U.S. FOOD & DRUG ADMINISTRATION

Human Foods Program

FDA'S Response to Peer Review 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)

February 2026

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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

[*]On October 1, 2024, FDA's Center for Food Safety and Applied Nutrition became the Human Foods Program.

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.

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. SUMMARY OF PEER REVIEWER COMMENTS AND FDA RESPONSE

We have split the original report into two parts, considering the suggestion from one reviewer (see question 7 below for additional details). Part one of the report (hereafter referred to as part 1) focuses on preharvest pathogen exposure assessment and predicts the concentration of pathogens on produce crops at the time of harvest. Part two of the report (hereafter referred to as part 2) extends the risk models in the first report and added postharvest modules and quantifies the potential public health risk (i.e., number of illnesses). A detailed response to each charge question is provided below.

Question 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?

Summary:

Overall, the reviewers agreed that all aspects of the risk assessment charge were adequately addressed, stating "The draft risk assessment addresses the outlined topics detailed in the Charge," "All the relevant aspects of the Risk Assessment Charge are properly addressed," and "Each aspect of the risk assessment described in the charge is adequately addressed in the methodology section of the risk assessment." One reviewer suggested including discussion on the potential of internalization and their impact on the predicted risk. Another reviewer noted that the risk assessment model was primarily based on lettuce and risk estimates cannot be applied broadly to all produce without considering differences post-harvest handling, washing, processing, storage consumption. The reviewer stated that it is not clear what risk estimates were used for calculating relative risk and the use of predicted average number of illnesses per lettuce field as model output needs clarification.

FDA Response:

We thank the reviewers for their comments.

Regarding potential pathogen internalization, we reviewed the Wright et al. (2017) study suggested by the reviewer. While internalization and growth of *E. coli* O157:H7 was observed on model species *Nicotiana benthamiana*, the authors reported lack of bacterial proliferation in the apoplast of edible plants including spinach and lettuce leaves. This finding is also consistent with Erickson et al., 2010 where no internalized *E. coli* O157:H7 was detected in any of the leafy green leaves. Therefore, in the risk assessment, potential internalization of pathogens was not considered, and we focused on pathogen transfer via splash as the route of contamination.

We agree with the reviewer that the risks specific to lettuce cannot be applied broadly to all produce without considering the differences among different types of produce that have varying growth environments, physical structures, harvesting methods, post-harvest processing, and storage conditions. In our risk assessment, to account for the differences among different produce types, we developed separate models for lettuce (sold as whole or fresh-cut), onion, and cantaloupe. Lettuce, onion, and cantaloupe also serve as examples of produce commodities that typically grow above the ground, in the ground, and on the ground, respectively. Using data and

information specific for each commodity, model outputs specific to each commodity were obtained from the three models. We modified the summary and conclusions section to clarify that results and conclusions were specific for the evaluated commodities.

> "The BSAAO risk assessment model and the risk estimates are limited to three example produce commodities (lettuce, onion, and cantaloupe), three pathogen/untreated BSAAO combinations (STEC O157 in untreated bovine manure, STEC non-O157 in untreated bovine manure, and *Salmonella* in untreated poultry manure), and geographically to the U.S."

Regarding the model output, we use mean predicted number of illnesses per field as the output and use it to calculate the relative risk between treated and untreated BSAAO scenarios. The predicted number of illnesses were calculated at the per field level, to ensure a constant unit between the risk estimates from treated and untreated BSAAO scenarios as both scenarios use the same farm specifications and have the same number of produce (i.e., lettuce, onion, and cantaloupe) crops per field. We added additional description in section 2.4.1 of part 2 to clarify:

> "Number of illnesses per lettuce field (20,000 lettuce heads) was calculated as the model output for these application interval scenarios and for the baseline models. The predicted number of illnesses were calculated at the per field level to ensure a constant unit between the risk estimates from untreated BSAAO scenarios and the treated BSAAO baseline models as both scenarios use the same farm specifications and have the same number of lettuce heads per field. Predicted number of illnesses per field from the untreated BSAAO scenarios with different application intervals were compared with results from the baseline models (i.e., treated BSAAO) and relative risks (as the ratio of results from untreated BSAAO scenarios to the baseline models' results) were calculated to provide an estimate of the impact application intervals could have on the estimated risk associated with the consumption of produce from soils amended with untreated BSAAO."

Question 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?

<u>*Summary*</u>*:* The reviewers provided several specific comments on the description of data and modeling approach for the risk assessment model. Specific comments were listed below.

Several reviewers suggested adding tables that list all model input parameters for each module and adding flow charts that show modules of the risk assessment for all produce products included in the model (fresh-cut lettuce, whole lettuce, onions, fresh-cut cantaloupe).

One reviewer suggested adding justifications for selection of method used to describe initial pathogen concentration in manure, clarification on concentration unit for *Salmonella* concentrations in manure, justification for the use of Weibull model for survival of *Salmonella* in amended soils, and justification for the use of die-off rates for pathogen survival on produce crops.

Another reviewer noted that specific modules could be better described to provide additional details such as definitions of transfer probability, transfer coefficient, and how distance was considered in the pathogen transfer model.

FDA Response:

We thank the reviewers for their comments.

We agree with the reviewers that data, modeling approach, and computational details for specific modules could be enhanced and we have revised the methodology sections to improve the clarity of the model. We added two tables that listed all parameters for the processing module (Table 1 in part 1) and the transportation, storage, and consumption modules (Table 2 in part 2) in addition to the existing tables for the pre-harvest modules (Table 2-5 in part 1). We also added the conceptual model framework for lettuce (Fig. 1 in part 1 and part 2), onion (Fig. 3 in part 1 and Fig. 2 part 2), and fresh-cut cantaloupe (Fig. 4 in part 1 and Fig. 3 in part 2) models to show the different modules included in the risk assessment.

