Final Report

External Letter Peer Review of FDA's

Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)

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I. INTRODUCTION

Versar Global Solutions (Versar), an independent Food and Drug Administration (FDA) contractor, coordinated an external letter peer review of the *Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)* report. The peer review was conducted for FDA's Center for Food Safety and Applied Nutrition (CFSAN).

Versar conducted an independent search for scientific experts with expertise that included Microbiology, Risk Assessment/Modeling, Food Science, and Produce Agriculture. As a result of this search, Versar identified and contacted eight experts. Of these, Versar received five positive responses expressing interest and availability to participate. The remaining three experts were not interested or available during the peer review timeframe. For each interested and available peer reviewer, Versar evaluated their qualifications and conducted conflict of interest (COI) screening to ensure that the experts had no COI.

Peer Reviewers:

Orlo (Bob) Ehart, MS
National Association of State Departments of Agriculture (NASDA)

Mr. Ehart has extensive experience in the food and agriculture sector and is respected nationally for his knowledge and expertise in this subject area. He is currently the Senior Policy and Science Advisor for NASDA where he started as the Animal and Plant Health Safeguarding Coordinator. Mr. Ehart has experience in agriculture science, regulatory science, and food and agriculture policy and communications.

Kostas Koutsoumanis, PhD
Aristotle University of Thessaloniki, Greece

Kostas Koutsoumanis is currently serving as a Professor, Head of Laboratory of Food Microbiology and Hygiene and Head of the Department of Food Science and Technology in Aristotle University of Thessaloniki, Greece. He received his B.S. degree in Agriculture Engineering from the Agricultural University of Athens, Greece, in 1997 and Ph.D. (Food Science) degree from the same University in 2000. After serving as a Research Associate in the Department of Animal Sciences at Colorado State University he took a Lecturer position in the Department of Food Science and Technology at Aristotle University of Thessaloniki in 2002 and promoted to Assistant Professor in 2007, Associate Professor in 2013 and Professor in 2017.

Jade Mitchell, PhD
Michigan State University

Jade Mitchell is an Assistant Professor in the Department of Biosystems and Agricultural Engineering at Michigan State University. She received a PhD degree in Environmental Engineering and MS in Civil Engineering from Drexel University. She also holds a BS (Civil and Environmental Engineering) from the University of Pittsburgh. Her work includes risk prioritization for chemical and food safety as well as bioterrorism response.

Fernando Perez Rodriguez, PhD
University of Córdoba, Spain

Fernando Perez Rodriguez undertook his degrees in Biological Science and in Food Science and Technology from the University of Córdoba (UCO) in 1999 and 2002, respectively. He completed his PhD in UCO (2007), which dealt with quantitative microbiological risk assessment and cross contamination in foods. He has published over 100 peer reviewed papers concerning predictive microbiology, quantitative risk assessment and food modelling.

Donald W. Schaffner, PhD
Rutgers University

Donald W. Schaffner is the Department Chair, a Distinguished Professor at Rutgers University, and Extension Specialist in Food Science. His research interests include quantitative microbial risk assessment, predictive food microbiology, handwashing and cross-contamination. He has authored more than 190 peer-reviewed publications, and numerous book chapters and abstracts. He holds a B.S. in Food Science from Cornell University and a MS and PhD in Food Science and Technology from the University of Georgia.

II. CHARGE TO REVIEWERS

Introduction

Biological soil amendments of animal origin (BSAAO) are a potential source of contamination of produce with pathogens that can cause human illness. Some produce farms use untreated BSAAO. On November 27, 2015, FDA published a final Produce Safety Rule entitled "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption," (80 FR 74354), which is codified at 21 CFR part 112. FDA reserved one of the provisions in the final rule's Subpart F (Biological Soil Amendments of Animal Origin and Human Waste) for potentially setting a quantitative application interval standard and anticipated locating such a future standard in that provision. As finalized, the Produce Safety Rule establishes that there is no minimum application interval required when untreated BSAAO are applied in a manner that does not contact covered produce during or after application (§ 112.56(a)(1)(ii)), and the minimum application interval is [reserved] when applied in a manner that does not contact produce during application and minimizes the potential for contact with produce after application (§ 112.56(a)(1)(i)).

This risk assessment is being developed to inform policy decisions regarding produce safety, including the reserved provision in the Produce Safety Rule. The examination of current science, development of a predictive model, and modeling results provided by this risk assessment are among the tools that the FDA will use to evaluate current and potential new policies, programs, and/or mandatory or voluntary practices designed to minimize the risk of human illness associated with the consumption of produce grown in growing areas amended with untreated BSAAO that are potentially contaminated with enteric pathogens such as *Escherichia coli* O157:H7, non-O157 Shiga-toxin producing *E. coli* (STEC), or *Salmonella.*

Charge:

Conduct a quantitative risk assessment to evaluate the risk of human illness associated with produce from growing areas amended with untreated BSAAO that are potentially contaminated with enteric pathogens such as *E. coli* O157:H7, non-O157 STECs, or *Salmonella* and the impact of a time interval between application of untreated BSAAO and crop harvest, on the predicted risk.

This risk assessment will take into account available data and information on relevant steps in the produce food safety continuum including:

- field specifications and agricultural timeline
- prevalence and concentration of pathogens in untreated BSAAO
- manure application and initial contamination condition in amended soils
- pathogen survival (and growth) in soils amended with untreated BSAAO as impacted by different agricultural and ecological conditions
- transfer of pathogens from amended soils to produce crops
- pathogen survival on produce crops grown in the field
- cross-contamination during produce processing (e.g., washing)
- pathogen survival and growth during storage and transportation
- consumption

- dose-response and risk characterization

The risk assessment will focus on lettuce as the case study for produce that grows above the ground. The risk assessment will modify the model developed for lettuce to evaluate the risk associated with produce grown in the ground or on the ground and the impact time intervals may have, using onions and cantaloupe as case studies.

The FDA will consult with USDA on the design of field experiments, including collection and analyses of data, needed to fill data gaps to inform this risk assessment.

Charge Questions for Peer Reviewers:

1. Given the Risk Assessment Charge provided above, are there aspects of the Risk Assessment Charge not addressed in the risk assessment report? If so, what specific aspect(s) of the charge remain to be addressed?
2. The risk assessment model contains modules that describe and characterize specific aspects and processes in the farm-to-table-to-illness continuum. These are described in the Methods section of the document.
   2.1 Have we adequately described our modeling approach, data included, and mathematical/computational details for each module and the overarching model? If not, what additional information should we provide?
   2.2 In developing the quantitative model, we collected, reviewed, analyzed, and included relevant data, as appropriate, for various modules including initial prevalence and levels in untreated manure, pathogen survival in amended soils, pathogen transfer from soils to produce crops, and pathogen survival on produce crops grown in the fields. In a number of cases, FDA commissioned studies specifically designed to fill data gaps and these data were used to develop the relevant modules in the model.
      2.2.1 Are any of the data used not appropriate for any of the modules? If so, please explain which data should not be included and explain your reasoning.
      2.2.2 Are there data not yet used but that should be considered? If so, please provide reference to the data and explain why the additional data might enhance the specific modules of the risk assessment.
   2.3 Are the modeling approaches, methods, and assumptions we used for the model modules and overarching model appropriate for the purpose of this risk assessment? If not, please explain your reasoning and provide alternatives for FDA to consider. Please be specific and provide references, as appropriate.
      2.3.1 The study uses the estimated risk associated with application of treated BSAAO (compost) as a reference for comparison. Is application of treated BSAAO (compost) appropriate as a baseline for comparison? If not, what alternative baseline would you suggest FDA to consider?
      2.3.2 The dose-response relationship of Shiga-toxin producing *Escherichia coli* (STEC) non-O157 strains is not well understood. Given the lack of specific data, the model assumed that the dose-response relationship of STEC non-O157 is the same as STEC O157 strains. Is this choice appropriate given the information available? If not, what alternative dose-response relationship or adjustment to the STEC non-

O157 dose-response relationship would you recommend FDA consider using? Please explain your reasoning and provide appropriate references.

3. We developed a set of overarching scenarios to address the risk assessment charge. Are there additional scenarios we should include to address the risk assessment charge? If yes, please describe those scenarios.
4. We ran a large number of scenarios to address the charge and present results graphically and in tables. Are there additional or alternative strategies you think we should utilize to better communicate the risk assessment results? Specifically, was the impact of different time intervals on the predicted risk for different scenarios as shown in Fig. 4 to Fig. 7 clearly presented?
5. We examined alternative distributions and models as part of our sensitivity analysis, including initial contamination conditions (i.e., prevalence and concentration of pathogens) in untreated bovine or poultry manure, survival of pathogens in amended soils, and survival rates of pathogens on produce crops grown amended soils. Are there additional alternative scenarios we should include as part of our sensitivity analysis? If yes, please explain your reasoning and provide details on scenarios for FDA to consider.
6. Are there key findings and conclusions that we present in the report not supported by the data used and outputs generated by the risk assessment? If so, please explain which findings and conclusions should be revised, and what alternative findings and conclusions should be considered.
7. Do you have any additional comments? Please share them in your review.

III. INDIVIDUAL REVIEWER COMMENTS

A. Reviewer #1

Comments on FDA's *Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)*

Reviewer #1

I. RESPONSE TO CHARGE QUESTIONS

1. ***Given the Risk Assessment Charge provided, are there aspects of the Risk Assessment Charge not addressed in the risk assessment? If so, what specific aspect(s) of the charge remain to be addressed?***

   The draft risk assessment addresses the outlined topics detailed in the Charge.

2. ***The risk assessment model contains modules that describe and characterize specific aspects and processes in the farm-to-table-to-illness continuum. These are described in the Methods section of the document.***

   2.1 Have we adequately described our modeling approach, data included, and mathematical/computational details for each module and the overarching model? If not, what additional information should we provide?

   The Methods section addresses the intended approach.

   2.2 In developing the quantitative model, we collected, reviewed, analyzed, and included relevant data, as appropriate, for various modules including initial prevalence and levels in untreated manure, pathogen survival in amended soils, pathogen transfer from soils to produce crops, and pathogen survival on produce crops grown in the fields. In a number of cases, FDA commissioned studies specifically designed to fill data gaps and these data were used to develop the relevant modules in the model.

   2.2.1 Are any of the data used not appropriate for any of the modules? If so, please explain which data should not be included and explain your reasoning.

   The data seems appropriate. The commissioned studies fill some of the gaps.

   2.2.2 Are there data not yet used but that should be considered? If so, please provide reference to the data and explain why the additional data might enhance the specific modules of the risk assessment.

   Gaps in knowledge certainly still exist; however, I am not aware of published studies that should be included in the risk assessment.

