle-confirmed outbreaks was egg-associated, with a range of 68% to 95%. In 1998, of the 18 outbreaks for which a vehicle could be confirmed, 15 (83%) were associated with eggs.[36]

Comment #14: The baseline data for the mean number of SE in contaminated eggs are grossly overestimated. Therefore, the SE levels at all other steps are also grossly overestimated. Research has established that naturally contaminated eggs contain minimal (2 to 10) SE cells in each contaminated egg. **Estimating 9.1×10^6, is a gross overestimation of the levels of SE and makes the entire model inaccurate.**

Reply: The commenter misconstrued the baseline value of 9.1×10^6. As was stated in the original text, immediately before the table in which the value is presented:

> It should be noted that most eggs are not capable of supporting bacterial growth, either in the layer house or during on-farm storage; thus most of the eggs would have the same number of bacteria with which they were contaminated, generally no more than 1,000. *If just a few bacteria grow to high levels, however, the mean number of bacteria will reflect those high levels* [Emphasis added.]

Comment #15: Data for non-pasteurized shell eggs. *"It further estimates approximately 0.0003 or about 3 eggs in every 10,000 would be contaminated at lay."* The 1998 risk

assessment estimated that one in 20,000 eggs may be contaminated with SE. The mid 1990s were the peak of SE illnesses and since then, illnesses and egg associated outbreaks have declined. **We question the conclusion that 3 in 10,000 eggs are contaminated at lay when all epidemiological and field data indicate that SE contamination rates at lay have declined dramatically since the 1998 risk assessment was published.**

Reply: The proportion of contaminated eggs is determined primarily by three factors: 1) the proportion of infected flocks, 2) the proportion of infected hens in infected flocks, and 3) the proportion of contaminated eggs laid by infected hens. Each of these factors can be represented by an uncertainty distribution. Uncertainty was generally not evaluated in this assessment, however, because the global uncertainty in the model overwhelmed the uncertainty present in the epidemiologic evidence. This is because many of the inputs are based on very limited data. Rather the effect of individual inputs was evaluated with a sensitivity analysis.

Thus, each of the factors that determined the proportion of contaminated eggs was represented by the expected value of the underlying uncertainty distribution. This gives the single value of about 1 contaminated egg per 3,400 shown in the report. If uncertainty is modeled for these three proportions then 90% of the values for the proportion of infected flocks can range from about 7% to more than 40%, the proportion of infected hens in infected flocks can range from less than 1% to more than 20%, and the proportion of contaminated eggs laid by infected hens ranges from about 7% to about 11%.

The uncertainty in these three values gives a proportion of contaminated eggs that could be less than 1 contaminated egg in 20,000 eggs or more than 1 contaminated egg in 1,000 eggs. Because the model is anchored on the epidemiologic data, the estimated number of illnesses will be the same whether the true value is 1 per 20,000 or 1 per 1,000. Certainly, an estimate of 1 per 20,000 falls within the range of uncertainty. If there are relatively few contaminated eggs, then each contaminated egg accounts for a larger proportion of human illness. If there are relatively more contaminated eggs, then each contaminated egg accounts for a smaller proportion of human illness. In either case, however, decreasing the proportion of contaminated eggs by 1% would decrease the number of human illnesses by 1%.

Comment #16: UEP and UEA request additional clarification on how the Agency concluded that 350,000 illnesses each year are due to raw shell eggs and 200,000 illnesses each year are due to pasteurized shell eggs.

Reply: All of the information for how the estimates presented in the risk assessments were determined is presented in the body of the accompanying report. The numbers of predicted illnesses are revised in the current report. As for “additional clarification” FSIS is happy to meet with individual stakeholders to help them understand what is included in the report.

Comment #17: This section [Risk Characterization, p 153] is confusing and contains information that is not clear and does not reflect industry practices. The statement *"storage temperature after processing was set at 3 different values: 45, 53, and 60° F"* is misleading. Processing refers to pasteurization of egg products. **Liquid egg products are held at 40° F after processing.** Frozen egg products are held at freezer temperatures while dried egg products are held at slightly cooled or room temperatures. It is appropriate to model *shell* eggs stored at the three reference temperatures prior to washing, packing and breaking. Table 5-5 states the number of estimated human illnesses that would occur at different times of refrigerated storage after pasteurization. The point at which pasteurization occurs is an important factor and could change the data presented in the table.

Reply: In an effort to remove confusion, the sentence was changed to read "Egg storage time was truncated at 3 different values: 12 hours, 24 hours, and 36 hours."

We agree that the point at which pasteurization occurs is an important factor in the number of estimated illnesses. Indeed, the purpose of Figure 5-1 (titled "Number of estimated human illnesses after each step in model if eggs were immediately consumed") was to illustrate this fact.

Comment #18: Figure 5-17 overestimates the mean number of SE at the layer (107) and therefore throughout the process. FSIS should correct this baseline information based on published research, or provide justification for the use of numbers this high. The pasteurization process would reduce the mean number of SE cells to well under the 1000 cells indicated. We also question the growth rate of 50 percent for injured cells. Research in this are is required prior to making such an assumption.

Reply: We respectfully suggest, similar to comment #14 above, that the commenter misconstrued the graph. We realize the idea of 1000 cells after pasteurization is counterintuitive, but it is nevertheless correct. As explained in the original text.:

> Intuitively, 3 $\log_{10}$ pasteurization or a 3-$\log_{10}$ reduction would be expected to reduce the number of SE by 99.9%. [……..]. If just a few bacteria grow to high levels, however, the mean number of bacteria will reflect those high levels.

The growth rate of 50 percent for injured cells has been removed. Research has established that injured cells grow slower than non-injured cells; however, because the injury phenotype is not heritable, every subsequent generation of cells would be expected to grow as wild-type, a fact which quickly negates the effect of injury on the rate of bacterial population growth.

Comment #19: UEA believes FSIS has gravely erred in its discussion of the number of illnesses estimated in the risk assessment attributed to egg products. Egg products are required to be pasteurized under the Egg Products Inspection Act (EPIA) which is enforced by FSIS. **In the 34 years since the EPIA went into effect, we are aware of no reported illnesses or outbreaks of salmonellosis due to pasteurized egg product.** For the agency responsible for the safety of egg products to estimate that thousands of

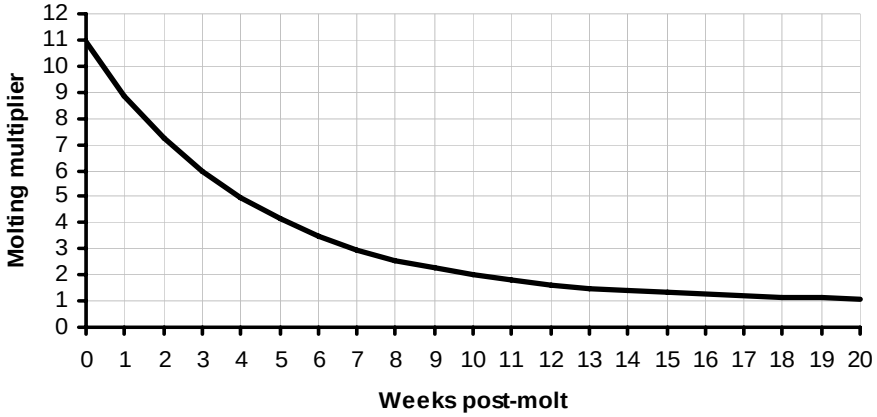
illnesses each year are due to egg products without any epidemiological evidence is a disservice to the egg industry and consumers. We believe that the history of the safety of egg products should be considered and the illness estimates should be reevaluated. We commend the writers of the risk assessment for acknowledging the lack of epidemiological data of illnesses due to egg products. The next step is to develop a realistic estimate of illnesses due to egg products that is consistent with the epidemiological data and the food safety record of the egg products industry. UEA is in the process of administering a survey to their members on practices related to egg pasteurization. UEA will submit this data to FSIS in the near future.