Regarding the initial pathogen concentration in manure, we followed the approach described by Jay-Russel et al. (2023). As described in Appendix A in part 1, Jay-Russel et al. (2023) found a significant correlation between concentration of *E. coli* O157:H7 in manure samples and the prevalence of positive manure piles. Based on results from linear regression, a normal distribution was derived where the mean was expressed as a function of prevalence of positive piles. When compared to observation from sampling, predictions from the derived normal distribution were consistent with the enumeration data. Therefore, we followed the same approach and derived normal distributions to describe pathogen concentrations from each of the three regions when a significant correlation was identified between concentration and prevalence. We added additional details for normal distribution derivation in appendix A in part 1:

> "A linear regression analysis was conducted and results indicate a significant ($p < 0.05$) correlation between the concentration and the number of positive in a set of 7 samples: the greater the number of positives, the higher the mean concentration. Based on results from the linear regression, a normal distribution was derived where the mean was expressed as a function of prevalence of positive piles to describe the concentration in a positive pile (log10 MPN/g):
>
> $$\text{Normal}(-0.78+1.74 \times \text{prevalence}, 1.16)$$
>
> Predictions from the derived distribution is generally consistent with the enumeration data, where the median level for STEC O157 is significantly higher in a pile when 4 positive samples were detected than when 3 positive samples were detected. Similarly, the median level for STEC O157 is significantly higher in a pile when 7 positive samples were detected than when 4, 3 or 1 positive sample were detected."

Regarding *Salmonella* concentrations, we revised Table 2 in part 1 so that units are now consistent. We also stated that MPN and CFU was used interchangeably in the risk assessment model as suggested.

Regarding *Salmonella* survival model, we fitted the survival data from Bardsley et al. (2021) to log-linear survival model and the Weibull survival model. Goodness of fit was compared between the two models using the Akaike Information Criterion (AIC) values and Weibull model

was chosen given the generally lower AIC values. We added additional descriptions to clarify the model selection:

> "In this BSAAO risk assessment, data from the survival trials conducted by Bardsley et al. (2021) was fitted to log-linear and Weibull survival models. Goodness of fit of the two survival models was compared based on the Akaike Information Criterion (AIC) values. Curve fitting using the Weibull models generally resulted in lower AIC values and was therefore chosen to estimate the survival of *Salmonella* in soils amended with untreated BSAAO."

We also added description to clarify that Bardsley et al. (2021) reported *Salmonella* survival curves following inoculation in amended soils under daily irrigation (12 strains) or weekly irrigation (3 of the 12 strains). We fitted all the survival curves to the Weibull model and used the data to account for strain variability in *Salmonella* survival in amended soils (see additional details in Appendix D in part 1).

Regarding modeling of pathogen survival on crops, based on our literature search, pathogen survival patterns vary greatly among studies. Some survival data sets indicated a log linear die-off pattern while other studies showed more complex patterns such as biphasic die-off or curvature. Given the complexity of survival patterns observed among survival studies, instead of fitting data to a specific form of survival model, we described pathogen survival on crops using the daily die-off rate calculated as: (the initial level on crops - final level at the end of survival trial)/the number of days between the first and last sampling. The simplicity in its form also allows for integration of the crop survival module to other modeling components, especially for connecting to the transfer models that calculate pathogen transfer during irrigation or rainfall events that occur during pathogen survival on crops. We added additional details to section 2.6 of part 1:

> "Survival data retrieved from the literature search showed a variety of survival patterns, including log-linear die-off, biphasic die-off, or curvature. Diversity of STEC O157 survival patterns on contaminated field lettuce was also reported by McKellar et al. (2014). Considering the complexity of survival patterns observed from survival studies, instead of fitting the data to a specific form of survival model, we described pathogen survival on crops using a daily die-off rate (log CFU day^{-1}) calculated as:
>
> $$D_r = \frac{C_{p0} - C_{pl}}{DPI} \qquad \text{Equation (6)}$$
>
> where D_r is the daily die-off rate (log CFU day^{-1}); Cp0 is the initial number of pathogens on crops (log CFU); Cpl is the number of pathogens on crops on the last sampling day (log CFU); DPI is the number of days post initial inoculation. Given its simplicity, using daily die-off rates also allows for integration of the crop survival module to other modeling components, especially for the transfer models that calculates pathogen transfer during irrigation or rainfall events that occurs during pathogen survival on crops."

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.

Summary:

Overall, the reviewers stated that the data used are generally appropriate for this risk assessment. One reviewer noted that using only one study for *Salmonella* die-off on lettuce could bias results and suggested adding information from other studies that didn't meet the inclusion criteria to improve representativeness and better define variability and trends. Another reviewer raised concerns about the lack of seasonal dimension in the data and models used in the risk assessment. Specifically, the reviewer stated influence of season on pathogen presence in manure and on pathogen survival in amended soils should be included and how season dimension was taken into account in model simulations should be provided. The reviewer also noted that additional risk factors associated with presence of pathogens in manured soils are not taken into account in the risk assessment model.

FDA Response:

We thank the reviewers for their comments and for providing references.