   2.3 Are the modeling approaches, methods, and assumptions we used for the model modules and overarching model appropriate for the purpose of this risk

assessment? If not, please explain your reasoning and provide alternatives for FDA to consider. Please be specific and provide references, as appropriate.

I believe the modeling approaches, methods and assumptions are appropriate. Other reviewers are better qualified to provide more specific comments.

2.3.1 The study uses the estimated risk associated with application of treated BSAAO (compost) as a reference for comparison. Is application of treated BSAAO (compost) appropriate as a baseline for comparison? If not, what alternative baseline would you suggest FDA to consider?

Treated BSAAO is a reference for comparison. No BSAAO application might also be a potential useful reference.

2.3.2 The dose-response relationship of Shiga-toxin producing Escherichia coli (STEC) non-O157 strains is not well understood. Given the lack of specific data, the model assumed that the dose-response relationship of STEC non-O157 is the same as STEC O157 strains. Is this choice appropriate given the information available? If not, what alternative dose-response relationship or adjustment to the STEC non-O157 dose-response relationship would you recommend FDA consider using? Please explain your reasoning and provide appropriate references.

This area seems ripe for additional research. STEC, whether O157 or non-O157, are not alike; some appear to be super-bugs and others not so much. And then there are variables in dose-response. And when more than one E. coli is present, competition is another variable. Since more science is yet unavailable, the explanation of the assumption is necessary and appropriate. To attempt to predict science would not be consistent with this risk assessment.

3. We developed a set of overarching scenarios to address the risk assessment charge. Are there additional scenarios we should include to address the risk assessment charge? If yes, please describe those scenarios.

I am a huge supporter of using scenarios to explain, predict or better understand the circumstances in which the known scientific knowledge is being applied to a situation. As a result, more scenarios can always be justified.

4. We ran a large number of scenarios to address the charge and present results graphically and in tables. Are there additional or alternative strategies you think we should utilize to better communicate the risk assessment results? Specifically, was the impact of different time intervals on the predicted risk for different scenarios as shown in Fig. 4 to Fig. 7 clearly presented?

From a scientific perspective, the figures and tables are clear and provide useful communication of your efforts to explain the scenarios. I assume the risk assessment will

be used by the FDA when/as it develops the requirements for biological soil amendments of animal origin, Subpart F of the PSR.

5. ***We examined alternative distributions and models as part of our sensitivity analysis, including initial contamination conditions (i.e., prevalence and concentration of pathogens) in untreated bovine or poultry manure, survival of pathogens in amended soils, and survival rates of pathogens on produce crops grown amended soils. Are there additional alternative scenarios we should include as part of our sensitivity analysis? If yes, please explain your reasoning and provide details on scenarios for FDA to consider.***

   I appreciated the inclusion of prevalence and concentration in the analysis, as this will be a variable in real farming conditions.

6. ***Are there key findings and conclusions that we present in the report not supported by the data used and outputs generated by the risk assessment? If so, please explain which findings and conclusions should be revised, and what alternative findings and conclusions should be considered.***

   The paper follows the outline/charge developed for this risk assessment and explains the overall project.

7. ***Do you have any additional comments? Please share them in your review.***

   I commend FDA for this effort. There is important value that it has/will have within scientific circles. It is likely much of what I am mentioning below doesn't modify the risk assessment; however, it may appropriately apply to its use. Is it appropriate for the risk assessment to indicate where/how the risk assessment fits into the greater scheme of developing public policy? For example, the regulated community/communities will likely not immediately grasp where this effort fits in the wider policy efforts of FDA, e. g., the agency will be developing a standard for the use of BSAAOs – treated and untreated – in the growing of covered produce. The standard will need to address use of BSAAOs in, for example, above ground crops; in ground crops; on ground crops; crops grown on plastic, and without; crops grown with irrigation, without irrigation; type of irrigation used; crops grown with ag water, without ag water. It further will address whether or when the presence of organisms of public health significance is acceptable. If the decision is that these thoughts address issues beyond the scope of this risk assessment, I would like to propose that FDA consider creating a separate document describing/summarizing the risk assessment created for farmers/regulated communities so that its relevance and future use is understood.

   I have a few additional thoughts about some additions that I think are and/or would be helpful. First, the regional approach helps recognize differences in growing conditions, which is helpful. The risk assessment references many scientific studies, as it obviously should and is greatly appreciated; however, I did not have access to these papers, nor the time to cross-reference any studies – not a criticism, just merely a fact. Some of my comments may have been answered had I also reviewed the referenced studies. In

addition, I may have missed an adequate description that already exists in the risk assessment; I apologize if that is the case.

I couldn't always tell – sometimes it seems the studies focused on presence or absence of pathogens – some studies have reported specific levels that are present. Either are fine; however, mere presence of a pathogen may – or may not – constitute a risk.

In the "summary" – it states: The virtual produce production systems in the model were created with specifications and agricultural practices reflecting typical commercial produce farm operations in the US. I would prefer "…reflecting **some** typical commercial produce farm…" (*Emphasis added only for clarity*)

Data from actual production? I don't believe any studies are from actual production – most research in this area isn't; however, where possible – and supported by in-farm field research – would bolster the value of the risk assessment.

The following thoughts are comments that might improve the understanding of the science behind the risk assessment or are comments related to but perhaps not included in this risk assessment, e. g., the following thoughts may be known to the authors of the risk assessment but were not included – may not need to be included.

Several terms are used or reported in the risk assessment that I would like to have defined/further discussed in the risk assessment, e. g., is there universal use and understanding of the terms used? It isn't required that the terms mean the same thing in all instances; however, knowing there are/may be differences is useful. Some include:

- amended soil – (how so? – incorporated in the same manner? how much material was added to the soil – e. g., a uniform amount/variable amounts, depending on the study?)
- whole lettuce – (commercial production removes outer leaves – in the research were outer leaves removed?),
- fresh cut processing – (how so? – e. g., were research activities consistent with industry practices?)
- irrigation/rainwater – (what type? – furrow, drip, micro, seepage, subsurface, center pivot, sprinkler, terraced, smart…– on plastic – on incorporated soil – simulated rain – natural rain – if included in any study, what characteristics – how much? How long of a rain event? – were the irrigation practices the same in all studies referenced in the risk assessment? They don't need to be; however, the specifics may inform the science.)
- transfer/splash – (under what conditions? The risk assessment mentions one study where 3 m was the splash distance – and an alternative of 4 m was also considered. There are several papers that seem to also contain research on splash – were other distances mentioned/researched? Was the crop growing on plastic? Directly on amended soil? How incorporated? At what stage(s) of growth were the plants when splash was measured?)

Do these terms mean the same thing in each of the studies referenced? They don't need to; however, differences should be identified.

Industry practices are not identified; however, some may mitigate risks while others may contribute to some risks, e. g., types of irrigation practices; grazing practices in nut tree "orchards – support another societal goal – reduced uses of pesticides – but untreated BSAAOs are present at harvest. Some organic practices likewise may mitigate and/or contribute to risks of microbial contamination. These practices are not the subject of this risk assessment but may help define risk associated with the use of BSAAOs in produce production.

II. Specific Observations

No comments provided.

B. Reviewer #2

Comments on FDA's *Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)*

Reviewer #2

I. RESPONSE TO CHARGE QUESTIONS

1. ***Given the Risk Assessment Charge provided, are there aspects of the Risk Assessment Charge not addressed in the risk assessment? If so, what specific aspect(s) of the charge remain to be addressed?***

   All the relevant aspects of the Risk Assessment Charge are properly addressed. However, pathogen internalization has been reported in lettuce (https://doi.org/10.1111%2F1751-7915.12596). Given that the phenomenon could affect survival in plant, processing steps and subsequent growth, this factor should be discussed and the related modeling assumptions, justified.

2. ***The risk assessment model contains modules that describe and characterize specific aspects and processes in the farm-to-table-to-illness continuum. These are described in the Methods section of the document.***

   2.1 Have we adequately described our modeling approach, data included, and mathematical/computational details for each module and the overarching model? If not, what additional information should we provide?

   Specific modules should be better described. The assumptions for using different distributions or how distribution parameters were derived or calculated should be clarified. Some other details are missing. For example, a better definition of "transfer probability", "pathogen transfer coefficient" and "transfer coefficient", and how the distance was considered in Equation 5. The number of lettuces in a radius of 3 m should be provided.

   Section 2.6 presents how data were collected, and which studies were excluded to estimate the die-off rate, but there is no detail on the way this was modeled (log-linear approach, etc.) and integrated with the previous step. A table with the empirical distributions or the main statistics should be provided. Only one study is used for *Salmonella*. This implies that the distribution could be solely based on those data. In this case, the distribution represents something different from what is defined in *E. coli*, in which empirical distributions were built on various data sets. This is an very important source of uncertainty and it should be clearly noted in the corresponding table.

   Although processing and transport-consumption modules are based on previous FDA works and models, it is not clear whether parameters are defined with the same value or modified with new data. For example, for b and Tmin for growth. The document should be standalone and provide this information for the different models and microorganisms (in Appendix).

Equations 11 and 12 should be written as ODE (with the suitable mathematical annotation) and solved properly as such using a suitable algorithm.

For the sake of understanding the product distribution on the fields for the onion and cantaloupe scenarios, a figure could be provided similar to Fig 1 elaborated for lettuce.

2.2 In developing the quantitative model, we collected, reviewed, analyzed, and included relevant data, as appropriate, for various modules including initial prevalence and levels in untreated manure, pathogen survival in amended soils, pathogen transfer from soils to produce crops, and pathogen survival on produce crops grown in the fields. In a number of cases, FDA commissioned studies specifically designed to fill data gaps and these data were used to develop the relevant modules in the model.

2.2.1 Are any of the data used not appropriate for any of the modules? If so, please explain which data should not be included and explain your reasoning.

In section 2.6, it is mentioned that only one study was used for describing Salmonella die-off on lettuce at the crop. Using only one study could significantly bias results. Probably, adding information from other studies, though with fewer sampling days, could increase representativeness and better define variability and trends

2.2.2 Are there data not yet used but that should be considered? If so, please provide reference to the data and explain why the additional data might enhance the specific modules of the risk assessment.

Robin C. McKellar, Fernando Peréz-Rodríguez, Linda J. Harris, Anne-laure Moyne, Burton Blais, Ed Topp, Greg Bezanson, Susan Bach, Pascal Delaquis, Evaluation of different approaches for modeling Escherichia coli O157:H7 survival on field lettuce, International Journal of Food Microbiology,184,2014,74-85, https://doi.org/10.1016/j.ijfoodmicro.2014.04.026.