Reply: Please see the reply to comment #3 above.

Comment #20: *Annex B. Distribution of* Salmonella *prevalence in hens and eggs. Page 5.* The flock prevalence estimate was based on proven methods from several data sources, and then multiplied by a factor of two. We question the need to multiply the estimate by a factor of 2 due to false negative testing. If false negative testing is a problem, then the method should be validated. There is no scientific justification for multiplying a well established estimate by two just because one reference in 1995 stated so. Environmental testing was not common in 1995 and it is very common in 2004. The method the FDA recommends has been through at least one revision in recent years. We strongly suggest you evaluate the current state of environmental testing methods prior to using a multiplication factor of two to adjust for "false negative" results.

Reply: The basis for the factor of two to adjust for false negatives in environmental testing is found in data from the study conducted by Schlosser et al. (1999), as cited in the risk assessment report. Approximately 48% of infected flocks were found positive on a single test. A single flock test usually consisted of collecting separate swab samples from each manure bank, each egg belt, and other surfaces in the poultry house. In the field trial, 12 flocks' environments were sampled weekly for 12 consecutive weeks. Eight of the flocks had at least one positive test result during the 12 weeks of sampling. Among these 8 flocks there were 46 positive results from 95 environmental collections. Assuming these 8 flocks were positive for all 12 weeks, the above result implies an approximate 50% false negative rate. We evaluated the testing procedures used by Schlosser et al. (1995) and from the National Animal Health Monitoring Survey (NAHMS) and found the sampling and culturing procedures comparable. Because the NAHMS survey data were used in the risk assessment it seemed logical to use the data from Schlosser et al. to adjust for a false negative rate. Whether tests administered in 2004 have been improved to correct for false negatives (as implied by the commenter) is in this case not germane to the NAHMS survey data.

Comment #21: *Annex B. Distribution of* Salmonella *prevalence in hens and eggs. Page 28. Molting factors.* We disagree with FSIS's reasoning regarding the percent of positive eggs post molt. We strongly suggest that FSIS collect data on this before assuming that 100 percent of eggs from molted hens are SE positive for the first week after the end of the molt. Not all molted hens are exposed to SE and certainly not all eggs will contain SE. Experimental research studies have demonstrated an increase in the susceptibility of



SE infection after a molt, yet no field studies have demonstrated the same susceptibility. The timing also needs to be clarified because hens do not lay eggs during a molt. If the "first week of infection and molt" means the first week that egg production resumes after a molt, the document should state that. Another important factor is that significant numbers of producers within the egg industry have adopted a non-feed withdrawal molt and the susceptibility of the hens to SE may therefore be dramatically reduced in these flocks (Seo, KH, Holt, PS, Gast, RK Comparison of *Salmonella* Enteritidis infection in hens molted via long-term feed withdrawal versus full-fed wheat middlings. *Journal of Food Protection*, 64(12), 2001, 1917-1921.) Research has also demonstrated that vaccine use may protect hens during a molt from SE infection (Holt, PS, Gast, RK, Kelly-Aehle, S. Use of a live attenuated *Salmonella* typhimurium vaccine to protect hens against *Salmonella* Enteritidis infection while undergoing molt. *Avian Diseases*, 47, 2003, 656-661.)

USDA scientists do not agree that an induced molt necessarily leads to increased post-molt shedding of SE in field conditions. We are attaching a letter from Jean Guard Bouldin, DVM, PhD., a distinguished Agricultural Research Service scientist at the Southeast Poultry Research Laboratory in Athens, GA. She notes the large-scale epidemiological comparisons that can be made between the United States, where induced molting is common, and the European Union, where it is not permitted, and states, "The epidemiological outcome strongly suggests that molting does not impact food safety associated with the problem of egg contamination, because Europe has a much worse problem than does the United States." We suggest FSIS review Dr. Bouldin's letter in its entirety, and consult with her and other experts in this area.

Reply: We agree with the commenter that "Not all molted hens are exposed to SE and certainly not all eggs will contain SE." Rather, the model assumes that flocks that are exposed to SE have a higher prevalence of contaminated eggs for no more than 20 weeks following return to production. The chart below demonstrates the assumed molting multiplier for different weeks after return to production. The x-axis shows the number of weeks after molt. The y-axis shows the associated increased risk. The chart shows that: 1) Hens in the immediate post-molt period have a much higher frequency of shedding SE-contaminated eggs than hens that have not been molted, 2) hens that are 20 or more weeks post-molt have the same frequency of shedding SE as hens that have not been molted, and 3) overall, hens in the 20 week post-molt period have about 3 times the frequency of shedding SE than hens that have not been molted.
Data used to construct this chart came not from experimental studies but from the *Salmonella* Enteritidis Pilot Project. The Pilot Project examined eggs laid by naturally

infected hens for the presence of SE. The results, as detailed in table B8 of the report, showed that the prevalence of SE in eggs from non-molted hens was 0.02% (14/6700) whereas the prevalence of SE in eggs from molted hens was 0.05% (39/7400). The data from the Pilot Project further showed that the percentage of SE-positive eggs was greatest for 0-10 weeks post-molt, declining thereafter (see table). The effect of molting on SE-positive egg production was not investigated post-20 weeks.

DATA USED TO DETERMINE MOLTING EFFECT ON PERCENT SE-POSITIVE EGGS.

Relation to Molt	Weeks Pre- or Post-molt	No. Flocks	No. Eggs Tested	SE-Contaminated
Pre-	-20 to -16	3	7,000	4
Pre-	-15 to -11	9	16,000	1
Pre-	-10 to -6	12	23,000	4
Pre-	-5 to 0	12	21,000	5
Post-	0 to 5	6	9,000	13
Post-	6 to 10	8	19,000	13
Post-	11 to 15	9	18,000	2
Post-	16 to 20	10	28,000	11

We also agree that "significant numbers of producers within the egg industry have adopted a non-feed withdrawal molt and the susceptibility of the hens to SE may therefore be dramatically reduced in these flocks . . ." Unfortunately there are no field studies that show the effect of molting birds under these conditions.