Regarding *Salmonella* survival on crops, we agree with the reviewer that die-off rate based on a single study may bias results. In the risk assessment, as described in 2.7.4 in part 1 and 2.5 in part 2, we conducted an uncertainty analysis for *Salmonella* survival on crops to specifically evaluate its potential impact on model outputs. In the uncertainty analysis, we loosened the inclusion criteria so that information and data from additional studies were analyzed and included. Empirical distributions were derived based on retrieved data from the literature following a similar approach to that for STEC O157. Details on additional studies included in the uncertainty analysis are provided in Appendix E in part 1, and uncertainty analysis results are presented in section 3.4 of part 1 and in section 3.2 of part 2. We also added additional descriptions in the first report to clarify that we performed additional analysis to account for the potential uncertainty associated with *Salmonella* survival rate on crops:

> "In the model, daily die-off rates for *Salmonella* were calculated based on data from Islam et al. (2004b). Additional scenarios were conducted to evaluate the uncertainty of *Salmonella* survival on crops (see section 2.8.4 and Appendix E for details)."

We added the seasonal dimension to the risk assessment model as suggested by the reviewer. First, we evaluated the seasonal impact on pathogen presence in manure following the approach described in Jay-Russell et al. (2023). Pathogen prevalence data from all three regions were categorized by season (winter-spring from December to May vs. summer-fall from June to November) based on sample collection date. Then the impact of season on pathogen prevalence in manure was evaluated using Donner and Rao-Scott test. If impact of season was significant, separate distributions were derived to describe prevalence for each season. The following text was added to reflect the changes:

> "We also considered the potential seasonality of pathogen prevalence in untreated BSAAOs. Pathogen prevalence data were categorized by sampling season (winter-spring from December to May *vs.* summer-fall from June to November) based on the date when samples were collected. Then considering the clustering effect, the impact of sampling season on pathogen prevalence in manure was evaluated using Donner and Rao-Scott test. If impact of sampling season on prevalence was significant (Table 1), separate distributions

were derived to describe prevalence for each season. Table 2 summarized the generated distributions that best represent the prevalence of pathogens in manure piles from each region considering seasonality.

Table 1. Impact of sampling season on prevalence of pathogens in untreated BSAAO.

Region	Pathogen	p – values[1]
West	*E. coli* O157:H7	0.036, 0.001
	Salmonella	0.350, 0.356
South	*E. coli* O157:H7	0.183, 0.142
	Salmonella	0.0001, 0.0002
Mid-Atlantic	*E. coli* O157:H7	NA[2]
	Salmonella	0.005, 0.006

[1]p-value for the Donner test and the Rao-Scott test, where $p \leq 0.05$ indicates a significant difference in pathogen prevalence between summer-fall and winter-spring season.
[2]None of the samples were positive for *E. coli* O157:H7

Table 2. Derived distributions for prevalence of pathogens in untreated BSAAO.

Region	Pathogen	BSAAO type	Prevalence	Distribution	Reference
West	*E. coli* O157:H7	Bovine manure	Summer-fall: 23.3% Winter-spring: 9.7%	Summer-fall: Beta(0.070, 0.486) Winter-spring: Beta(0.19, 10.201)	Jay-Russell et al., 2018; Jay-Russell et al., 2025
	Salmonella	Poultry manure	52.9%	Beta(0.231, 0.571)	Jay-Russell et al., 2018; Jay-Russell et al., 2025
South	*E. coli* O157:H7	Bovine manure	56.7%	Beta(0.534, 2.227)	Baker et al., 2019
	Salmonella	Poultry manure	Summer-fall: 55.6% Winter-spring: 14.8%	Summer-fall: Beta(0.546, 1.760) Winter-spring: Beta(0.110, 2.611)	Dunn et al., 2022
Mid-Atlantic	*E. coli* O157:H7	Bovine manure	<0.6%*	NA	Gartley et al., 2018; Litt et al., 2025
	Salmonella	Poultry manure	Summer-fall: 88.2% Winter-spring: 60.0%	Summer-fall: Beta(0.572, 0.346) Winter-spring: Beta(0.284, 0.666)	Gartley et al., 2018; Litt et al., 2025

*Estimation based on 0 positive out of 161 samples."

In addition, we also added:

"Seasonality in concentrations of pathogens in positive manure samples was evaluated and no significant difference was found between concentrations in samples collected from summer-fall season vs. winter-spring season; thus, we used the same concentration distribution for the same region regardless of difference in prevalence."

Secondly, the survival model developed by Pang et al. (2020) was used in our risk

assessment to estimate survival of STEC O157 in amended soils. The Pang et al. (2020) model was developed using the survival data from Sharma et al. (2019) (the study that reviewer suggested to include). As described in section 2.4 of part 1, the Pang et al. (2020) model included a variety of agricultural and environmental factors to predict survival of STEC O157 in amended soils under dynamic conditions using a random forest approach. Therefore, impact of season and other seasonal factors such as temperature, precipitation, and soil moisture were incorporated as model variables (as listed in Table 4 in part 1). During model simulation, environmental data including ambient temperature, precipitation, and soil moisture for each specific region were retrieved and used to calculate the environmental variables during the production dates (i.e., from manure application to harvest) for each simulated season (winter-spring vs. summer-fall). We added additional description in section 2.4 in part 1 to clarify that the survival model was derived using data from the longitudinal study by Sharma et al. (2019) and the survival model incorporated impact of season and other environmental factors as model variables:

> "The survival model used in this risk assessment to estimate the survival of STEC O157 in soils amended with untreated BSAAO adapted the machine learning predictive model by Pang et al. (2020) that was developed based on the longitudinal field survival trial data from Sharma et al. (2019). The predictive model considered the impact of various agricultural and environment variables, such as soil amendments application methods (surface or tillage), amendment type (e.g., dairy or poultry manure), season, ambient temperature, precipitation, and soil moisture content, to predict the concentration of *E. coli* O157:H7 in amended soil over time under dynamic field conditions."