2.3 Are the modeling approaches, methods, and assumptions we used for the model modules and overarching model appropriate for the purpose of this risk assessment? If not, please explain your reasoning and provide alternatives for FDA to consider. Please be specific and provide references, as appropriate.

In general, the modelling approaches and assumptions are appropriate to the purpose of the risk assessment. However, some aspects should be better justified and substantiated. The assumption of clustering and its definition in this context is unclear. The overall prevalence is reported (8.1 % for E coli), but there is no description of if this prevalence is per pile or sample. In the document, the explanation seems to exclude the unit “pile” and relies on “sample”. However, in the field, manure could proceed from different piles or only one. This is unclear. For only one pile, if it is negative, the whole field is negative. With different piles, it could vary, some of them could be negative and others positive, and during the application, in the positive ones, there could be some negative portions and therefore some grid units that are not contaminated. Clustering could be happening at two levels (pile and sample). From this perspective, the situation that is

represented by the commissioned work and then translated to the model could be in line with a hierarchical approach (hierarchical betabinomial), modelling different variability levels. In this respect, according to what is explained, if each iteration from the betabinomial represents a field, model could produce several fields (iterations) without contamination, thus removing from the simulation the pile-to-pile variability. This comment does not mean that the approach should be changed, but at least, better described, including a comparison with the model accounting for pile-to-pile variability.

The simulation unit is also an issue. As mentioned above, simulation iterations seem to represent fields, however, there are several inputs, especially in the earlier modules that are referred to other units such as ggrid or Cgrid. One iteration of these inputs refers to only one simulated grid. With this, model variability is being collapsed, and removed from the model output.

Splash effects from irrigation and rainfall are considered, using a default radius (increasing that could represent a more intense rain event), while runoff water from rainfall or extreme weather conditions is not considered. These phenomena are expected to occur more frequently and consequently impact food safety and microbial risk in lettuce and other crops. Unlike splash effects, runoff water could modify the number of contaminated grid units, amplifying the impact of a contaminated pile, even with lower concentrations. Please, consider the following paper as an example of this factor in risk assessment, although the context could be a bit different: 10.1016/j.jenvman.2021.113627

It should be clarified why no modified atmosphere packaging is included in the growth model for both pathogens as lettuce (not processed) is usually presented in this format. In general, the processing and storage steps should be better explained. Maybe a table or general graphical representation could help. The assumption made for simulating transfer to cantaloupes by splash based on the model developed with lettuce data should flag that those transfers and the effect of model variables (e.g. distance) could be different.

2.3.1 The study uses the estimated risk associated with application of treated BSAAO (compost) as a reference for comparison. Is application of treated BSAAO (compost) appropriate as a baseline for comparison? If not, what alternative baseline would you suggest FDA to consider?

According to the risk assessment charge, treated BSAAO could be a suitable baseline, as it is a general practice, and provide a reference value to compare the effect of non-treated BSAAO.

2.3.2 The dose-response relationship of Shiga-toxin producing Escherichia coli (STEC) non-O157 strains is not well understood. Given the lack of specific data, the model assumed that the dose-response relationship of STEC non-O157 is - same as STEC O157 strains. Is this choice appropriate given the information available? If not, what alternative dose-response relationship or adjustment to the STEC non-O157 dose-response relationship would you recommend FDA consider using? Please explain your reasoning and provide appropriate references.

The assumption is a pragmatic approach in the absence of detailed, specific data for non-O157 strains. However, this may not fully capture the nuances and variations between different non-O157 strains. There are previous works that have developed and applied dose-response models for STEC, despite the lack of data. Briefly, a top-down approach has been generally used, by collecting national data for attack rates, STEC contribution and secondary infection rates. This information was later used to adjust existing dose-response models. In some specific cases, *Shigella dysenteriae* and entero-pathogenic *E. coli* (EPEC) dose–response curves were used as starting points to derive STEC-specific dose-response models.

- https://doi.org/10.1080/10807039.2013.862065
- 10.1371/journal.pone.0290182

3. ***We developed a set of overarching scenarios to address the risk assessment charge. Are there additional scenarios we should include to address the risk assessment charge? If yes, please describe those scenarios.***

   The proposed scenarios are relevant to the risk assessment charge and could provide a sufficient benchmark for subsequent decision-making. However, it could be advisable, for updated versions, considering weather condition changes and their impact on microbial survival and cross-contamination in soil and plants. The conclusions on application intervals could be different. Internalization is another important aspect to be considered in potential scenarios to elucidate its contribution to final risk.

4. ***We ran a large number of scenarios to address the charge and present results graphically and in tables. Are there additional or alternative strategies you think we should utilize to better communicate the risk assessment results? Specifically, was the impact of different time intervals on the predicted risk for different scenarios as shown in Fig. 4 to Fig. 7 clearly presented?***

   The graphs (Fig. 4 -7), as presented, are effective means to convey results in this respect. For the sake of clarity and readiness of the document, it would be useful to include a table of scenarios in M&M, and another for uncertainty (as already presented).

5. ***We examined alternative distributions and models as part of our sensitivity analysis, including initial contamination conditions (i.e., prevalence and concentration of pathogens) in untreated bovine or poultry manure, survival of pathogens in amended soils, and survival rates of pathogens on produce crops grown amended soils. Are there additional alternative scenarios we should include as part of our sensitivity analysis? If yes, please explain your reasoning and provide details on scenarios for FDA to consider.***

   As explained in previous comments, assumptions should be detailed, and the arguments and justification presented. Therefore, keeping in line with that, sensitivity analysis to assess the impact of the different modeling approaches should be added to the document, as appendices. This could help readers understand the reasoning behind the chosen modeling strategies and better assess the uncertainty and limitations of the model and model results, which is critical in decision-making.

6. ***Are there key findings and conclusions that we present in the report not supported by the data used and outputs generated by the risk assessment? If so, please explain which findings and conclusions should be revised, and what alternative findings and conclusions should be considered.***

   The major contribution of fresh-cut lettuce to risk by *Salmonella* as compared to whole lettuce should be supported by a deeper analysis of simulated data.

7. ***Do you have any additional comments? Please share them in your review.***

   As a general comment, M&M and model should be better described, and assumptions supported, underlining those aspects most contributing to the model uncertainty. The uncertainty table could be improved by mentioning the expected impact of each source of uncertainty. In some cases, it could be based on the simulations performed with the upper and lower bounds, and others based on a more qualitative assessment (low, medium or high).

II. Specific Observations

Page	Paragraph/Line	Comments
11	Table 2	Please, specify how the empirical distribution was computed
14	Lines 13	Grid is not defined in terms of lettuce head. The adequacy of using -1.4 log CFU as the detection limit (or prevalence) will depend on how much lettuce is in a grid. Make a simple calculation, such as the average (flat rate), it is 20 g/grid (considering 4000 m2 , 20,000 lettuce heads, 400 g/head and 400,000 grids)
16	Equation 5	Cti (per lettuce head) is now the unit in simulation but before was field. It is unclear which is simulating in the model steps: field, lettuce head, etc. If model changes units, it should be reflected in the modeling and simulation set-up: Are the model simulated at different levels (entities)? A general simulation scheme for the QMRA model could help to follow the model logic
90	Line 1	Using the binomial, the probabilities calculated (51.9%, 32.1 %...) do not match those provided in the document.
21	Line 4	d is the consumed *integer* dose of pathogens (CFU) ….
27	Lines 5-6	It is not clear why it is the mid-Atlantic region mentioned here specifically. Is it an example?
	Line 11	Why only plus standard error (why not prediction error). On SE means that it is only covering 88 % values. The reason for considering this instead a more complete representation 3x SE should be added to the explanation.
	Fig. 2	Graphs do not show the expected increase in levels for short application intervals. When new amendment is added to the crop, an increase would be expected. It would be useful to see and assess those peaks.
32	Line 1	Is there any speak due to the application of compost (in short application interval scenarios)?
36	Line 9	Whole lettuce is mentioned in the Introduction and then here, differentiating between whole and fresh-cut. However, in M&M, this is not mentioned at all. How were these two pathways and products modelled? Is the different market/consumption levels/contributions for

Page	Paragraph/Line	Comments
		each considered? Pathogen growth could be also affected by the type of product.
36	Line 16 and followings	The explanation why fresh-cut lettuce had increased Salmonella risk as compared to whole lettuce is unclear, and not conclusive.
90	Line 7	The method for deriving the beta-binomial should be provided. It could explain for the parameter values.
90		The use of MNP adds some extra layer of uncertainty and variability which is not quantified. If so, it should be listed and made clear as a source of uncertainty in the model.

C. Reviewer #3

Comments on FDA's *Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)*

Reviewer #3

I. RESPONSE TO CHARGE QUESTIONS

1. ***Given the Risk Assessment Charge provided, are there aspects of the Risk Assessment Charge not addressed in the risk assessment? If so, what specific aspect(s) of the charge remain to be addressed?***

   None.

2. ***The risk assessment model contains modules that describe and characterize specific aspects and processes in the farm-to-table-to-illness continuum. These are described in the Methods section of the document.***

   2.1 Have we adequately described our modeling approach, data included, and mathematical/computational details for each module and the overarching model? If not, what additional information should we provide?

   Yes, except as noted below.

   2.2 In developing the quantitative model, we collected, reviewed, analyzed, and included relevant data, as appropriate, for various modules including initial prevalence and levels in untreated manure, pathogen survival in amended soils, pathogen transfer from soils to produce crops, and pathogen survival on produce crops grown in the fields. In a number of cases, FDA commissioned studies specifically designed to fill data gaps and these data were used to develop the relevant modules in the model.

   2.2.1 Are any of the data used not appropriate for any of the modules? If so, please explain which data should not be included and explain your reasoning.

   See comments below.

   2.2.2 Are there data not yet used but that should be considered? If so, please provide reference to the data and explain why the additional data might enhance the specific modules of the risk assessment.

   None.

   2.3 Are the modeling approaches, methods, and assumptions we used for the model modules and overarching model appropriate for the purpose of this risk assessment? If not, please explain your reasoning and provide alternatives for FDA to consider. Please be specific and provide references, as appropriate.

   Yes, except as noted below.

2.3.1 The study uses the estimated risk associated with application of treated BSAAO (compost) as a reference for comparison. Is application of treated BSAAO (compost) appropriate as a baseline for comparison? If not, what alternative baseline would you suggest FDA to consider?

Yes.

2.3.2 The dose-response relationship of Shiga-toxin producing Escherichia coli (STEC) non-O157 strains is not well understood. Given the lack of specific data, the model assumed that the dose-response relationship of STEC non-O157 is the same as STEC O157 strains. Is this choice appropriate given the information available? If not, what alternative dose-response relationship or adjustment to the STEC non-O157 dose-response relationship would you recommend FDA consider using? Please explain your reasoning and provide appropriate references.