We disagree with the opinion expressed in the letter by UEP that a conclusion can be drawn regarding the low prevalence of SE in the United States where molting is permitted and the high prevalence of SE in Europe where molting is not permitted. From an epidemiologic standpoint, to determine whether an association exists between the molting practices in the US and Europe and the incidence of SE infection in these two sites it is necessary to perform (i) studies of group characteristics using ecological studies and (ii) studies of individual characteristics using case-control and cohort studies. If such studies reveal an association, it is then necessary to determine whether the association is causal or spurious. Short of doing so, one could argue equally strongly (albeit equally unfounded), for example, that if molting were allowed in Europe their problem with SE would be much worse and if molting were not allowed in the U.S. their problem with SE would not be as bad.

Comment #22: MPN is an established scientific method for food microbiology. There is a lack of scientific evidence on "clustering" of *Salmonella* cells in egg products, and nod scientific evidence that clustering protects cells during pasteurization. The use of the MPN method, negates any effect of clustering if the method is performed correctly. Multiplying the levels determined by the Weibull distribution by a factor of 3 grossly overestimates the amount of *Salmonella* present in egg products prior to pasteurization and causes the results of the risk assessment model to be inaccurate.

Reply: The factor of 3 has been removed.

Comment #23: Page 7. *"If the eggs are about ten days or more old, then about 20% of the infected eggs might have experienced yolk membrane breakdown and have high levels of* Salmonella *Enteritidis (SE) (reference TA Cogan, Personal Communication, 2002). Supposing 100 eggs have high levels, on average 10^9 cells per egg, the contribution to the number of* Salmonella *from these eggs would be about 10^{11}."*

Published research has established that yolk membrane breakdown occurs at approximately 21 days when eggs are stored at room temperature (Humphrey, Contamination of egg shell and contents with *Salmonella* enteritidis: a review. 1994 *International Journal of Food Microbiology* 21:31-40). When eggs are refrigerated, yolk membranes remain intact for 70 days or longer according to research from ARS (Jones and Musgrove, 2004 http://www.ars.usda.gov/is/AR/archive/jun04/egg0604.htm). We question the statement that in 10 days, 20 percent of eggs have experienced yolk membrane breakdown and have high levels of SE. In naturally contaminated SE positive eggs, levels of 10^9 have not been documented. SE contaminated eggs only occur in rare circumstances and the SE levels are very low. Yolk membrane breakdown only occurs after 3 weeks of storage at room temperature, according to well accepted studies by Humphrey.

Reply: Yolk membrane breakdown is at present a construct meant to represent the transition from a period of low or no growth to a period of rapid growth. It may consist of leakage of nutrients from the yolk into the surrounding albumen or migration of bacteria into the yolk. The occurrence of YMB does not appear to be a bright line. Rather, older eggs are more likely to be able to allow rapid growth than younger eggs. Nevertheless, the report cites reasons why younger eggs may be able to allow rapid bacterial growth.

Comment #24: Finally, we do not think it is appropriate to use personal communication as an authority on the same level as published, peer reviewed studies in this risk assessment.

Reply: We agree. Please see our above reply regarding the personal communication between UEP and Dr. Bouldin (reply to comment #21).

Comment #25: Enough published scientific data is available and should be utilized. In fact a study was published in 2001 by Cogan et al, in the International Journal of Food Microbiology (Oct 22;70(1-2):131-41) titled "Growth of *Salmonella* enteritidis in artificially contaminated eggs: the effects of inoculum size and suspending media." The level of inoculation found to best simulate naturally contaminated eggs was two cells per egg. Significant time at high temperatures is necessary for two cells to reach levels at 10^9. We believe the assumptions are incorrect.

Reply: It is not clear those assumptions to which the commenter has referred. Regarding the issue of a two-cell inoculum, we were not confident, even accounting for a possible distribution of actual number of cells, that the inoculum was, on the average, 2 cells as designated. We agree that inoculum level could have had an effect on the results. This

concern is manifested in the inconsistency of the results that are associated with this inoculum level, when compared to the other results. On Table E4 of Annex E, it can be seen that at 30ºC the percentage of eggs showing large relative growth is greater, by a small amount, for the eggs inoculated with 2 cells versus those inoculated with 25 cells (30% versus 23%); on the other hand, at 20ºC, the relationship was reversed by a substantial amount (7% versus 30%). Note also that for the eggs inoculated with 25 cells, the percentage showing large growth was greater at 20ºC (30% versus 23% at 30ºC). Accounting for the eggs that actually had no cells being inoculated would increase the actual percentages by small amounts which would accentuate the inconsistencies even more. We could explain these data (and the inconsistency) by hypothesizing that a temperature effect only occurs for low initial levels and that otherwise, there is no temperature effect. And to fit the data well, we could have constructed a model that had this property – that the likelihood of large relative growth was temperature and level dependent when the temperature was below 30ºC (how much below would be anyone's guess), and when the levels were below 25 cells (again how much below is not known. But our concern was that the results at 20ºC, with such a low level of inoculation could be incorrect for reasons connected with the actual experiment, and consequently it was decided not to use these data for the inoculm of 2 cells. Even if the results represented the situation accurately the bias introduced by deleting these data would not be large: it is only the one cell – at 20ºC – that had significantly lower proportion of egg showing growth.

Comment #26: *Recommendations from UEP and UEA.* UEP and UEA respectfully make the following suggestions for improving the draft risk assessments.

1. Incorporate comments received from FDA, CDC and the reviewers into the draft risk assessments and re-release them for public comment.
2. Add information on *Salmonella* vaccine use to the risk assessments.
3. Make all surveys and data collected by FSIS available to the public.
4. Always use the most recent information available.
5. Re-evaluate all illness estimates.
6. Re-evaluate baseline information on SE and *Salmonella* contamination rates in shell eggs and egg products.
7. Eliminate the use of personal communication from the references, or make a transcript of that communication publicly available.
8. Re-evaluate all industry and scientific information used as assumptions in the draft risk assessment.
9. Cite documented evidence of actual illnesses due to pasteurized egg products or develop a methodology that appropriately considers the effect of legally mandated pasteurization.
10. Explain the process of methods used when using "weighted" estimates or multipliers throughout the risk assessment. Avoid using multipliers unless scientifically justified.

Thank you for the opportunity to submit these comments. UEP and UEA appreciate FSIS's consideration of our views.

Reply: Replies to each of the items included in the 10 points outlined in the comment may be found above in the responses to comments from UEP.

Michael Foods

Comment #1: *Comment period not sufficient and important information not provided.* The comments and submitted materials are offered for further consideration by the scientific staff responsible for preparing the risk assessment reports. Unfortunately, the public has been given a very short span of time to review the extensive draft assessments and the supporting materials presented in the Annexes. It would seem appropriate to allow additional time for review given that it is obvious that the FSIS has spent at least two years and utilized an extensive team of scientist including experts in statistics, risk assessment, microbiology, and food processing to prepare the risk assessments presented October 22, 2004. It is not reasonable to expect that individuals, impacted industry, or trade associations have had the time to conduct reviews and assemble appropriate expertise to comment in detail within the time allocated for public comments (Federal Register, Vol. 69, No. 192, October 5, 2004).