Lastly, to account for the seasonality in pathogen presence in BSAAOs and pathogen survival in amended soils, two produce growing seasons were simulated for each region: (1) summer-fall season where BSAAO application occurs on March 1st; and (2) winter-spring season where BSAAO application occurs on August 1st. During model simulation, pathogen prevalence in manure data and environmental data for pathogen survival specific for each growing season were used to generate outputs specific to each growing season. We added additional description on how season dimension was taken into account in model simulations in section 2.7.2 of part 1:

> "In addition, to account for the potential seasonality in pathogen presence in BSAAOs and pathogen survival in amended soils, two produce growing seasons were simulated for each region: (1) summer fall season where BSAAO application occurs on March 1st; and (2) winter spring season where BSAAO application occurs on August 1st. Specifically, pathogen prevalence/concentration data (as described in Section 2.2) and environmental data for pathogen survival in amended soils (as described in section 2.4) for each growing season were used to generate outputs specific to each growing season."

We also updated/added results from additional model simulations that consider seasonality in the results section.

We thank the reviewer for suggesting additional factors affecting pathogen presence in manure and for providing a reference (Pires et al., 2023). Pires et al. (2023) evaluated the potential impact on STEC non-O157 presence in soil samples post manure application of various farm management practice (e.g., manure type, application method, and soil type) and environmental factors (weather data such as precipitation and temperature). In our risk assessment, we used pathogen prevalence and level data from FDA commissioned manure survey studies (Jay-Russel et al., 2018; Jay-Russell et al, 2023; Gartley et al., 2018; Baker et al.,

2019; Dunn et al., 2022; Litt et al., 2023) which were designed specifically for evaluating pathogen presence in untreated manure. These manure survey studies were conducted in a variety of farms or facilities from three geographic regions with different agricultural practices and environmental conditions. Therefore, collected data was representative of potential pathogen prevalence and levels in manure under a variety of agricultural practices (e.g., geographic locations, farm/facility type, cattle type, and pile type) and environmental conditions (various seasons and weather conditions) similar to those evaluated in the Pires et al. (2023) study. Furthermore, factors similar to those evaluated and identified in Pires et al. (2023) were also considered in our risk assessment model through the use of the machine learning survival model for STEC O157 in amended soils (Pang et al., 2020) that included a variety of environmental and agricultural factors such as temperature, precipitation, soil moisture, amendment type, soil management, and season.

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.

Summary: One reviewer provided a reference on modeling *E. coli* O157:H7 survival on contaminated field lettuce. Another reviewer stated that strain variability was ignored in the risk assessment model and provided several references on strain variability in pathogen behavior.

FDA Response:

We thank the reviewers for their comments and for providing references.

We added the suggested reference for McKellar et al. (2014) on *E. coli* O157:H7 survival on lettuce to section 2.6 of part 1:

> "Survival data retrieved from the literature search showed a variety of survival patterns, including monophasic die-off (log-linear), biphasic die-off, or curvature. Diversity of STEC O157 survival patterns on contaminated field lettuce was also reported by McKellar et al. (2014)."

We do not agree with the reviewer that strain variability was ignored in the risk assessment; however, we recognize that additional data are available on strain variability in the survival of *Salmonella* spp. and STEC in amended soils, and we have revised the pathogen survival module to take into account the additional data.

As described in section 2.4 of part 1, survival of *Salmonella* was described using Weibull models derived from the study by Bardsley et al. (2021), which investigated the die-off of 12 different *Salmonella* strains and characterized the impact of strain, soil-type, and irrigation regimen on *Salmonella* survival. Considering the irrigation frequency used in our risk assessment (5-7 days), we used strain specific survival data from trials that implemented a weekly irrigation regimen for model development. Strains used in trials implementing a weekly irrigation regimen including *Salmonella* Braenderup, *S.* Meleagridis, and *S.* Newport. We added additional descriptions in section 2.4 of part 1 to clarify our considerations of strain variability on *Salmonella* survival:

> "Bardsley et al. (2021) investigated the survival of 12 different strains of *Salmonella* in soils amended with poultry litter and characterized the impact of strain, soil-type, and irrigation regimen on *Salmonella* survival";

and:

"Given the irrigation frequency used in the risk assessment (5-7 days), survival data for three *Salmonella* strains *S*. Braenderup, *S*. Meleagridis, and *S*. Newport that implemented a weekly irrigation regimen were used for model development. *S*. Braenderup, *S*. Meleagridis, and *S*. Newport strains also showed significantly slower die-off rates compared to other strains evaluated in the survival trials (Bardsley et al., 2021). Therefore, to take into account the strain variability in *Salmonella* survival in amended soils, a total of 6 survival datasets (3 *Salmonella* strains × 2 soil type) were used for model development, representing worst-case contamination scenarios."

As described in the response to Question 2.1 above, we added description in section 2.8.2 of part 1 to indicate that we evaluated additional data to more fully account for strain variability in *Salmonella* survival in amended soils.

For STEC O157, we used strain specific data obtained from Murphy et al. (2024) in uncertainty analysis for survival of STEC O157 in amended soils (Section 2.8.2 of part 1). The study by Murphy et al. (2024) investigated survival of 12 *E. coli* strains (including 5 strains for STEC O157, 4 strains for STEC non-O157, and 3 strains for generic *E. coli*). We used survival data specific to the five different STEC O157 strains to derive the Weibull survival models to account for strain variability in STEC O157 survival in amended soils. In addition, as described in section 2.7.3 of part 1, we evaluated the risk associated with STEC non-O157 in untreated BSAAO in model scenario analysis. Survival of STEC non-O157 in amended soils was described using Weibull models with parameter values derived based on data specific to the four STEC non-O157 strains reported by Murphy et al. (2023) following a similar approach described in section 2.4 of part 1. We modified/added additional descriptions in the first report to clarify how strain variability was taken into account in the model:

"Specifically, data specific for four STEC non-O157 strains from the survival trials conducted by Murphy et al. (2024) was retrieve and used to derive Weibull model parameters to describe STEC non-O157 survival in amended soils while accounting for variability."

and:

"In addition, we also tested the impact of using an alternative modeling approach for STEC O157 survival in amended soils while considering the impact of strain variability. Specifically, we developed Weibull survival models with parameter values derived based on specific survival data for five STEC O157 strains from the greenhouse study by Murphy et al. (2024) and then obtained risk estimates using the developed Weibull STEC O157 survival models."