Yes, this is appropriate, but the authors must still explain the dramatically larger number of non-0157 illnesses predicted, see below.

3. ***We developed a set of overarching scenarios to address the risk assessment charge. Are there additional scenarios we should include to address the risk assessment charge? If yes, please describe those scenarios.***

   No

4. ***We ran a large number of scenarios to address the charge and present results graphically and in tables. Are there additional or alternative strategies you think we should utilize to better communicate the risk assessment results? Specifically, was the impact of different time intervals on the predicted risk for different scenarios as shown in Fig. 4 to Fig. 7 clearly presented?***

   No, except, as noted below.

5. ***We examined alternative distributions and models as part of our sensitivity analysis, including initial contamination conditions (i.e., prevalence and concentration of pathogens) in untreated bovine or poultry manure, survival of pathogens in amended soils, and survival rates of pathogens on produce crops grown amended soils. Are there additional alternative scenarios we should include as part of our sensitivity analysis? If yes, please explain your reasoning and provide details on scenarios for FDA to consider.***

   No.

6. ***Are there key findings and conclusions that we present in the report not supported by the data used and outputs generated by the risk assessment? If so, please explain which findings and conclusions should be revised, and what alternative findings and conclusions should be considered.***

   See comments below.

7. Do you have any additional comments? Please share them in your review.

See comments below.

II. Specific Observations

Page	Paragraph/Line	Comments
6		Content: "FDA is conducting a risk assessment to quantify the potential for human illness" Comment: meaning this risk assessment that I'm reading now?
6		Content: "This risk assessment also considers available data and information on other relevant steps in the produce food safety" Comment: Meaning this risk assessment that I'm reading now?
7		Content: "Modelling components of" Comment: Modeling with two Ls is the European spelling, the American spelling is one L.
8		Content: "Overhead sprinklers are spaced around the field with 10 m between sprinklers." Comment: How common are overhead sprinklers for lettuce irrigation in the United States?
10		Content: "Therefore, separate analyses were performed using data from the three U.S. regions where manure survey studies were conducted: west, south, and Mid-Atlantic." Comment: How common is overhead irrigation in these three regions?
11		Content: "For Mid- Atlantic region, we assumed that STEC O157 levels in this region is the same as the levels in the other two regions, and the distribution of STEC O157 levels for this region was estimated using an empirical distribution (Table 2)." Comment: I don't understand how this sentence can be true given that the mean value in the mid-Atlantic is different than the main value in the other two regions as shown in the table. I also don't understand why the references are different for the mid-Atlantic if the levels were assumed to be the same as the other regions.
13		Content: "Specifically, ambient temperature, precipitation, and soil moisture data for each region were retrieved and used to calculate the environmental variables during the production dates (i.e., from manure application to harvest). Environmental variables used in the survival model are listed in Table 3. " Comment: Given that these data change constantly from season to season and year to year, it is unclear exactly how these data were used, and then applied in model form to make predictions.
14		Content: "Given the irrigation frequency used in the risk assessment (5-7 days), survival data from trials that implemented a weekly irrigation regimen were used for model development. This yielded a total of 6 survival datasets (3 Salmonella strains ×

Page	Paragraph/Line	Comments
		2 soil type). These survival datasets were fitted to the Weibull survival model and parameter values were generated for each Salmonella strain soil type combination (Table 4)." Comment: Given that there are only data for three different strains and those data seem to vary significantly both within and between soil types, how did the authors address survival of other Salmonella strains or other soil types? Or is the risk assessment only valid for these strains and soils?
15		Content: "In the BSAAO risk assessment model, we assumed that pathogen transfer mechanisms for amended soils are the same as those for animal feces in the field and data from the above-mentioned feces transfer studies were used to quantify the number of pathogens transferred from amended soils to crops during irrigation and rainfall events." Comment: given that animal feces in a field are likely to be on the surface of the soil, but soil amendments may be mixed with the soil, how did the authors address this difference?
15		Content: "The overall presence of positive lettuce heads after irrigation is 58% and the average calculated transfer coefficient is 0.035%." Comment: which pathogen? Which region? I don't understand how this sentence connects to the rest of the paragraph.
17		Content: "A thorough literature search was conducted to gather information and data to describe the" Comment: It's not necessary to say "thorough". The authors did a literature search.
19		Content: "estimated using the following equations developed by Baranyi et al. (1995):" Comment: Equation 13 was not developed by Baranyi.
20		Content: "The relationship between temperature and maximum growth rate of pathogens (Eq. 13) was described using the square root model of Ratkowsky et al. (1982), where b is a constant and Tmin is the theoretical minimum temperature for microbial growth. " Comment: Which specific growth models were used or what were the parameters used? If this can be found in other publications, these should be cited.
22		Content: "For the baseline models, number of illnesses was estimated using: (1) a "zero-day" interval between application of BSAAO and harvest (hereafter referred to as application intervals), reflecting the current Produce Safety Rule minimum required interval for treated BSAAO that meets treatment requirements (21 CFR §112.54(b));" Comment: see my comment elsewhere in this document, noting that no grower would apply soil amendments, and harvest the same day because it does not make financial sense.

Page	Paragraph/Line	Comments
22		Content: "2.10.3 Risk associated with STEC non-O157 in untreated bovine manure We also evaluated the risk associated with STEC non-O157 in untreated bovine manure using data from the commissioned studies (Jay-Russel et al., 2018; Gartley et al., 2018; Baker et al., 2019)." Comment: it seems odd to mention this component of the study at this point in the document. It would make much more sense to talk about this when the authors are addressing the other pathogens in section 2.2.
23		Content: "A virtual 1-acre onion field consists of 40 beds with one row of onions per bed spaced 0.05 m apart was created" Comment: there's some weird issues with the tense going on in the sentence. It needs to be read carefully and corrected.
24		Content: "The average calculated transfer rate is 0.2 (range from 0.08 to 0.33) and a triangular distribution derived based on these values was used to describe the proportion of pathogens transferred from the contaminated soil grids onto the onion surface that come in direct contact in the model. " Comment: Unclear what the authors are doing. Are they using a triangular distribution or are they using an average? Where are the data coming from for this distribution?
24		Content: "Given the lack of data to quantitatively assess the influence of post-harvest processes on pathogen contamination on onions, the risk model assumed an 80% to 99% with a mostly likely value of 99% reduction of STEC O157 on onions during post-harvest processes" Comment: Where are the data coming from for this assumption? if there's no data and the authors are just making up these numbers, they need to provide some justification.
24		Content: "A virtual 1-acre cantaloupe field consists of 40 beds with one row of cantaloupe spaced 1 m apart was created" Comment: same weird tense/grammar issue as before
24		Content: "Transfer of pathogens from amended soil to cantaloupes were modeled " Comment: transfer was modeled, not transfer were modeled
25		Content: "Considering that cantaloupes (grow on the ground) generally" Comment: Awkward phrasing in this sentence. Is there a word missing? Are the parentheses misplaced?
25		Content: "we assumed that the transfer rates for cantaloupes are 1/3 of those for onions." Comment: This just seems like a made-up number. What is the justification? Are the authors assuming that 1/3 of a cantaloupe is underground?
25		Content: "Table 5 summarized source of uncertainties and assumptions in the risk assessment"

Page	Paragraph/Line	Comments
		Comment: The tense throughout the document is quite weird. The authors switch from past tense to present tense. The whole document needs a careful edit for uniform tense. Generally, speaking work that was done in a manuscript is presented in the past tense, but if discussing the tables and figures, this is the present tense, hence this should read “Table 5 summarizes…”
25		Content: "Field specification variables and post-harvest model parameters have minimal impact when comparing the risk estimates associated with untreated BSAAO versus treated BSAAO and were therefore excluded from uncertainty analysis." Comment: What is the source of the assumption that these have minimum impact? Can the authors provide a citation?
26		Content: "Field specifications are representative of typical produce growing operations in the U.S." Comment: Given that typical produce growing operations specifications very considerably across the country, I’m not sure how one set of values can be truly representative.
27		Content: "For mid-Atlantic region, an upper bound value of 1.2% (200% of the original value) and a lower bound value of 0.3% (50% of the original value) were used." Comment: why the difference for the mid-Atlantic region?
27		Content: "For initial concentration of other pathogen/region combinations described by empirical distributions in the original model simulations (Table 2)," Comment: again, why is the mid-Atlantic region treated differently? Also, it’s a bit strange to change the order from the previous paragraph. In the previous paragraph mid-Atlantic region is discussed last and in this paragraph it’s discussed first.
28		Content: "impact of using an alternative modeling approach for STEC O157 survival in amended soils. Specifically, we developed Weibull survival models with parameter values derived from the survival data of STEC O157 strains from the greenhouse study by Murphy et al. (2023) and then obtained risk estimates using the developed Weibull STEC O157 survival models." Comment: this would appear to contradict an earlier statement that the authors did not use greenhouse data. This needs to be addressed uniformly and consistently throughout the document.
28		Content: "In uncertainty analysis, we increased the splash radius to 4 m and tested its impact on the predicted risk." Comment: Is the decision to change from 3 m to 4 m arbitrary? What’s the reason for using 4 m here?
28		Content: "Given the lack of studies that meet the criteria for inclusion for Salmonella survival on crops, we loosened the

Page	Paragraph/Line	Comments
		inclusion criteria described in section 2.6 and included survival data from growth chamber/laboratory studies" Comment: It seems a little weird to specify exclusion criteria in one part of the document, and then loosen them in another part. It would be clearer if these criteria or uniform throughout the document.
29		Content: "example, compare to predicted level" Comment: grammar, “compared”.
30		Content: "Fig. 2. Predicted E. coli O157:H7 (left) and Salmonella (right) concentration in amended soils over time with an application interval of: (A) 45-day; (B) 60-day; (C) 90-day; and (D) 120-day." Comment: It seems weird that the E. coli data do not seem to be models but seem to be actual observations while the salmonella data are models. Also, it appears the authors are using a dash redline and a dotted blue line. I think it might improve readability to use solid lines for all data.
31		Content: "E. coli O157:H7*" Comment: The meaning of the * is never defined
31		Content: "Fig. 3 displayed the estimated concentration of STEC O157 and" Comment: again, see earlier comment about tense. This should be figure 3 displays (present tense).
32		Content: "pathogen transfer from splash events (indicated by spikes observed from the curves shown in Fig. 3) and then started declining over time indicating subsequent die-off of pathogens on" Comment: I don’t see any obvious spikes in figure 3. Can the authors indicate which panel and which time correspond to a spike or perhaps indicate with an arrow or other symbol? Also, as relates to my earlier comment since rain events which caused splash will be randomly distributed, how did the authors address the variation expected by these splash events in their modeling?
32		Content: "Larger application intervals" Comment: I think the authors mean longer not larger.
34		Content: "The baseline model estimated an average of 0.004 illnesses" Comment: Obviously, this is not a real number of illnesses. What is the denominator for this number? In other words, how many servings is this based on? Is this an annual risk, etc.?
34		Content: "increased to 0.013 illnesses per field."