Reply: No response needed..

Comment #2: It would also be appropriate to provide information used in preparation of the drafts, but not available to the public. They include (1) several citations of "Personal Communications" used in support of develop of assumptions or analysis of data; (2) the complete presentation of the "Base Line Study" used to anchor the risk assessment for pasteurized egg products; and (3) studies conducted by Research Triangle Institute for the FSIS and used to support development of assumptions and analysis of data for the assessments.

Reply: These materials are available through the docket.

Comment #3: *Comments Regarding Salmonella Enteritidis (SE) in Shell Eggs:* **Assumptions for growth of SE in pasteurized shell eggs using the estimators built from evaluation of temperature and the "Yolk Membrane Breakdown" hypothesis overestimate should be reevaluated**. In the draft risk assessment the there is a brief discussion (Chapter 3 Exposure Assessment, pages 38-39) about the "yolk membrane breakdown" hypothesis that acknowledges the event(s) or process(s) is not well understood or defined. The hypothesis relates some change in the yolk membrane that that allows rapid growth of SE in an egg that also appears to be related to the storage temperature history of an egg. It is well accepted that both albumen quality and yolk membrane integrity are affected by temperature history. For shell eggs that have not been treated to stabilize the albumen for example by refrigeration, oiling the shell, thermostabilization, water glass treatment, or pasteurization it is expected that there will be deterioration of the yolk membrane as indicated by reduction in yolk index or weakness and fragility of the membrane.

Reply: We agree that albumen quality and yolk membrane integrity are affected by temperature history. As stated in the risk assessment report:

> It is hypothesized that, as the egg ages, the yolk membrane deteriorates so it ceases to completely separate nutrients in the yolk from the albumen. This deterioration depends

on the internal temperature of the egg: high temperatures hasten the rate of deterioration, while low temperatures lessen it.

The model does have an option of using a set of equations that predicts less deterioration of the yolk membrane at pasteurization temperatures. Nevertheless, using this set of equations would not affect the possible regulatory decisions.

Comment #4: Shell eggs that have been treated to stabilize interior quality of the egg as measured by albumen quality (Haugh Units) will also have associated maintenance of the yolk membrane quality. Oiling the shells, theromostabilization, water glass treatment, and pasteurization of shell eggs stabilize the albumen quality and yolk membrane quality. Stadelman's (1986) chapter, The preservation of quality in shell eggs. In Egg Science and Technology, 3rd edition. The Haworth Press Inc., Binghamton, NY. gives a good overview of the relationships of albumen and yolk quality and methods to preserve quality. Schuman et al. (1997) in Journal of Applied Microbiology Vol. 83, 438-444, presented results showing that pasteurization of shell eggs did not harm the albumen quality as measured by Haugh Units or yolk membrane quality as measured by yolk index.

Reply: We are unaware of data from industry that provides sufficient information on the extent and specific parameters of egg shell oiling.

Comment #6: Earlier unpublished research conducted at the University of Missouri-Columbia on pasteurization of shell eggs reported Haugh Units after four weeks of storage at 22.2°C for pasteurized eggs that would indicate albumen quality equal to that of USDA Grade A or AA eggs (report entitled Thermal Destruction of *Salmonella Enteritidis* in Shell Eggs, prepared by H. R. Ball is attached).

Quality Attributes of Thermally Treated Shell Eggs with and without Oiling after Four Weeks Storage at 22.2 °C (72°F) and 7.2 °C (45°F).

Treatments	**Albumen pH**	**Albumen pH**	**Haugh Units**	**Haugh Units**
No Oil	**22.2 °C (72°F)**	**7.2 °C (45°F**	**22.2 °C (72°F)**	**7.2 °C (45°F)**
No Heat	9.3	9.2	20	60
56.75°C, 36 min.	9.2	8.9	78	82
57.5 C° 23 min.	9.2	9.1	74	82
Oiled				
No Heat	8.0	8.1	58	70
56.75°C, 36 min.	7.9	8.2	80	80
57.5 C° 23 min.	8.0	8.1	81	82

Schuman et al. (1997) reported Haugh Unit values after treatment in a 58°C water bath of 80.7 Haugh Units. That observation determined within hours of heat treatment is similar

to the Hugh Unit data above. As noted in Schuman et al. (1997), the thermal treatments improve Haugh Units but had no effect on yolk index.

Reply: Please see above reply.

Comment #7: The Missouri data and Schuman et al. (1997) show a positive effect on the indicator of albumen quality with no effect on yolk index. The data also shows that the effect is maintained through at least 4 weeks of storage at 72^{o}F. Since the bulk of the prior literature shows positive relationships between maintenance of albumen quality and yolk membrane quality for eggs in general, it could reasonably be assumed that the positive albumen quality result that occurs with shell egg pasteurization also maintains yolk membrane quality.

Reply: The equations that determine YMB estimate very rapid breakdown at pasteurization temperatures. Ultimately, this issue awaits additional data.

Comment #8: Maintenance of egg quality indicators even at temperatures above 45^{o}F argue against acceptance of assumptions for growth of SE in pasteurized shell eggs using the estimators built from evaluation of temperature/time and the "Yolk Membrane Breakdown" hypothesis for non-pasteurized shell eggs.

Reply: See reply to comment #7 above.

Comment #9: Assumptions for SE surviving shell egg pasteurization should be reviewed and lowered.

Reply: All assumptions inputted into the model have been extensively reviewed. If there is already a net benefit derived from current model assumptions, increasing the modeled lethality would not be expected to affect the regulatory decision.

Comment #10: The United States Department of Agriculture, Agriculture marketing Service and the U.S. Food and Drug Administration have jointly established a requirement that shells eggs designated as pasteurized must be subjected to a treatment that yields a minimum 5-log reduction of viable salmonellae (Federal Register 62(185):49955-49957. Docket PY-97-008). Because of this existing regulation it is not clear why time an effort was devoted to evaluation of 3-log reduction processes. Those process would not be by regulation pasteurization processes or be expected to have creditability for food safety. In contrast to the 3-log reduction approach, the portions of the risk assessment for pasteurized egg products seemed to refer to USDA Egg Products Inspection regulation minimums in those discussions.

Reply: The risk assessment was designed for use as a policy tool. It can be used to examine risk scenarios for any log reduction values.

Comment #11: Some of the discussions in the draft report and Annex discuss the lack of information about the lethality of SE located in other portions of the egg other than the

center of the yolk. Schuman et al. (1997), Hou et al. (1996) (Food Microbiology, 13, 93-101.), and Brackett et al. (2001) (Journal of Food Protection 64, 934-938) report destruction of SE in the center of the yolk, assumed to the worst case situation because of potential high numbers and slowest portion of egg to heat. Although the heating medias were different in Schuman et al. and Bracket et al., the time-temperature curves were essentially the same with essentially equal lethality reported.

Reply: We agree. The data cited by the commenter refer to cells in the yolk. As noted below, the assessment models a net 5 $\log_{10}$ reduction of SE throughout the egg.