We reviewed the articles suggested by the reviewer on strain variability for survival in manure amended soil. Among the suggested references, the study by Bardsley et al. (2021) was already implemented in our risk assessment model as the data source for *Salmonella* survival model development (as described in section 2.4 of part 1 and in our response above). The study by Topp et al. (2003) monitored survival of two *E. coli* strains for a short period of time as the study focused on "persistence of *E. coli* within a relatively short time frame of a few days or weeks following application to soil." At the end of the trials, observed *E. coli* levels in manured soils were still relatively high and potential long term survival patterns were not well characterized given the length of monitoring. The study by Franz et al. (2011) investigated the variation in manure-amended soil survival capability among 18 STEC O157 strains. The study used a single sandy soil sample with standardized water availability maintained in soil. We

included the survival curves from the 18 strains from Franz et al. (2011) in the uncertainty analysis of our risk assessment. Weibull parameters reported in Franz et al. (2011) were adjusted to account for the potential difference in survival under a weekly irrigation regimen. Weibull parameters based on the adjusted values were used in uncertainty analysis for STEC survival models. We added description on the analysis using Franz et al. (2011) data in section 2.8.2 and in appendix D of part 1.

We also reviewed the references provided by the reviewer on strain variability for growth in food related conditions. The primary objective of our risk assessment was to compare the model outputs between untreated BSAAO vs. treated BSAAO and evaluate the impact of time interval between application and harvest on this comparison. Considering that post-harvest modules (e.g., processing, transportation, and storage) in the risk assessment are the same for both treated and untreated BSAAO scenarios, post-harvest module parameters were excluded from uncertainty analysis due to their limited impact on the comparison between untreated and treated BSAAO risk estimates (as described in section 2.5 of part 2). This was also suggested in a comment later in the review (Reviewer#5 for charge question 7). Therefore, potential strain variabilities in pathogen behavior during post-harvest stages (such as growth during transportation and storage) were not considered in the risk assessment.

We also reviewed the references provided by the reviewer on the survival of pathogens in manure amended soils. While we agree that various factors can affect the survival of STEC O157 and *Salmonella* in amended soils, we do not agree with the reviewer that these factors were not addressed in the risk assessment. The survival model for STEC O157 in amended soils adapted the machine learning survival model developed by Pang et al. (2020) that implemented the longitudinal field study data conducted by Sharma et al. (2019). As described in section 2.4 of part 1, the Pang et al. (2020) model incorporated a wide range of factors as model variables to predict the concentration of STEC O157 in amended soils over time. Variables in the Pang et al. (2020) model includes farm management practice variables (soil management, amendment type, and depth of manure application) and a series of environmental variables such as season, ambient temperature, precipitation, and soil moisture (as shown in Table 4 of part 1). By using the Pang et al. (2020) model, given the comprehensiveness of the underlying survival data on non-pathogenic *E. coli* and virulence-attenuated STEC O157 strains under field conditions from the multi-year longitudinal study by Sharma et al. (2019) and the machine learning models' ability to predict STEC O157 survival in amended soils under dynamic agricultural and environmental conditions, we are confident the potential impact of various factors affecting survival of STEC O157 in amended soils was well addressed in our risk assessment.

Furthermore, we derived alternative Weibull survival models (described in Appendix D of part 1) to represent the variability in the survival of STEC O157 and non-O157 strains using data reported in the greenhouse study by Murphy et al. (2024) that compared the survival of non-pathogenic *E. coli* strains and that of pathogenic STEC strains under the same conditions. We added results in section 3.4 of part 1 and in section 3.3 of part 2 to indicate that the pathogen contamination and risk estimates for application interval of 60, 90, and 120-days are lower based on the alternative Weibull survival model than the random forest survival model (based on data for non-pathogenic *E. coli* strains) is not unexpected because of the faster die-off of pathogenic STEC strains than the non-pathogenic *E. coli* strains observed under greenhouse conditions. For *Salmonella*, we derived survival models based on the greenhouse study by Bardsley et al. (2021). We acknowledge that greenhouse study cannot truly mimic the diversity of field conditions.

However, data from greenhouse studies (Bardsley et al., 2021; Murphy et al., 2024) provide valuable insights into the magnitude of differences in survival between pathogenic and non-pathogenic strains in greenhouse that simulated several key field conditions. this limitation was compensated in Bardley et al. (2020) study by taking into account strain variability, impact of soil type, and impact of irrigation regimen on *Salmonella* survival in amended soils. Therefore, we believe Bardsley et al. (2020) study was appropriate to estimate *Salmonella* survival in amended soils in our risk assessment. As we stated in section 3.3.1 of part 1, future longitudinal field data is needed to further investigate *Salmonella* survival (e.g., by using appropriate non-pathogenic surrogate) under field conditions and to incorporate impact of additional environmental factors.

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.

<u>*Summary*</u>: Reviewers generally indicate that the modeling approaches, methods, and assumptions are appropriate for the purpose of this risk assessment.

One reviewer commented that some aspects can be better justified and substantiated, including clarification on whether pathogen prevalence in manure was at "sample" level or "pile" level, clarification on simulation unit, consideration of runoff, consideration of modified atmosphere packaging, and the assumption made for simulation transfer to cantaloupes by splash based on lettuce data should be stated.