Page	Paragraph/Line	Comments
		Comment: Now the authors are making clear that this is illness is per field. Again, it would be useful to remind the reader of how many servings come from a field in this scenario.
34		Content: "Compare to scenarios using a zero-day" Comment: Grammar error. This should be “compared“.
34		Content: "where BSAAO was applied on the same day of harvest," Comment: It is important to point out that no grower would ever apply soil amendments on the same day as harvest. Soil amendments are intended to improve crop yield, and it does not make financial sense to apply them on the day of harvest. I understand why the authors are including this variable in the simulation, but I think this is going to strike any growers that read this document as “odd” at best and “FDA is incompetent” at worst. I think the authors need to do a better job of justifying why this is a baseline scenario even though it would never occur in the real world.
41		Content: "Overall, STEC non-O157 scenarios resulted in higher predicted number of illnesses when compared to the estimates from STEC O157 scenarios." Comment: These data are orders of magnitude higher. This does not make sense if the dose response relationship is the same. Why are the STEC non-O157 scenarios predicting dramatically higher numbers of illnesses?
42		Content: "Table 10. Predicted average number of illnesses associated with STEC non-O157 from consumption of fresh-cut or whole lettuce grown in a field (20,000 lettuce heads) amended with treated or untreated BSAAO. " Comment: These numbers seem wildly different for untreated soil amendments relative to the O157:H7 tables above. These numbers seem more in line with Salmonella data rather than E. coli data from Tables 8 and 9.
51		Content: "The predicted number of illnesses associated with untreated BSAAO varied greatly by region." Comment: Why? Is this primarily due to differences in pathogen prevalence and concentration in soil amendments in different regions? Is it something else? Is it a combination of factors?
52		Content: "reflecting typical commercial produce farm operations in the U.S. " Comment: this is not exactly true. The authors assumed overhead irrigation, which, while it is predominantly used in western states for leafy green production, that’s not true with other regions. More importantly, onion irrigation technique can vary by region. Cantaloupes are almost never irrigated using overhead irrigation. This calls into question the validity of those findings.

D. Reviewer #4

Comments on FDA's *Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)*

Reviewer #4

I. RESPONSE TO CHARGE QUESTIONS

1. ***Given the Risk Assessment Charge provided, are there aspects of the Risk Assessment Charge not addressed in the risk assessment? If so, what specific aspect(s) of the charge remain to be addressed?***

   Each aspect of the risk assessment described in the charge is adequately addressed in the methodology section of the risk assessment.

2. ***The risk assessment model contains modules that describe and characterize specific aspects and processes in the farm-to-table-to-illness continuum. These are described in the Methods section of the document.***

   2.1 Have we adequately described our modeling approach, data included, and mathematical/computational details for each module and the overarching model? If not, what additional information should we provide?

   a. Justification for selection of the method used to treat initial pathogen concentration when significant correlations with prevalence of positive samples exists should be included in the report since other approaches exists.
   b. Variability in concentrations of pathogens in manure samples cited are described in MPN/g for *Salmonella* then reported in Table 2 as CFU/g. While it is possible for them to be equivalent, the unit is method dependent and sometimes differences are related to other factors so the units should be reported so that they are consistent with the study that generated the data. If equivalent in these cases, the report should state that.
   c. Sec. 2.4 Report should specify whether alternative survival models were fit to the *Salmonella* or justify its selection. If alternatives were tested, did the Weibull model have the best fit using an appropriate model fit criterion?
   d. Sec. 2.6 While caution was taken to exclude studies reporting die off rates based on observations less than 10 days, direct use of the rates from such studies should be further analyzed and modeled to determine if a single rate (based on an assumed model form) is appropriate. Biphasic decay is commonly observed and could result in significant differences in the predicted number of remaining pathogens especially after longer periods of time. Further since geographic region which affects temperature and UV exposure vary and affect die-off rates, could the data be categorized and applied for by region like the prevalence rates (e.g. West, South, mid-Atlantic).

   2.2 In developing the quantitative model, we collected, reviewed, analyzed, and included relevant data, as appropriate, for various modules including initial prevalence and levels in untreated manure, pathogen survival in amended soils, pathogen transfer from soils to produce crops, and pathogen survival on produce

crops grown in the fields. In a number of cases, FDA commissioned studies specifically designed to fill data gaps and these data were used to develop the relevant modules in the model.

2.2.1 Are any of the data used not appropriate for any of the modules? If so, please explain which data should not be included and explain your reasoning.

The data used are appropriate for this risk assessment and the related assumptions required about its applicability are reasonable.

2.2.2 Are there data not yet used but that should be considered? If so, please provide reference to the data and explain why the additional data might enhance the specific modules of the risk assessment.

See above.

2.3 Are the modeling approaches, methods, and assumptions we used for the model modules and overarching model appropriate for the purpose of this risk assessment? If not, please explain your reasoning and provide alternatives for FDA to consider. Please be specific and provide references, as appropriate.

The models and approaches utilized are comprehensive and appropriate. FDA could consider the following items which are not formally incorporated in the risk assessments models though the importance of such additions need to be tested through sensitivity analysis:

- Transportation from field to cooling may affect growth that is not accounted for in the models.
- Implications from leafy green outbreaks regionally distant from harvest locations resulting in longer cold storage times may also impact the number of VBNC or cells in persister states. These cells could pose risks but are unaccounted for and not considered in the models utilized.

2.3.1 The study uses the estimated risk associated with application of treated BSAAO (compost) as a reference for comparison. Is application of treated BSAAO (compost) appropriate as a baseline for comparison? If not, what alternative baseline would you suggest FDA to consider?

Yes, application of treated compost is an appropriate baseline for comparison. Pathogen survival after compost treatment is documented. A more conservative alternative baseline would not be appropriate as some risk is inherent due to the nature of the pre-harvest environment.

2.3.2 The dose-response relationship of Shiga-toxin producing Escherichia coli (STEC) non-O157 strains is not well understood. Given the lack of specific data, the model assumed that the dose-response relationship of STEC non-O157 is the same as STEC O157 strains. Is this choice appropriate given the information available? If not, what alternative dose-response relationship or

adjustment to the STEC non-O157 dose-response relationship would you recommend FDA consider using? Please explain your reasoning and provide appropriate references.

Given the uncertainty in the dose-response relationship for non-O157 *STEC*, it is reasonable to use the same model as STEC O157 strains as a conservative estimate of virulence.

3. ***We developed a set of overarching scenarios to address the risk assessment charge. Are there additional scenarios we should include to address the risk assessment charge? If yes, please describe those scenarios.***

   The scenarios address the charge as written. Intervals selected for comparison are reasonable. Additional scenarios for onions and cantaloupe represent reasonable extremes for consideration.

4. ***We ran a large number of scenarios to address the charge and present results graphically and in tables. Are there additional or alternative strategies you think we should utilize to better communicate the risk assessment results? Specifically, was the impact of different time intervals on the predicted risk for different scenarios as shown in Fig. 4 to Fig. 7 clearly presented?***

   Bar graphs containing the relative risks for each time interval are a clear way to convey the results of each set of scenarios. Fig 4 to Fig 7 are clear and communicate the results as reported.

5. ***We examined alternative distributions and models as part of our sensitivity analysis, including initial contamination conditions (i.e., prevalence and concentration of pathogens) in untreated bovine or poultry manure, survival of pathogens in amended soils, and survival rates of pathogens on produce crops grown amended soils. Are there additional alternative scenarios we should include as part of our sensitivity analysis? If yes, please explain your reasoning and provide details on scenarios for FDA to consider.***

   No. The sensitivity analysis seems adequate. As suggested above, further investigation of die-offs rates should be considered for STEC where enough data is available given the long time intervals.

6. ***Are there key findings and conclusions that we present in the report not supported by the data used and outputs generated by the risk assessment? If so, please explain which findings and conclusions should be revised, and what alternative findings and conclusions should be considered.***

   None

7. ***Do you have any additional comments? Please share them in your review.***

   No comments provided.

II. Specific Observations

Page	Paragraph/Line	Comments
12	2nd sentence in section 2.4	Missing the word "model" after predictive.
14	Last sentence/last paragraph	Move the following text to the end of Sec 2.6 rather than describing twice. "If the predicted number of pathogen cells in a field grid falls below 1 CFU (corresponds to approx. -1.4 log CFU/g considering an average amount 25g of manure in a field grid), a Bernoulli process is used to determine if the grid becomes negative due to pathogen die-off based on the predicted number of pathogen cells in the field grid, i.e., Bernoulli(Cgridj), where Cgridj is the number of pathogen cells (<1 CFU) in the field grid j."
17	Last sentence /last paragraph	Start a new paragraph at "A lettuce head…." to describe this process separately.

E. Reviewer #5

Comments on FDA's *Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin (BSAAO)*

Reviewer #5

I. RESPONSE TO CHARGE QUESTIONS

1. ***Given the Risk Assessment Charge provided, are there aspects of the Risk Assessment Charge not addressed in the risk assessment? If so, what specific aspect(s) of the charge remain to be addressed?***

The charge is to "Conduct a quantitative risk assessment to evaluate the risk of human illness associated with produce from growing areas amended with untreated BSAAO that are potentially contaminated with enteric pathogens such as E. coli O157:H7, non-O157 STECs, or Salmonella and the impact of a time interval between application of untreated BSAAO and crop harvest, on the predicted risk". The developed model is mainly based on lettuce. In the R&D section the risk of STEC in onions and Salmonella in cantaloupe is presented although the methodology is not described. In the conclusion section the results on the risk in lettuce are actually generalized to produce. However, the risk of E. coli and Salmonella contamination on lettuce cannot be extrapolated and generalized for the entire food group of produce because different types of produce have varying growth environments, physical structures, and harvesting methods that influence their susceptibility to contamination. Lettuce, which grows close to or directly on the ground, is highly susceptible to direct contact with soil, water, and any pathogens present in untreated manure. Its broad, leafy surface area increases the likelihood of retaining contaminated particles. In contrast, produce such as root vegetables like carrots grow underground, benefiting from some level of natural protection provided by the soil, which can act as a barrier against pathogen transfer. Additionally, fruits that grow above ground, such as tomatoes, have reduced direct exposure to potential soil-borne contaminants and are often less susceptible to direct contact with pathogens in untreated manure. The post-harvest handling, washing, processing, storage consumption etc. also vary widely across different types of produce, further affecting the risk. Therefore, the risks specific to lettuce are shaped by its unique characteristics and cannot be applied broadly to all produce without considering these significant differences.