Comment #12: Because of conduction heating, heat is transferred from the media through the shell, shell membranes, albumen, yolk membrane and final to center of the yolk. Although time-temperature profiles have not been fully developed for the different portions of the eggs during pasteurization processes evaluated, it is logical to assume that those portions nearest the heating media reach temperatures of the media from one to five minutes or less than time required to reach temperature in the center of the yolk.

Reply: We agree. The risk assessment models the effect of a net 5 $\log_{10}$ reduction in *Salmonella*. Additional modeling could be done to depict a gradient effect from the outside to the center of the egg. This would be necessary, however, only if economic benefit does not show a net benefit given the 5 $\log_{10}$ reduction. If there is already a net benefit, increasing the modeled lethality would not be expected to affect the regulatory decision.

Comment #13: The University of Missouri studies described above and attached, showed up to 6-log reductions of SE inoculated on the surface of the yolk membrane in less than 27 minutes in 57.5 ^{o}C water bath, less than 32 minutes in 56.7 ^{o}C water bath, and less than 45 minutes in 56 ^{o}C water bath.

Reply: No response needed..

Comment #14: Schuman et al. (1997) reported center yolk temperatures of 55.3 to 56.2 ^{o}C and log reductions of 4.3 and 4.83 respectively for eggs held 35 minutes in a 57 ^{o}C water bath.

Reply: No response needed.

Comment #15: Bracket et al. (2001) reported center yolk temperatures of 56.12 and 56.18 ^{o}C and log reductions of 6.13 and 6.21 for eggs held in humid heated air for 30 minutes at 57.2 ^{o}C.

Reply: No response needed.

Comment #16: The residence time of 27 minutes in a 57.5 ^{o}C water bath used in the Missouri study is approximately the come-up time required to achieve pasteurization temperatures if the pasteurization process is define by time at temperature in the center of

the yolk. The risk assessment draft did not adequately define shell egg pasteurization as used in the context of the report. Definition including time and temperature as well as minimum required log reduction at a specific location in the egg should be included.

Reply: The risk assessment models the effect of a net $5\log_{10}$ reduction of SE in whole shell eggs. To model the effect of time and temperature throughout the matrix of the egg it would be essential to have better information on the location of SE within the egg.

Comment #17: When considering the conductive nature of heat transfer in water immersion or humid air heating, if minimum 5-log reduction processes defined for center yolk are used it is unlikely that there would be any survivors in any portion of the egg outside of the yolk.

Reply: We largely agree, though with a subtle distinction. If minimum 5-log reduction processes defined for center yolk are used, it is likely that a 5-log reduction will be applied throughout the egg proper. This is not the same, however, as it being unlikely that there "would be any survivors" in the egg.

Comment #18: Based on reported D-value of approximately 2 minutes at 56.7 ^{o}C for pH 8.8 egg white (UEA/AEB, 2002, International Egg Pasteurization Manual), an optimum egg white pH for best visual qualities of pasteurized shell eggs) and assuming that the temperature of the egg white from shell to the yolk membrane was at 56 ^{o}C in 30 minutes (Bracket et al. 2001) or 35 minutes (Schuman et al. 1997) the log reductions would be 15 and 17.5 respectively for SE in the albumen.

Reply: Please see reply to comment #12 above.

Comment #19: If 5-log reduction process for center yolk, i.e., time and temperature at center of the yolk, are used there will always be 2-3-log reduction occurring in the yolk as the center yolk temperatures are approaching the process control temperature. Seven to 8-log reductions would be expected for the total process with no survivors in the albumen.

Reply: No response needed.

Comment #20: Given the above and assuming that minimum legal pasteurization process must deliver a 5-log reduction minimum in the center of the yolk, it seems reasonable that the assumptions for survival of SE after pasteurization should be lowered. At this time there are only two producers of pasteurized shell eggs. The process used by Michael Foods, Inc. is defined for a 5-log minimum reduction in the center of the yolk.

Reply: See the reply to comment #18 above.

Comment #21: *Comments Regarding Salmonella spp. In Liquid Egg Products:* **50,000 illnesses attributable to *Salmonella spp*. In liquid egg products seems unreasonable.**

Given that there have been no documented illnesses attributable to *Salmonella spp*. from pasteurized liquid egg products it seems reasonable to question the assumptions used to develop the estimate of 50,000 illnesses per year. The estimates seemed to be anchored based on the incidence of *Salmonella spp*. positive egg white samples found in the base-line study. There could also be some fundamental issues with assumptions used to estimate numbers surviving, growth post-pasteurization, and portions of egg consumed in prepared foods.

Reply: We agree that the estimate of 50,000 illnesses from *Salmonella* in egg products was high. Due to several factors discussed throughout this document, including removal of the 3X factor for clumping and examining multiple sets of time-temperature data, the number of predicted illnesses is reduced.

Comment #22: The draft report and Annex discuss the broad assumptions that equal portions of each type of egg would be consumed and that the analysis did not deal with food formulation or preparation practices that in themselves would not allow illnesses to develop.

Reply: The analysis considered cooking, an important preparation practice that if done properly significantly reduces the likelihood of illness.

Comment #23: Although the base line study reported finding positive samples, we do not know if the producing plants would have also determined that the product was contaminated and held for rework or disposal. The study did not consider the possibility that a significant portion of positive product could be detected at the plant level and not allowed to move to distribution. If the risk assessments can use assumptions of survival of *Salmonella spp*. and subsequent growth, it would seem reasonable to also use assumptions that quality and food safety programs would prevent a portion of positive product from moving to market.

A correction for intervention of quality programs should be included in the determination of risk.

Reply: The base line study sampled egg product prior to pasteurization. It was assumed that this entire product was subsequently pasteurized. In addition, FSIS also routinely collects post-pasteurization samples to monitor the pasteurization process. These samples suggest that pasteurization is not always 100% effective in eliminating viable *Salmonella*. It is possible that industry may sample product and repasteurize product with positive samples. In this case the effective lethality would be higher than modeled. Information on the proportion of product that undergoes multiple pasteurization was unavailable but could be incorporated into the model.

Comment #24: *The base line study as reported lacks critical information.* At this time the details of the base line study have not been fully presented. For example one must assume at this time that all samples were sent as liquid samples with sufficient

refrigeration and insulation to keep them at temperatures less than 40°F. That detail has not been discussed.

Reply: Samples were sent refrigerated and as liquid. Upon receipt, sample temperatures were determined and those either <0°C or >10°C were discarded. Assuming a sample was held at a temperature of 10°C for 24 hours at pH 6.8, the $\log_{10}$ colony-forming-units/ml would be expected to increase no more than 0.04. See:

> Pathogen Modeling Program, at http://www.arserrc.gov/mfs/PATHOGEN.HTM
>
> T.P. Oscar, Growth Kinetics of *Salmonella* Isolates in a Laboratory Medium as Affected by Isolate and Holding Temperature: Journal of Food Protection (1998) 61(8):964-968
>
> T.P.Oscar, Response Surface Models for Effects of Temperature, pH, and Previous Growth pH on Growth Kinetics of *Salmonella typhimurium* in Brain Heart Infusion Broth: Journal of Food Protection (1999) 62(2):106-111.