Another reviewer noted that transportation from field to cooling may affect growth and was not accounted for in the models. The reviewer also commented that longer cold storage times may impact the number of VBNC or cells in persister states and the potential impact of these cells on risk was not considered in the models.

A third reviewer stated that modeling approaches, methods, and assumptions are not fully appropriate for several aspects of the model. Specifically, the reviewer noted that survival model for *Salmonella* in amended soils did not consider the effect of temperature, humidity and precipitation, suggested assessing the Sharma et al. (2019) data for modeling of STEC O157 survival in amended soils, suggested clarification on growth model parameters and temperature conditions during transportation/storage, clarification on the use of illness per field as model output, suggested second order Monte Carlo simulation and analyzing the overall model uncertainty, and clarification on how seasonal dimension was incorporated in the model.

<u>FDA Response:</u>

We thank the reviewers for their comments.

Regarding comments on pathogen prevalence in manure, raw data were reported for individual samples (whether the samples were positive, and if positive, at what population size) and the manure pile from which the sample was taken, as described in Appendix A of part 1. After considering the clustering effect, data analysis was conducted at the pile level, and the region-specific prevalence distributions (Table 2 in part 1) were for pile level prevalence. In our model, in each iteration, percentage of contaminated manure being applied onto the field was

described using the pile level prevalence (described by distributions in Table 2 in part 1). As a result, after application of manure, some grids become contaminated while others remain uncontaminated (as determined by equation 1 in part 1), and percentage of contaminated grids across the entire field represents the prevalence of contaminated manure. For example, if pile level prevalence is 18%, then 18% of the manure being applied onto the field is contaminated, and ~18% of grids will be contaminated after application. We added additional description in section 2.2 and 2.3 of part 1 to clarify that pile level prevalence was calculated and pile to pile variability was simulated during model simulation.

Regarding simulation units, simulation iterations represent fields in our risk assessment. Within a field, we break down the field into soil grids to explicitly model pathogen behavior following an agent-based modeling approach. During simulation, each soil grid is assigned contaminated or uncontaminated based on field level prevalence distributions (as described by equation (1) in part 1, and contaminated grids were assigned a concentration level based on region-specific contamination level distribution (Table 3 in part 1). Therefore, one iteration refers to one simulated field where field level variability is represented by all soil grids from the field as a whole.

We evaluated the potential impact of run-off on model outputs as suggested by the reviewer. We considered a scenario where run-off occurred immediately after manure application to the field and run-off spread pathogens in the amended soils evenly to the entire fields. We added descriptions on simulating run-off event in the method section (section 2.7.3 of part 1 and 2.4.2 of part 2) and added results from the analysis in section 3.3.1.2 of part 1 and 3.1.1.3 of part 2.

Reviewers provided several comments on the potential influence of transportation and storage conditions, use of modified atmosphere packaging, presence of VBNC cells, and lag phase during pathogen growth on risk estimates. The primary objective of our risk assessment is to compare the model outputs between untreated BSAAO vs. treated BSAAO as influenced by length of application intervals. To evaluate the impact of application intervals on these comparisons, both untreated BSAAO and treated BSAAO scenarios use the exact same post-harvest modules adapted from previously published risk assessments (Mokhtari et al., 2018; Mokhtari et al., 2021; Pang et al., 2022). Therefore, while we agree with the reviewers that post-harvest factors such as transportation and storage conditions, use of modified atmosphere packaging, presence of VBNC cells, and lag phase during pathogen growth and can affect absolute risk estimates, their influence on the relative risk comparisons between untreated BSAAO and treated BSAAO scenarios is limited as both scenarios have the exact same post-harvest modules. Thus, these abovementioned post-harvest factors were not considered in the risk assessment model to minimize the uncertainties in post-harvest modules as suggested in a comment later in the review (Review #5 for charge questions 7). Instead, we focused on the impact of application intervals on relative risk comparisons between untreated and treated BSAAO.

Regarding comments on the survival model for *Salmonella* in amended soils, we developed Weibull models based on data from the survival trials conducted by Bardsley et al. (2021). Bardsley et al. (2021) invested survival of 12 *Salmonella* strains under greenhouse conditions that mimic the temperature and humidity during produce growing season. In addition to strain variability, the study also invested the impact of irrigation regimen and soil type on *Salmonella* survival in amended soils. While we acknowledge that the greenhouse study cannot truly mimic

the diversity of conditions encountered during field production (e.g., temperature, humidity, and precipitation), this limitation was compensated in Bardley et al. (2020) study by using 12 different *Salmonella* strains and taking into account the impact of soil type and irrigation regimen on *Salmonella* survival in amended soils. Therefore, we believe Bardsley et al. (2020) study was appropriate to estimate *Salmonella* survival in amended soils in our risk assessment. As stated in section 3.3.1 of part 1, we acknowledge that future longitudinal field data is needed to further investigate *Salmonella* survival under field conditions and to incorporate impact of additional environmental factors.

Regarding the comments on survival model for STEC O157 in amended soils, we used the Pang et al. (2020) model that was derived based on Sharma et al. (2019) data that was suggested by the reviewer. Using data from the longitudinal field trials by Sharma et al. (2019), the Pang et al. (2020) model incorporated a wide range of factors as model variables to predict the concentration of STEC O157 in amended soils over time. As shown in Table 4 in part 1, Pang et al. (2020) model considered a wide range of variables including farm management practice variables (soil management, amendment type, and depth of manure application) and a series of environmental variables such as season, ambient temperature, precipitation, and soil moisture. By using the comprehensive survival data under field conditions from the multi-year longitudinal study by Sharma et al. (2019), the Pang et al. (2020) model was able to accurately predict STEC O157 survival in amended soils under dynamic agricultural and environmental conditions. We added additional description in section 2.4 of part 1 to clarify that the STEC O157 survival model in amended soils was derived based on data from Sharma et al. (2019).