I understand that assessing the risk for the whole group of produce has certain difficulties but this should be clarified and agreed between the risk managers and risk assessors and reflected in the risk management question (charge)

Another point is that the charge asks for the risk of human illnesses. Considering that the charge asks for consumption to be taken into account, risk should be express in relation to the population (e.g. annual number of illnesses in US). The assessment however provides the predicted average number of illnesses per lettuce field. This is a very "strange" output which makes the risk management decision difficult.

The second part of the question is related to the impact of a time interval between application of untreated BSAAO and crop harvest, on the predicted risk. For this the

assessment provides the relative risk but it is not clear if this is also related to the predicted average number of illnesses per lettuce field.

2. The risk assessment model contains modules that describe and characterize specific aspects and processes in the farm-to-table-to-illness continuum. These are described in the Methods section of the document.

2.1 Have we adequately described our modeling approach, data included, and mathematical/computational details for each module and the overarching model? If not, what additional information should we provide?

The modeling approach, data included, and mathematical/computational details for each module and the overarching model are not adequately described. The methodology section is very general and important information are missing. Even looking at the scripts it is very difficult to understand the methodology and the reasoning behind it. The clarity of the modeling approach and the mathematical/computational details for each module could be enhanced by including comprehensive Tables that list the various model parameters, along with a brief description of each, and the corresponding equations or mathematical details, presented on a module-by-module basis.

The following should be included in the methodology section:

a) The conceptual model (or flow diagram as called in the Pang et al paper) showing the different modules for all produce products included in the assessment (fresh-cut lettuce, whole lettuce, onions, cantaloupe). In Pang et al paper a conceptual model is presented for whole and fresh cut lettuce starting from the incoming lettuce heads (see below)

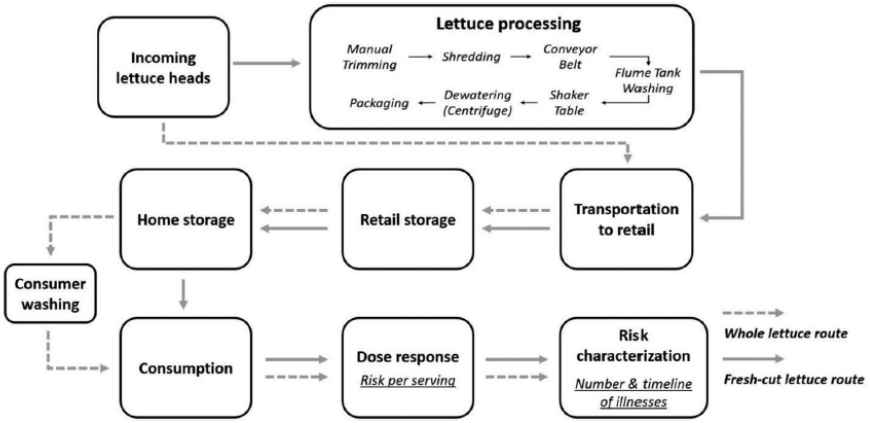

FIGURE 1 Flow diagram of the FDA leafy green quantitative risk assessment- epidemic curve model (FDA-LG QRA-EC). Solid arrows represent the supply chain for whole lettuce; Dashed arrows represent the supply chain for fresh-cut lettuce

The authors should include a similar one including the additional modules at previous stages. Similar graphs should be included for onions and cantaloupe.

b) For each module a Table with all models used, input parameters, their description, units, values and references.

Transparency is very important for regulatory science. The main objective of the methodology section is to assure that anyone can reproduce the assessment.

2.2 In developing the quantitative model, we collected, reviewed, analyzed, and included relevant data, as appropriate, for various modules including initial prevalence and levels in untreated manure, pathogen survival in amended soils, pathogen transfer from soils to produce crops, and pathogen survival on produce crops grown in the fields. In a number of cases, FDA commissioned studies specifically designed to fill data gaps and these data were used to develop the relevant modules in the model.

2.2.1 Are any of the data used not appropriate for any of the modules? If so, please explain which data should not be included and explain your reasoning.

My main concern here is the lack of seasonal dimension in the data and models used. For the assessment the prevalence and concentration of pathogens in untreated BSAOO were described with a distribution (for each region) without taking into account the seasonal effect which we know that can be significant. Following are the results of **Alam and Jurek (2006)** showing the effect of season on E. coli 0157 prevalence in cattle feces.

J. Food Prot., Vol. 69, No. 12 SEASONAL PREVALENCE OF *E. COLI* O157:H7 IN CATTLE FECES

TABLE 1. *Prevalence of* E. coli *O157:H7 in cattle feces during the study period*

Month, 2004–2005	No. of samples tested	No. positive	% prevalence	Mean temp (°C)[a]
August	76	9	11.8	22.1
September	160	12	7.5	21.7
October	160	9	5.6	14.4
November	190	9	4.7	7.1
January	134	12	8.9	−2.3
February	171	31	18.1	3.9
Total	891	82	9.2	NA[b]

[a] Based on a daily average (data are recorded hourly by the K-State Research and Extension Weather Data Station).
[b] NA, not applicable.

The lack of seasonal effect in prevalence and concentration can lead to erroneous risk estimated through various paths including the respective effect of season in the survival of the pathogens in BSAAO on the fields. Indeed, **Sharma at al., (2019)** reported that spatiotemporal factors influence survival durations of E. coli more than amendment type, total amount of E. coli present, organic or conventional soil management, and depth of manure application. Overall, the of the above study data show that spatiotemporal factors like site and season may have more influence than manure type in supporting survival of E. coli beyond 90 days in amended soils in the Mid-Atlantic United States. The following graph of **Sharma at al., (2019)** shows the impact of season.

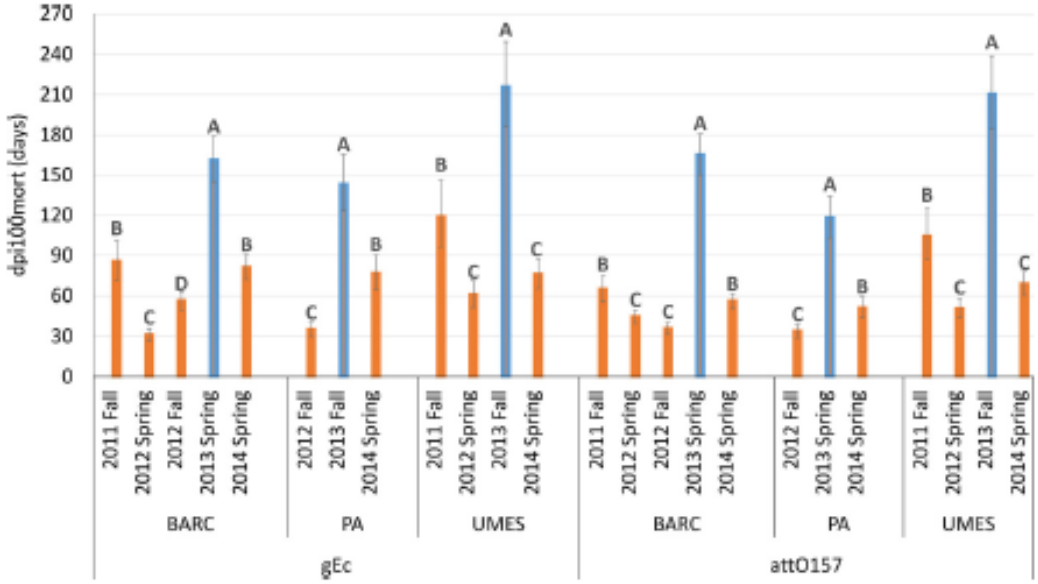

FIG 4 Within each inoculum (gEc or attO157) and site, seasonal dpi100mort values (survival durations) denoted by different capital letters are significantly ($P < 0.0001$) different from each other. dpi100mort values for 2013 seasons (blue bars) at each site had significantly greater dpi100mort values than seasons in other years.

By combining the above Table and graph it is obvious how important the season effect can be.

Furthermore, it not clear if the season dimension was taken into account in the exposure assessment simulating crops growth at different seasons over the year.

Apart from season there are additional risk factors associated with the prevalence of pathogens in manured soils which are not taken into account in the assessment. **Pires et al., 2023** ("Risk factors associated with the prevalence of Shiga-toxin-producing Escherichia coli in manured soils on certified organic farms in four regions of the USA) reported that farm management practices (previous use with livestock, presence of animal feces on the field, season of manure application) soil characteristics, manure application method, snowfall and time-variant predictors (year and sampling day) affected the presence of STEC.

2.2.2 Are there data not yet used but that should be considered? If so, please provide reference to the data and explain why the additional data might enhance the specific modules of the risk assessment.

The assessment completely ignores the variability in the behavior of different strains. However, it is well known that strain variability plays an important role in risk assessment.

Following are some papers providing data on the strain variability in the behavior of Salmonella and E. coli
Strain variability for survival in manure amended spoil
https://journals.asm.org/doi/full/10.1128/aem.00745-11
https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2021.590303/full

https://academic.oup.com/femsec/article/44/3/303/523554
https://globalbiodefense.com/2016/03/21/salmonella-0157-stec-strain-survival-variability-studies/ (project)

Strain variability for growth in food related conditions
https://www.ars.usda.gov/ARSUserFiles/80720500/Poultry/15.pdf
https://www.sciencedirect.com/science/article/pii/S0740002023000242
https://www.sciencedirect.com/science/article/pii/S0740002010000808
https://www.sciencedirect.com/science/article/pii/S0740002014001622
https://link.springer.com/article/10.1023/A:1010087808314

The following paper present an example of a stochastic approach for integrating strain variability in modeling Salmonella enterica growth
https://www.sciencedirect.com/science/article/pii/S0168160511003758

There are also many papers with data on the impact of various factors on the survival of Salmonella and E. coli in manure amended soil which are not addressed in the assessment. Following are only some of them:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC127522/
https://www.sciencedirect.com/science/article/pii/S0362028X22103509
https://www.mdpi.com/2077-0472/11/1/14
https://journals.asm.org/doi/10.1128/aem.01791-08
https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2021.781357/full
https://www.centerforproducesafety.org/amass/documents/researchproject/350/CPS%20Final%20Report%20-%20Warriner.pdf
https://www.frontiersin.org/journals/sustainable-food-systems/articles/10.3389/fsufs.2021.674767/full
https://europepmc.org/article/med/18218027

In general, it seems that assessment did not include a systematic literature review as the starting point for data collection.