Comment #25: For samples having the higher estimates of *Salmonella spp*. we do not have confirmation that temperature of the samples were known at time of reception.

Reply: Please see above reply.

Comment #26: The discussions in the Annex and draft report indicate that there could be uncertainty in the uniformity of sampling which could impact on the results.

Reply: Correct. Assumptions about uniformity are made for estimating the percentage of SE-positive hens by transovarian infection:

> The uniformity assumption implicitly made is that at any time, 1/8 of the infected hens (over an 8 week period) will be just recently infected and laying (potentially) a high percentage of infected eggs. At the same time, this assumption suggests the other 7/8th of the hens will not be laying a larger percentage of eggs (4.1%). Furthermore, the percentage of positive eggs was not decreasing for the later 7 weeks, thus it is not possible to guess or extrapolate the time when the percentage of infected eggs would be negligible. For modeling purposes, 8.615% (based on 54 positive results from 592 eggs tested) is assumed. Uncertainty of this percentage is determined assuming that these results were generated from a trinomial distribution, albumen, yolk and inner shell membrane, with n = 592.

and for the percentage of annual molted flocks:

> Using the uniformity distribution assumption, it is assumed that 10% of the molted flocks will be producing SE-positive eggs for each of the 10 weeks, i.e. 2.2% of all flocks will be molted and considered to be producing a greater frequency of SE-positive eggs each week for 10 weeks.

Comment #27: We do not know the pasteurization process associated with the samples, minimally the temperature and hold time. This is especially critical for understanding the data relative to survival of *Salmonella spp*. reported in egg white samples. Did the processes include use of pH adjustment as permitted as a process aid? Were they with or without hydrogen peroxide? Were they after hot room treatment for dried whites?

Reply: A pH of 8.8 was used as in the model as a default to determine lethality in egg white in the egg products model. Using a pH of 9.3 resulted in much higher lethalities. Unfortunately, information was not available on the proportion of egg white pasteurized at different pH values.

Comment #28: Other critical information would be total aerobic plate count for the raw egg samples prior to pasteurization and relation to estimated content of *Salmonella spp*.

Reply: Because these risk assessments focused on salmonellae, total aerobic plate counts was not deemed a critical input. Furthermore, direct measurments of *Salmonella* were available through the FSIS baseline study for liquid egg products.

Comment #29: *New data describing the pH effect on lethality of Salmonella spp. in white based egg substitute.* During the public meeting where the risk assessments were presented, there was an invitation for additional information on several topics that relate to effectiveness of pasteurization processes. The pH of egg white has been recognized as being important to lethality of egg white pasteurization processes. The UEA/AED (2002) study reported lethality at pH values 7.8, 8.2, 8.8, and 9.3. D-vales for egg white at 9.3 were significantly lower than those for the lower pH values. The lower pH values are more consistent with fresher egg generally used for processing. The UEA/AEB (2002) report indicated a pH effect with lethality generally higher as pH increased.

Included with these comments are an internal report and raw data evaluating the effect that pH of an egg substitute (98% egg white) has on lethality of *Salmonella spp*. As pH increased from 8.2 to 9.0, D_{135oF} decreased from 1.02 to 0.69 minutes. The results provide additional information that generally supports the understanding that lethality of egg white based liquid egg products is enhanced at higher useful pH values. This provides an approach that has long been recognized as an effective aid to pasteurization of whites.

As noted above, knowing the details of the pasteurization processes applied to the egg white samples that were positive for *Salmonella spp*. would be useful. It would also be useful to understand the general use of pH control for assisting egg white pasteurization.

Reply: A pH of 8.8 was used in the model as a default to determine lethality in egg white in the egg products model. Using a pH of 9.3 resulted in much higher lethalities. Unfortunately, information was not available on the proportion of egg white pasteurized at different pH values.

Comment #30: *Concluding Comments:* The USDA, FSIS staff and others contributing to the risk assessments have devoted may hours to data collection and data analysis. The drafts provide an excellent base for discussion with the intent to enhance public safety by reducing the risk of illnesses due to *Salmonella spp.* It is our conclusions that time to study the drafts should be extended and that the additional disclosure of some of the critical data be provided.

We also believe that the estimated illnesses attributed to pasteurized shell eggs and pasteurized liquid egg products are over stated. Specific points of concern and suggested reasons for reconsidering some of the assumptions used are presented above.

It is difficult for me to adequately review the "science" and "statistical" theories used in developing the various equations to assign risk. However there seems to be some opportunity to further enhance the understanding of the characteristics of pasteurized shell eggs and egg products that may allow building of assumptions that are more closely related to on going experience and science/technology of the products under study.

Reply: We thank the reviewer for the helpful and thoughtful comments.

U.S. Poultry and Egg Association

Comment #1: Because of the long length and complexity of the subject draft risk assessment, the allowed thirty days for comment was inadequate. That short time frame limits the extent and possible benefit of public comment in the final preparation of this document. In fact, some of the annex documents of the draft were not available to the public until as recently as three weeks.

Reply: No response needed.

Comment #2: It is my understanding that the draft which has been submitted for public comment does not incorporate changes suggested by either FDA or CDC. I am also uncertain as to whether the draft includes the suggestions made by the peer reviewers listed in the document. It would have been very helpful if the public had been given a draft that had been updated to reflect such previous input.

Reply: The revised risk assessment models and accompanying reports take into account comments received from (i) federal agencies, (ii) peer reviewers, and (iii) public comment. Itemized responses to all comments received on the risk assessments are included in this document.

Comment #3: I attended the hearing on this risk assessment via webcast and was not there in person, however I thought I heard Dr. Schroeder answer a question from the audience related to the numbers of human illnesses that had been related to the consumption of pasteurized egg products. He answered that he did not know of any. His answer is supported by the first paragraph on page 197 of the draft where it is written: "Historically, pasteurized egg products have been a very safe food. There have been no outbreaks linked to the consumption of egg products and consumption of pasteurized egg products does not appear as a risk factor in case control studies of foodborne illness."

Reply: The commenter is correct. The portion of the public meeting discussion to which he refers is found on pages 67, 68, and 69 of the meeting transcript (see http://www.fsis.usda.gov/PDF/SERA_Meeting_Transcript_102204.pdf). The comment made by Dr. Schroeder was in response to a question posed by a representative of the United Egg Producers. Our reply to the discrepancy between the number of estimated illnesses for *Salmonella* in egg products and extant epidemiologic surveillance data is as follows (taken from reply to Comment #3 from the United Egg Producers above):

> "We agree the estimated number of annual illnesses from *Salmonella* in egg products was an overestimate, a belief we stated at the public meeting. The question is not *whether* the estimate is too high but *why* it is too high. Was the model flawed and/or were the data put into the model flawed? We have reason to believe it was the latter.
>
> As part of their comments to the docket the Food Processors Association submitted data described as representative of the industry for time and

temperature of egg product pasteurization. These data indicate reductions of *Salmonella* in egg products higher than those modeled in the original assessment. As such, when inputting these revised data into the model, the predicted number if annual illnesses from *Salmonella* in egg products reduces significantly, from about 40,000 to 3,000. While we cannot state definitely whether the data submitted by FPA are representative of the industry, they are in line with statements from UEP at the public meeting.