We added a table that listed all parameters used in the transportation, storage, and consumption modules as suggested by the reviewers (Table 2 in part 2). The table detailed the definition of the parameters, units, values or distributions used for the parameters, and the references from which these were derived.

As mentioned in our previous response, we calculated the predicted number of illnesses at per field level to ensure a constant unit between the risk estimates from treated and untreated BSAAO scenarios as both scenarios use the same farm specifications and have the same number of produce (i.e., lettuce heads, onions, and cantaloupes) crops per field.

Considering the spatial resolution and complexity of the risk assessment model, the underlying model code was written to be launched on parallelized processors using a high-performance computing cluster (FDA's Human Foods Program HPC support). Even on a high-performance computing cluster, given the numerous modeling scenarios (e.g., pathogen, season, region, application interval, and produce type), simulations took months to complete. Additionally, the number of HPC nodes available for model simulation was heavily limited as we competed with other agency priorities. Therefore, a full scale second order Monte Carto is not practical given the resource availability. To assess model uncertainty, we performed sensitivity analysis to identify the most influential model parameters and focused on the impact of individual model parameters on the model outputs. Following the approach by Pérez-Rodríguez et al. (2017), Pouillot et al. (2020), and Pouillot et al. (2021), selected input variables were assigned an upper bound value or a lower bound value (or both). The model was rerun to evaluate the impact of such changes on model outputs.. In addition, we estimated the overall uncertainty range of the model by using combined upper or lower bound values for each considered model parameter. We also considered uncertainty in the comparison between treated

and untreated BSAAO with different application intervals by adding density plot comparison figures in the results section (Fig. 7 in part 2) as suggested by the reviewer. We added additional descriptions in section 2.8 of part 1 and 2.5 of part 2 to clarify.

As mentioned in our previous response, to account for the seasonality in pathogen presence in BSAAOs and pathogen survival in amended soils, two produce growing seasons were simulated for each region: (1) summer fall season where BSAAO application occurs on March 1st; and (2) winter spring season where BSAAO application occurs on August 1st. During model simulation, pathogen prevalence in manure data and environmental data for pathogen survival specific for each growing season were used to generate outputs specific to each growing season.

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?

Summary: Reviewers agree that using treated BSAAO (compost) as a reference is appropriate.

FDA Response: We thank the reviewers for their comments.

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.

Summary: Overall, reviewers agree that dose-response modeling approach for STEC non-O157 strains were appropriate and pragmatic. Reviewers also suggested references for the approach of integration when data becomes available.

FDA Response: We thank the reviewers for their comments and for providing additional references.

Question 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.

Summary: Reviewers agree that overarching scenarios are adequate for the charge.

FDA Response: We thank the reviewers for their comments.

Question 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?

Summary: Reviewers agree that data presentation is adequate. One reviewer suggested adding uncertainty boundary in the comparison figures.

FDA Response: We thank the reviewers for their comments. We added comparison figures using density distributions (Fig. 7 in part 2).

Question 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.

Summary: The reviewers agreed that our sensitivity analysis is adequate.

FDA Response: We thank the reviewers for their comments.

Question 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.

Summary: One reviewer noted that the observation of higher *Salmonella* risk associated with fresh-cut lettuce compared to whole lettuce should be supported by additional analysis of simulated data. Another reviewer raised concerns about conclusions being stated without expressing certainty/uncertainty associated with the conclusions.

FDA Response:

We thank the reviewers for their comments.

In our risk assessment, we predicted that risk estimates for STEC O157 (lower concentration on lettuce at the time of harvest prior to processing) contaminated fresh-cut lettuce were lower compared to whole lettuce while fresh-cut lettuce resulted in higher risk estimates for *Salmonella* (higher concentration on lettuce prior to processing) compared to whole lettuce. This prediction is consistent with our previous risk assessment study (Mokhtari et al., 2022). The underlying reason for this observation is beyond the scope of this risk assessment to evaluate the impact of application intervals on predicted risk associated with untreated and treated BSAAO. We have modified the section to clarify:

"Compared to fresh-cut lettuce (Table 5), risk estimates for STEC O157 contaminated whole lettuce (Table 4) are higher across all different application intervals. On the contrary, risk estimates for *Salmonella* contaminated whole lettuce (Table 6) are generally lower compared to the results for *Salmonella* contaminated fresh-cut lettuce (Table 5). This observation is consistent with our previous study that found fresh-cut processing resulted in

increased risk predictions when lettuce contamination was low prior to processing whereas fresh-cut processing reduced the predicted risk for lettuce heads that were contaminated at higher levels prior to processing (Mokhtari et al., 2022)."

Question 7

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

Summary: One reviewer mentioned that the methods section should be better described and the uncertainty table could include the expected impact of each source. Another reviewer suggested focusing on the uncertainties in the pre-harvest modules as both untreated BSAAO and treated BSAAO scenarios use the exact same post-harvest modules.

FDA Response:

We thank the reviewers for their comments.

We added additional details throughout the methods section to improve descriptions of data and modeling approaches. We also included additional tables that listed model parameters used in the processing module and transportation, storage, and consumption module. We also added the expected impact of each uncertainty source in Table 6 in part 1 and Table 3 in part 2 as suggested by the reviewer.

We agree with the reviewer that focusing on the uncertainties in the pre-harvest modules fits the purpose of our risk assessment to evaluate the impact of application intervals on the comparison of model outputs between treated and untreated BSAAO. As stated in section 2.5.1 of part 2, post-harvest model parameters have minimal impact when comparing the risk estimates associated with untreated BSAAO versus treated BSAAO (as each scenario uses the exact same post harvest module) and were excluded from uncertainty analysis of the risk assessment model.