2.3 Are the modeling approaches, methods, and assumptions we used for the model modules and overarching model appropriate for the purpose of this risk assessment? If not, please explain your reasoning and provide alternatives for FDA to consider. Please be specific and provide references, as appropriate.

The modeling approaches, methods, and assumptions used for the model modules and overarching model are not fully appropriate for the purpose of this risk assessment. There is a high number of assumptions and simplifications which do not completely encompass the whole complexity of the charge. Following are some weak points of the modelling approach.

a) **Survival model for Salmonella in soil:** The model used for the survival of Salmonella in soil does not take into account the effect of temperature, humidity and precipitation which are the most important factors for the survival. As a result, the predictions of the models on the survival of the pathogen are expected to be very far

from reality. There are many papers on the effect of temperature, humidity and precipitation on Salmonella survival in soil (see 2.2.2) which could be used for the development of the model.

b) **Survival model for E. coli:** I would recommend to assess also the Sharma model (Survival of Escherichia coli in Manure-Amended Soils Is Affected Spatiotemporal, Agricultural, and Weather Factors in the Mid-Atlantic United States which includes more factors compared to the Pang model including the seasonal effect

c) **Growth models:** The authors describe the growth model for E. coli but not for Salmonella, In the E.coli growth model its not clear what value for the Tmin parameter of the secondary model was used. Based on the Pang paper it seems that they used a single value for Tmin based on Koseki study, However, a single value does reflect the strain variability. The use of a distribution for the Tmin parameter is recommended to take into account the strain variability. It is clear also if the authors used the same E. coli Tmin for Salmonella which would not be appropriate. In addition, the Lag phase of the pathogens was not taken into account which can significantly affect the risk estimates. It need to be noted that the stressful conditions in the soil can significantly affect the physiological state of the cell leading a significant lag phase in the product before growth

d) **Temperature conditions during transportation and storage of lettuce:** The Time-temperature profiles for the transportation and storage steps are being outlined but neither the statistical method used to export the temperature distribution, nor the actual final probability distribution are provided. In addition, after checking the scripts it seems the it was assumed that whole lettuce is transported and stored under refrigeration as in the case of fresh-cut lettuce. However, the common practice for whole lettuce is to transported and stored at ambient temperature which can lead to significantly higher growth of the pathogen and thus impact the risk. This also refers to onion and cantaloupe.

e) **Consumption:** The 'consumption' module of the assessment seems underwhelming. A complete 'consumption' module would include the percentage of consumers that prefer to buy and consume whole lettuce in comparison with freshly cut lettuce, the frequency of whole and fresh cut lettuce and it would use these data to express the final result as "illnesses per population". As it currently stands, the model outputs "predicted average number of illnesses per lettuce field", instead of the number of illnesses per total population, which would overall be a more useful metric and actually it is not clear how consumption was incorporated in this calculation.

f) **Uncertainty Analysis:** The uncertainty analysis is the weakest point of the assessment. Based on the above comments it is clear that there many important uncertainty sources that were not taken into account in the assessment. For the rest sources the uncertainty analysis is actually limited to a sensitivity analysis using assumed bounds for the uncertain parameters (Tables 12, 13). However, the overall uncertainty around the outcome originated from all the individual uncertainty sources is not presented. Based on Tables 12 and 13 one could see that the overall uncertainty is expected to be very high. **An overall uncertainty analysis is necessary for the assessment. In addition, considering the second part of the charge the uncertainty analysis should include the comparison between the treated manure and the untreated with the different time intervals** between application and harvest. This is a challenging exercise but very important for the risk management question. A methodology for the above risk ranking is presented in an EFSA opinion

(https://www.efsa.europa.eu/en/efsajournal/pub/393). Following is the part of the opinion which I think is very relevant and applicable to this assessment.

First, a method is presented for comparing the risk associated with two food–pathogen combinations. For example, we have two combinations A and B in presence of uncertainty on the parameters used to assess the associated risk for consumers, which propagate through the model leading to uncertainties in risk estimates. In this case, risk calculations should reflect these uncertainties and so should the ranking. For simplicity of illustration, log-normally distributed uncertainty is assumed to be affecting directly the risks for A and B.

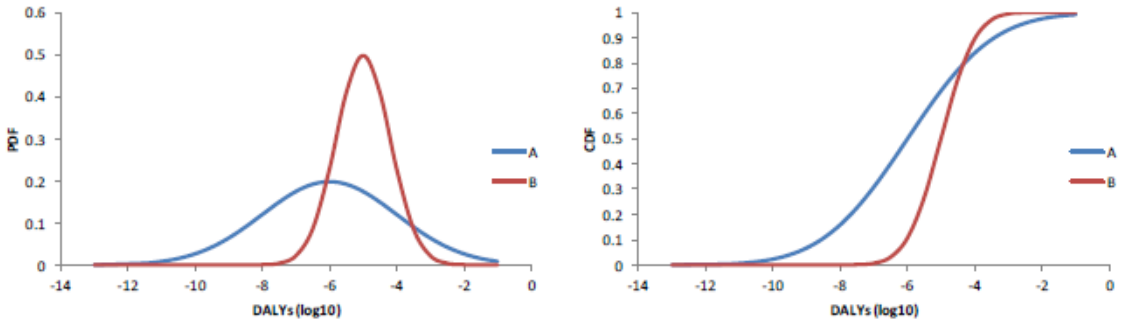

Figure 21: Probability density functions (PDF) and cumulative distribution functions (CDF) of the random variables DALYs for A and B. Above the 75th percentile B > A, below the 75th percentile A > B.

Examining the distributions of the DALYs associated with A and B in Figure 21, distribution A (DA) and distribution B (DB), respectively, one may observe that the DA is much more uncertain than DB but the expected value of DB is greater than DA. On the other hand, there is a range in which the DB percentiles are larger than the DA ones. For example, if one were to perform the ranking based on the DALYs 95th percentile values, the conclusion would be that combination A is more risky than B, contrary to what would happen if the rankings were based on the expected values.

The drawback of comparing the expected values or specific percentiles lies in the loss of information about the distribution. In order to give full account of the difference between the distributions of DA and DB one have to consider the random variable DA–DB whose PDF and CDF are shown in Figure 22.

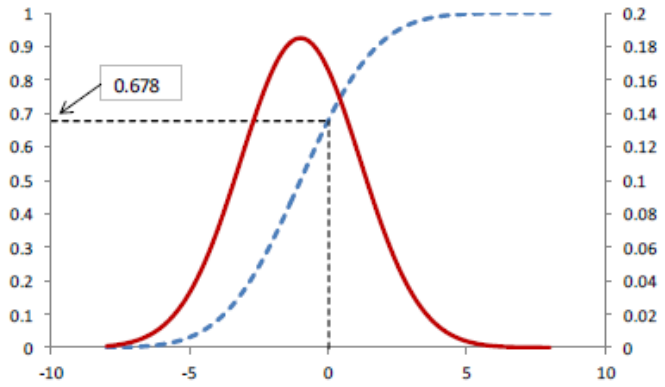

Figure 22: PDF and CDF of the random variable (DA–DB). The probability of DA–DB < 0 is 0.678.

In order to establish whether A is more risky than B, one can consider the probability rAB = 1 – P(DA–DB < 0) (0) that DA is greater than DB; for example, in the present case rAB = 1 – 0.678 = 0.322, which means that, with a probability of 0.322, A is more risky than B. To decide on the relative importance of the two combinations A and B, one may choose a threshold (T) ranging from 0.5 to 1 on the rAB value such that, if rAB is larger than T, then A is more risky than B, otherwise no conclusion can be drawn. Obviously, the lower the threshold, the higher the risk associated with the decision. However, the choice of a simple-valued threshold has some limitations when considering multiple combinations. These limitations can partially be overcome by referring the comparison to a threshold range [Tl, Tu] in such a way that for the two components A and B (Baraldi et al., 2009):

- if rAB > Tu, then A is more risky than B;
- if rAB < Tl, then B is more risky than A;
- if Tl < rAB < Tu, then A is equally risky to B.

To extend the method to systems with a large numbers of components, a procedure for successive ranking must be introduced to avoid the combinatorial explosion of pairwise comparisons using, for example, the Quicksort algorithm (Horae, 1962) implemented by Baraldi et al. (2009). Once the probability distributions have been specified, representative samples are drawn from these distributions using Monte Carlo sampling. The samples are drawn independently, and each sample is generated by drawing independently the value of each parameter.

After the sample of parameters values have been generated, the corresponding model output values are computed. If the computation of the model output is time consuming, this step may be difficult to carry out. In this case, the sample size (N) must be changed to a smaller value because of the computation time.

The last step of the analysis is to summarise the values of obtained outputs. Different quantities can be easily calculated. For example, when the model has a single output variable, estimates of the expected value and variance of can be computed. It is also useful to estimate the quartiles/percentiles associated with the distribution and the probabilities that the output variable is lower than some thresholds. A histogram representation of the output variable values can also provide more information than the summary statistics.

g) **Uncertainty and variability are not separated:** This is also very important for the outcome and requires a second order Monte Carlo simulation.

h) **It is not clear how the seasonal dimension was incorporated in the model.** It is stated in the methodology (section 2.1) that "Time for growth of lettuce plants (between planting and harvest) was assumed to be 45 days". This period can be in different seasons during the year with significantly different conditions (temperature, humidity, precipitation) which can significantly affect the risk. For example what is the percentage of lettuces plants in the different seasons and how did you simulate the seasonal dimension.

2.3.1 The study uses the estimated risk associated with application of treated BSAAO (compost) as a reference for comparison. Is application of treated BSAAO (compost) appropriate as a baseline for comparison? If not, what alternative baseline would you suggest FDA to consider?

The application of treated BSAAO (compost) is considered appropriate as a baseline for comparison for the risk assessment model.

2.3.2 The dose-response relationship of Shiga-toxin producing Escherichia coli (STEC) non-O157 strains is not well understood. Given the lack of specific data, the model assumed that the dose-response relationship of STEC non-O157 is the same as STEC O157 strains. Is this choice appropriate given the information available? If not, what alternative dose-response relationship or adjustment to the STEC non-O157 dose-response relationship would you recommend FDA consider using? Please explain your reasoning and provide appropriate references.