> [Regarding] minimum pasteurization requirements in your modeling. The industry puts in an additional factor of protection in that. Would [it] be more accurate to rerun that model with what the industry actually does, and would some industry data on those kind of fudge factors be helpful in the risk assessment?

We believe the revise risk assessment accurately incorporates the "additional factor of protection" or "fudge factors" referred to in the above comment. Thus, quite simply, it appears as if we significantly underestimated the $\log_{10}$ reductions of *Salmonella* in egg products currently achieved by industry at processing."

Comment #4: It would appear that it would be reasonable to base the need for additional or more stringent regulations concerning pasteurized liquid egg products or pasteurized eggs on the following:

-The numbers of illnesses that have been attributed to the consumption of pasteurized egg products.
-The incidence rate and levels of *Salmonella* Enteritidis bacteria found in pasteurized egg product.

Reply: No reply needed.

Comment #5: If this information was acquired and used, the actual level of risk would have a factual basis and there would be no need to utilize what might be termed "voodoo" statistics where the outcome of statistical assessments is assigned a much higher level of credibility than the input estimates justify.

Reply: The information to which the comment refers has been acquired and used in the risk assessment.

Comment #6: For example [see above comment], the multiplication factor of 3X that is applied to the estimates of the numbers of SE bacteria in pre-pasteurized egg products because clumping could account for negative results and could make the cells more resistant to the killing effects of pasteurization without supportive data to justify the correction factor and the 2X factor that is applied to the number of positive environmental cultures of poultry houses, when the testing protocols have been proven over time and widespread use to be sensitive and reliable detracts from the soundness of the assessment.

Reply: We agree with the commenter regarding the 3X factor. Though cell clumping is likely to occur, our use of the 3X factor was arbitrary and cannot be justified based on current data. This factor has been removed from the assessment and the predictions recomputed accordingly. As for the factor of 2X, we feel its use in the risk assessment is justified. Its basis is in data from the study conducted by Schlosser et al. (1999), as cited in the risk assessment. Approximately 48% of infected flocks were found positive on a single test. A single flock test usually consisted of collecting separate swab samples from each manure bank, each egg belt, and other surfaces in the poultry house. In the field trial, 12 flocks' environments were sampled weekly for 12 consecutive weeks. Eight of the flocks had at least one positive test result during the 12 weeks of sampling. Among these 8 flocks there were 46 positive results from 95 environmental collections. Assuming these 8 flocks were positive for all 12 weeks, the above result implies an approximate 50% false negative rate. We next evaluated the testing procedures used by Schlosser et al. (1995) and from the National Animal Health Monitoring Survey (NAHMS). We found the sampling and culturing procedures comparable. Therefore, because the NAHMS survey data were used in the risk assessment it seemed logical to use the data from Schlosser et al. to adjust for a false negative rate.

Comment #7: Another disappointing feature of the assessment report is the reliance upon SE numbers related to illnesses and outbreaks that were acquired during the height of the SE problem a decade ago, instead of the more recent information that reflects the significant progress that has been made by the industry and government in correcting the problem. There has been a continuing effort made to decrease the possibility of SE illness related to eggs and the more recent illness and outbreak numbers from CDC reflect that progress. There is no need or justification to base a current risk assessment on outdated information. The use of SE vaccines, assurances of SE-negative breeding stocks, emphasis on improved rodent control and biosecurity have all had a role in achieving the decline of SE illnesses related to eggs. The voluntary diversion of eggs to pasteurization from environmentally positive flocks has also lessened the likelihood of egg-related SE illness.

Reply: We agree with the commenter that various control measures have likely lessened the likelihood of egg-related illnesses. As was stated in the first paragraph of the Introduction:

> [Interventions including] good agricultural practices, such as voluntary quality assurance programs for egg production, refrigeration during transport to limit SE growth in eggs, and consumer education efforts aimed at cooking eggs fully, all of which likely contributed to the decline in SE infections reported to the Centers for Disease Control and Prevention (CDC) from 1996 to 1998.

It is a mistake to conclude that in the current risk assessment there was "reliance" upon data from past outbreaks and illnesses during the height of the SE problem a decade ago. Such data were used simply to place the genesis of the earlier (1998) risk assessment conducted jointly by USDA and FDA for *S.* Enteritidis in eggs in context. To wit:

> Shell eggs and egg products transmit *Salmonella* to humans. The period 1976 to 1995 saw an 8-fold increase in reported infections with SE, greater than 75% of which were associated with foods containing undercooked eggs. Based largely on these observations, Federal and State agencies worked with industry and consumers to implement farm-to-table interventions to reduce the risk of illness from SE in eggs. [Executive Summary, page 2.]

Lastly, though we too are encouraged by that the SE epidemic appears to have lessened since the mid-1990s, it would be derelict to thus assume it is no longer a problem.

Comment #8: Finally, the effectiveness of the current Egg Products Inspection Act implemented around 1970 became clear when egg [product] consumption and *Salmonella* illness became disassociated. It was a great accomplishment by USDA in protecting public health. The requirements of the Act may need slight adjustments as the products and processes change but I see no evidence in the risk assessment document that justifies a major expansion or overhaul of the existing regulation. Such actions would not be based on science, product contamination surveys, or actual human illness numbers related to SE in eggs.

Reply: The majority of this comment is related solely to policy and thus we do not address it here in reply to technical comments on the risk assessments.

Comment #9: It is very beneficial to conduct risk assessment exercises because they can define the areas where information exists and where more information is needed, therefore helping researchers plan studies that can provide data for use in future regulatory decision making. This assessment will likely be more beneficial if the concerns and input of those providing comments are seriously considered by FSIS.

Reply: We agree that risk assessment is extremely beneficial on this point and we encourage others to review carefully the research needs we have identified through conducting these risk assessments. Filling them will improve our knowledge of the public health problem that is eggborne salmonellosis.

SPARBOE Companies

Comment #1: We appreciate the opportunity to comment on the Food Safety and Inspection Service (FSIS) Docket No. 04-034N "Draft Risk Assessments of *Salmonella* Enteritidis in Shell Eggs and *Salmonella* spp. in Egg Products." We believe that a number of issues still need to be addressed. Among those issues/concerns we deem the following as most critical:

An oversight of the risk assessment is the omission of a *Salmonella* Enteritidis vaccination program as a control measure. With hundreds of flocks currently being vaccinated and after the results in Great Britain, we believe there is sufficient data to incorporate vaccination as a viable alternative to some other control measures.

Reply: We agree that vaccination is an important control measure. In response to this comment we offer the reply given to Comment #58 of Reviewer #5 above.