IV. SPECIFIC OBSERVATIONS

Summary: The reviewers provided specific comments and suggested edits/changes on the draft report.

FDA Response: We thank the reviewers for their comments and suggested edits. We have considered each in our revision of report.

One reviewer stated that overhead irrigation as the primary type of irrigation method may vary depending on region. For comparison purposes, overhead sprinkler was considered as the method of irrigation across all regions in our risk assessment model. As a conservative assumption, we assumed overhead irrigation for all regions as overhead irrigation poses greater potential risk of pathogen transfer from amended soils to produce crops grown in the field via splash during irrigation compared to other irrigation methods (e.g., drip irrigation). We modified the summary and conclusions section to clarify:

> "The virtual produce production systems in the model were created with specifications and agricultural practices reflecting some commercial produce farm operations in the U.S. While result trends are expected to be robust, quantitative results (e.g., the risk estimates) are expected to differ for produce farms with different specifications and following different agricultural practices (e.g., types of irrigation)."

The reviewer also stated that onion irrigation techniques vary by region and cantaloupes are almost never irrigated using overhead irrigation. To clarify, for onion scenarios, irrigation was not modeled, and pathogen transfer via direct contact with soil was modeled as the route of contamination. For cantaloupe scenarios, the model considered pathogen transfer from splash only during rain events but not irrigation. We added additional descriptions to clarify in the text.

The reviewer also raised concerns about the use of zero-day interval as a baseline model since no grower would apply soil amendments and harvest on the same day as it provides no financial benefit. This scenario reflects the current Produce Safety Rule minimum required interval of 0 days for application of treated BSAAO. Therefore, we think it is worth evaluating the risk associated with this theoretical zero-day interval scenarios as a reference for comparison. In addition, we also developed another treated BSAO baseline model as a reference for comparison that implements a realistic 45-day application interval. We modified section 2.7.1 of part 1 to clarify:

> "We developed a baseline model that implements a 0-day application interval (i.e., BSAAO application occurs on the same day of harvest (hereafter referred as the zero-day baseline model). A zero-day application interval reflects the current Produce Safety Rule minimum required interval of 0 days for treated BSAAO (21CFR112.56) that meets treatment requirements (21 CFR §112.54(b)). In addition to the zero-day baseline model, we also developed a baseline model that implements a 45-day interval between BSAAO application and harvest, reflecting recommended time interval by the California Leafy Green Products Handler Marketing Agreement (LGMA) regarding use of composted manure for leafy greens (LGMA, 2023)."

The reviewer also stated that the dramatically higher risk estimates for STEC non-O157 compared to STEC O157 needs explanation. This difference in risk estimates is likely due to the higher contamination prevalence and concentration of STEC non-O157 in raw manure observed

from the survey studies. For example, from the mid-Atlantic region sampling data, none of the 161 samples were found positive for STEC O157, whereas STEC non-O157 was found in over 19% of the 161 samples (Litt et al., 2023). Reported prevalence and concentration of STEC non-O157 in manure were also higher compared to those of STEC O157 for west region (Jay-Russel et al., 2018; Jay-Russel et al., 2023). Therefore, although both STEC O157 and STEC non-O157 model use the same dose response, the difference in initial contamination conditions contributed to the dramatical difference risk estimates. We added additional description to clarify in the results section:

> "Overall, STEC non-O157 scenarios resulted in higher predicted number of illnesses when compared to the estimates from STEC O157 scenarios, which is likely attributable to the higher prevalence and concentration of STEC non-O157 observed in untreated manure sampling data. For example, from the mid-Atlantic region sampling data, none of the 161 samples were found positive for STEC O157 whereas STEC non-O157 was found in over 19% of the 161 samples (Litt et al., 2025). Concentration of STEC non-O157 also ranges from -1.05 to 4.87 $\log_{10}$ MPN/g from west region as compared to -1.05 to 3.04 $\log_{10}$ MPN/g for STEC O157 concentrations (Jay-Russel et al., 2018; Jay-Russel et al., 2023)."

The reviewer requested explanation of why only data for three strains were picked for modeling survival of *Salmonella* in amended soils. The Bardsley et al. (2021) study investigated the survival of 12 *Salmonella* strains in two soils (clay-loam and sandy-loam) amended soil poultry litter. Among the survival trials of the twelve *Salmonella* strains, three strains were exposed to two different irrigation regimens (daily or weekly) while the other nine strains were exposed to only daily irrigation regimen. Given the significantly longer survival reported by Bardsley et al. (2021) in trials receiving weekly irrigation compared to trials receiving daily irrigation, survival data from the daily irrigation trials were excluded from modeling to avoid underestimating *Salmonella* survival considering our risk assessment model's 5-7 day irrigation frequency. In the uncertainty analysis, we added an alternative scenario to more fully represent strain variability in *Salmonella* survival, as described in our response to other comments (see FDA response to **Question 2.2.2**). To account for the potential variability observed from survival trials that only exposed to daily irrigation, survival data from daily irrigation trials were adjusted based on the observed differences in survival from the three strains that were exposed to both weekly and daily irrigation. Then Weibull model parameters were derived based on the adjusted survival data from nine strains exposed to only daily irrigation. Weibull model parameters, including those derived from the three strains that were exposed to weekly irrigation and parameters derived from the adjusted survival data of the nine strains that were exposed to daily irrigation, were combined to describe survival of *Salmonella* in amended soils as a part of model uncertainty analysis. We added descriptions in section 2.8.2 and details in Appendix D of part 1.

V. REFERENCES

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