Incorporating different dose-response models in the "dose-response" module to also include differences in strain variability concerning the infectivity of each strain would result in more accurate model predictions as well as decrease the overall model uncertainty around those predictions. See following the approach of Stathas et al, for Salmonella (https://www.sciencedirect.com/science/article/pii/S0963996924000309)

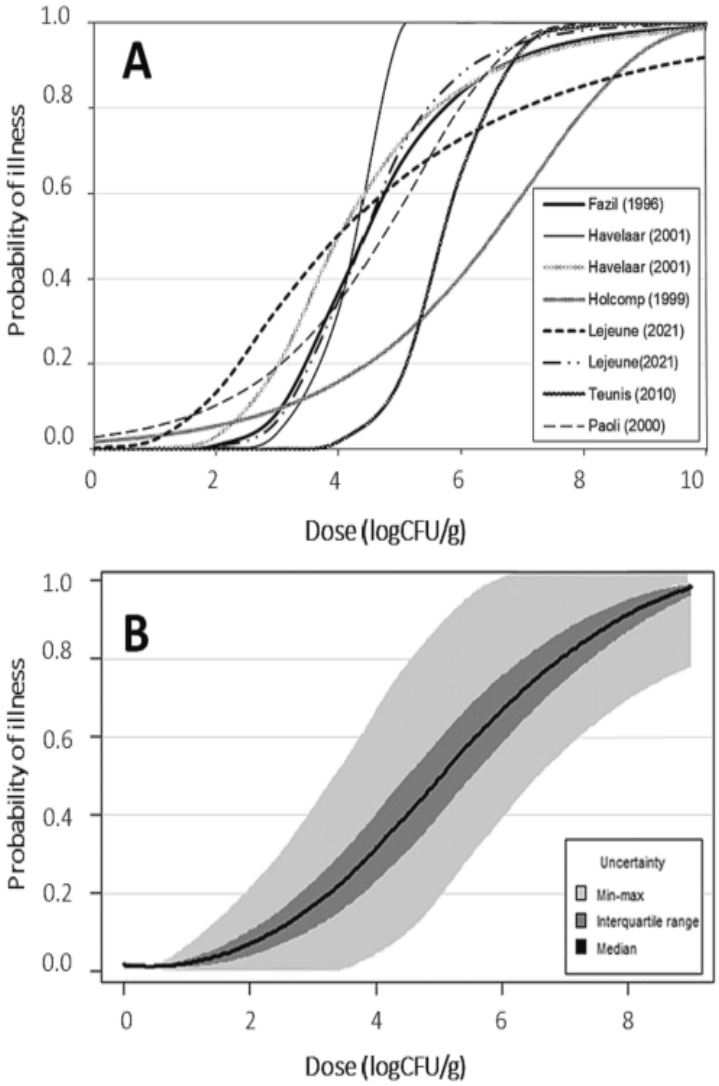

Fig. 2. A: Literature dose-response models for *Salmonella* spp. B: Dose-response model for *Salmonella* spp developed in the present study incorporating eight literature individual models using a Pert distribution for describing uncertainty.

5

3. ***We developed a set of overarching scenarios to address the risk assessment charge. Are there additional scenarios we should include to address the risk assessment charge? If yes, please describe those scenarios.***

   I think it is ok.

4. ***We ran a large number of scenarios to address the charge and present results graphically and in tables. Are there additional or alternative strategies you think we should utilize to better communicate the risk assessment results? Specifically, was the impact of different time intervals on the predicted risk for different scenarios as shown in Fig. 4 to Fig. 7 clearly presented?***

   Fig. 4 to Fig. 7 should include the uncertainty boundary with an indication about the probability of the different rankings (see EFSA opinion above)

5. ***We examined alternative distributions and models as part of our sensitivity analysis, including initial contamination conditions (i.e., prevalence and concentration of pathogens) in untreated bovine or poultry manure, survival of pathogens in amended soils, and survival rates of pathogens on produce crops grown amended soils. Are there additional alternative scenarios we should include as part of our sensitivity analysis? If yes, please explain your reasoning and provide details on scenarios for FDA to consider.***

   The set of overarching scenarios is enough to sufficiently address the risk assessment charge.

6. ***Are there key findings and conclusions that we present in the report not supported by the data used and outputs generated by the risk assessment? If so, please explain which findings and conclusions should be revised, and what alternative findings and conclusions should be considered.***

   There are concerns about all the key findings and conclusions of the assessment mainly due to the incomplete uncertainty analysis.

   For example, a key finding of the assessment is the following conclusion: "For lettuce, the predicted number of illnesses associated with STEC O157 in untreated bovine manure was reduced to below the risk estimates from the baseline models using treated BSAAO when a 120-day interval between application and harvest was used"

   The above conclusion is a very strong statement and can have a significant impact on the decision of the risk managers about a new legislation on the use of BSAAO. Thus it is very important to provide the uncertainty about this statement in a quantitative way.
   So the question should be **how certain you are (expressed with a probability)** that the predicted number of illnesses associated with STEC O157 in untreated bovine manure is reduced to below the risk estimates from the baseline models using treated BSAAO when a 120-day interval between application and harvest was used taking into account **the overall uncertainty of the model**.

   As stated in the conclusion it seems **that the risk assessors are 100% certain** that a 120-day interval between application and harvest for untreated BSAAO will lead to a lower risk compared to treated BSAAO.

   Is this 100% certainty a conclusion of the assessment?
   As described above to assess the level of certainty on this (and the others) conclusion you need to perform an appropriate quantitative analysis of the overall uncertainty (with all sources of uncertainty) taking which will probably require and Expert Knowledge Elicitation (EKE)

7. ***Do you have any additional comments? Please share them in your review.***

   Just a proposal base on my experience in answering risk management questions.

It seems to me that the important management question is actually the time interval between application and harvest for untreated BSAAO leading to an equal or lower risk compared to treated BSAAO.

Considering that the risk assessment modules after the income of the lettuce heads are the same for both treated and untreated BSAAO I think it would be wiser to translate the risk management question to an assessment question which is limited to the impact of the untreated BSAAO to the prevalence and concentration of the pathogens in income lettuce heads in order **to avoid all the uncertainties related to following modules**. In this case, if a tested time interval between application and harvest leads to an equal or lower prevalence and concentration of pathogens in the incoming lettuce heads compared to treated BSAAO then it is certain that the risk will be also lower.

II. Specific Observations

Page	Paragraph/Line	Comments
7	Paragraph 2	This paragraph should include a flow chart of the model that comprehensively summarizes all modules (inputs/outputs) and how each interacts with one another
10	Table 1 Headers	"Overall Prevalence (%)" should be "Mean Overall Prevalence (%)".
11	Table 2	While Table 1 has the distribution and the mean value as separate columns, Table 2 has them as one column. Either one should be selected to be standardized across all tables.
12	3rd line from the end	This should be "The predictive model considered…." instead of "The predictive considered…"
13	Table 3	There should be more descriptors of the environmental variables other than the units. For example, their values, or the equations used to calculate them together with the corresponding references.
14	Equation (4)	Bardsley et al. (2021) noted that throughout their study greenhouse conditions were maintained to replicate temperature and humidity during the spring growing season. This is why temperature is not a parameter of their model. This assumption greatly undermines the significance of seasonality on the field. Especially in the case of lettuce, which can be harvested from late spring through to winter, the temperature difference between seasons is so great that completely change the outcome of the risk. Temperature on the fields not being modelled here is a major constraint of the present risk assessment.
17	2nd line	Since there is an explicit claim that "A thorough literature search was conducted…" the literature search process should be described. Examples of the keywords, inclusion/exclusion.
17-18	Paragraph 2.6	The effects of strain variability should be included in the modelling of pathogen survival on produce crops grown in the field.

Page	Paragraph/Line	Comments
19-20	Paragraph 2.8	Normally, whole lettuce is not stored under refrigeration. Does the 'storage' module of the assessment pertain only to the processed lettuce? Please clarify in text.
19-20	Paragraph 2.8	This paragraph should include a table that comprehensively summarizes all model parameters of the transportation, storage, and consumption modules.
19-20	Paragraph 2.8	The effects of strain variability should be included in the modelling of transportation and storage
20-21	Paragraph 2.9	The effects of strain variability should be included in the modelling of dose-response and risk characterization. There are few mentions of this in page 41, but some information should also be stated here.
23	Paragraph 2.10.4	The conceptual framework of the onion and cantaloupe risk assessment models are not described in sufficient detail.
23	Paragraph 2.10.4	This paragraph should include a table that comprehensively summarizes all model parameters of the different scenarios.
23	Paragraph 2.10.4	This paragraph should include a flow chart of the two models that comprehensively summarize all modules (inputs/outputs) and how each interacts with one another.
25-26	Paragraph 2.11	The uncertainty analysis assigns upper and lower bound to selected input variables and reruns the model to evaluate the impact of such change on risk estimates. This does not provide any information regarding the overall uncertainty of the model.
25-26	Paragraph 2.11	The uncertainty analysis should consider the whole distribution of uncertainty of every variable, not just upper and lower bounds.
25-26	Paragraph 2.11	The distinction between Uncertainty and Variability should be more explicitly defined.
26	Table 5	This Table seems to present the 'assumption mapping' of the model, not the sources of uncertainty. Some of the parameters mentioned in the table are not actual sources of uncertainty, but rather sources for variability (for example: the initial prevalence/concentration of the pathogen).
28	Paragraph 2.11.4 1st and 2nd lines	What are the baseline inclusion/exclusion criteria and which are the alternatives? Does 'lack of studies' correspond to exactly 0 studies or "not enough studies so that a statistical summary could not be derived"?
28	Final line	What mathematical procedure was used to derive this empirical distribution?
27-29	Collectively on paragraphs 2.11.1 – 2.11.4	This paragraph should include a table that comprehensively summarizes all relevant model parameters and the equations used to model each.
34	1st – 3rd lines	Is there any advantage in expressing the model results as "Predicted average number of illnesses per lettuce field"? This is not an intuitive metric, since "probability of illness from consumption of a single dose" and/or "illnesses per population"

Page	Paragraph/Line	Comments
		are most commonly preferred. This comment is also applicable for the result presented in Table 9 and Table 10.
39	Figure 4	Consider adding colors and/or patterns in the bar plots to better differentiate the different scenarios.
40	Figure 5	Consider adding colors and/or patterns in the bar plots to better differentiate the different scenarios.
43	Figure 6	Consider adding colors and/or patterns in the bar plots to better differentiate the different scenarios.
45	Figure 7	Consider adding colors and/or patterns in the bar plots to better differentiate the different scenarios.
46	Before or after paragraph 3.3	Consider adding another paragraph for sensitivity analysis in order to elucidate the most influential parameters of the model. In that case, a tornado chart could also be included to better visualize the results. If the sensitivity analysis includes the model uncertainty, the new paragraph should be placed after paragraph 3.3. Otherwise, it should be placed before 3.3.