> "Vaccination does appear to be an effective mitigation for reducing infection of humans with SE from eggs. This point was also raised through public comments on the risk assessments.
>
> With respect to the risk assessment for *Salmonella* spp. in liquid egg products, we used data from FSIS baseline studies of liquid egg products completed in 2003. Vaccination is a control measure introduced prior to egg laying; thus, in this sense, it is "upstream" of shell eggs or egg products sampled at processing or retail. As such, the effect of vaccination, as currently practiced, *is* included in the risk assessments and has not been overlooked.
>
> With respect to the *S.* Enteritidis in eggs risk assessment, the effect of vaccination was not included in the shell egg risk assessment. Data were unavailable to assess the frequency of use or effect of use of current vaccines."

Comment #2: Another concern we have is with the estimated number of illnesses attributed to egg products each year, since there has not been any reported illnesses and no outbreaks in the 34 years of mandatory FSIS inspected pasteurization. The validation of the eggs products model states the number of illnesses due to pasteurized egg products is too high with the absence of epidemiological data. We agree with this assessment.

Reply: We agree with the commenter's point. The number of predicted illnesses is reduced in the revised model.

Comment #3: Sparboe Companies also requests that comments and review by the Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC), in addition to any outstanding comments from the peer reviewers be incorporated into the document prior to another public comment period. It will be very important to the industry to review the documents again after those changes have been made.

Reply: The revised risk assessment models and report have been updated based on comments from federal agencies, independent peer reviewers, and public comment.

APPENDIX I

Peer reviewers of the risk assessments for *Salmonella* Enteritidis (SE) in eggs and *Salmonella* spp. in egg products.

Peer Reviewer	Title and Affiliation	Expertise
Scott Ferson	Senior Scientist, Applied Biomathematics, Setauket, New York, United States of America	In addition to his position at Applied Biomathematics, Dr. Ferson holds an adjunct appointment at Stony Brook University's Marine Science Institute. His research focuses on developing reliable mathematical and statistical tools for risk assessment and on methods for uncertainty analysis. Dr. Ferson has authored scholarly papers, books, and software packages in environmental risk analysis and uncertainty propogation.
Tom Humphrey	Professor, Division of Food Animal Science, School of Clinical Veterinary Science, University of Bristol, Bristol, United Kingdom	Initially trained as a meat technologist, Professor Humphrey is head of the Bristol Foodborne Zoonoses Group, which studies *Salmonella* and *Campylobacter* in foods and food animals. His research uses traditional and molecular laboratory techniques and covers host welfare and infection; bacterial diversity; stress responses; and epidemiology.
Christine Little	Head of Section - Food & Environmental Investigations, Health Protection Agency Centre for Infections, Gastrointestinal Diseases Department, London, United Kingdom	Christine Little is a public health microbiologist with responsibility for the coordination of UK food, water and environmental surveillance programmes and public health investigations and provision of technical advice on food safety and food law. Dr. Little's research activites include microbiological risk assessment.
John Maurer	Associate Professor, Poultry Diagnostic and Research Center, University of Georgia, Athens, Georgia, United States of America	Professor Maurer's research areas include the molecular epidemiology of veterinary and foodborne pathogens and molecular detection of foodborne pathogens. He recently assisted in developing a risk assessment for macrolide use in food animals.
Maarten Nauta	Senior Scientist, Microbiological Laboratory for Health Protection, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands	Maarten Nauta is a mathematical biologist with expertise in microbiological risk assessment modeling, predictive modeling, and food chain risk assessment. He has published widely on topics including bacterial growth modeling, exposure assessment, dose-response, and other food safety issues.

APPENDIX II

The following letter was sent to each of the peer reviewers. The purpose of the letter was to provide a brief decription of the risk assessments and to assist in directing the review. that accompanied the review packages sent to the peer reviewers. The paragraph beginning "Specifically, you were identified" contains the charge to the reviewers. The letters sent to each of the five reviwers were identical, except for the charge. The paragraph containing the charge in the letter sent to those with expertise in microbiology, salmonellae, and food safety is labeled as "a," whereas the one in the letter sent to those with expertise in risk assessment modeling is labeled as "b."

May 10, 2004

Address here

Dear Dr. _____:

On behalf of my colleagues in the Risk Assessment Division, Food Safety & Inspection Service, U.S. Department of Agriculture, thank you in advance for your time and effort reviewing our recently completed draft document entitled *Draft Risk Assessment of the Public Health Impact of* Salmonella *Enteritidis in Shell Eggs and* Salmonella *spp. in Egg Products.*

As background, in 1996, together with the U.S. Food and Drug Administration we initiated a risk assessment for *S.* Enteritidis in eggs and egg products. Results of the assessment were deemed insufficient for evaluating FSIS risk management options for developing performance standards for both eggs and egg products. Since then, however, data have become available to develop a more robust risk assessment for *S.* Enteritidis in eggs. We also used these data to develop a risk assessment for *Salmonella* spp. in pasteurized liquid egg products. The purpose of these two assessments is to assist FSIS risk managers in evaluating egg handling and pasteurization performance standards for reducing the likelihood of *Salmonella* Enteritidis contamination in shell eggs and *Salmonella* spp. in egg products, and the subsequent risk of human illness, hospitalization, and death.

The risk assessment report consists of an Executive Summary, followed by Hazard Identification, Exposure Assessment, Hazard Characterization, Risk Characterization, and Research Needs. The body of the report is approximately 220 pages in length. In addition to this main report, we are including nine supplemental annexes (Annexes A through I). We have written these annexes to provide a detailed account of the rationale behind the risk assessment model. The annexes include detailed descriptions of the data and the analysis procedures used for determining many of the distributions and values of model parameters.

Please keep in mind that this report is being simultaneously reviewed by multiple scientific experts. We recognize that it is not reasonable to expect a lone individual to provide an in-depth critique on all aspects of the report. Few individuals possess expertise

in each of the individual scientific disciplines, including microbiology, food processing, public health, mathematical modeling, statistics, etc., which were required to conduct the assessments and assemble the report. To that end, please focus on those aspects of the report that fall under your area(s) of expertise.

(a) Specifically, you were identified as a potential reviewer for your expertise in issues related to *microbiology*, *salmonellae*, and *food safety*. Thus, although additional informed comments are always appreciated, you need not worry about reviewing the risk assessment model. Rather, please focus on questions such as: 1) Is the report clearly written? 2) Does it follow a logical structure and layout? 3) Does the background information sufficiently and accurately capture the current state of knowledge regarding *Salmonella* and egg safety? 4) Have all of the assumptions used in developing the assessments been clearly stated? 5) If so, is the rationale for these assumptions valid? Answers to these and similar questions will provide us valuable feedback as we revise the report.

(b) Specifically, you were identified as a potential reviewer for your expertise in issues related to modeling. Thus, although additional informed comments are always appreciated, you need not worry about reviewing the microbiological aspects of the report. Rather, please focus on questions such as: 1) Have the assumptions been appropriately modeled? 2) Does the model follow a logical structure and layout? 3) Are there programming errors within the model? 4) Are there ways to optimize the model? Answers to these and similar questions will provide us valuable feedback as we revise the report.

Again, on behalf of my colleagues, please accept our sincere thanks for agreeing to review this report. We look forward to receiving your comments.

Sincerely,

Carl M. Schroeder, Ph.D.
Risk Analyst