Human Foods Program

Risk Assessment of Foodborne Illness Associated with Pathogens from Produce Grown in Fields Amended with Untreated Biological Soil Amendments of Animal Origin – Part 2: Postharvest Exposure Assessment, Dose Response, and Risk Characterization

Human Foods Program

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1. INTRODUCTION

Human pathogens such as Shiga toxin producing *Escherichia coli* (STEC) O157:H7 and *Salmonella* can present in untreated biological soil amendments of animal origin (BSAAOs) such as raw bovine manure and raw poultry manure. Once introduced into the produce growing environment through soils amended with untreated BSAAO, human pathogens have been shown to survive for extended periods (Jiang et al., 2002; Islam et al., 2004a; Islam et al., 2004b) which can potentially cause human illness when contaminated produce is consumed (FDA, 2015).

To understand the complex interactions and significant factors associated with foodborne pathogen persistence in agricultural soils where untreated BSAAO has been applied, The U.S. Food and Drug Administration (FDA) conducted a risk assessment of foodborne illness associated with pathogens from produce grown in fields amended with untreated BSAAO. Preharvest modules of the BSAAO risk assessment model and the results from the preharvest exposure assessment were described in detail in part 1 of the risk assessment report. The current document here serves as part 2 of the risk assessment report and focuses on postharvest exposure assessment, dose response, and risk characterization.

2. METHODS

The preharvest modules of the BSAAO risk assessment model have been described in detail in part 1 of this report. Briefly, the preharvest modules mimicked certain commercial lettuce production operations in the U.S. and modelled pathogen (STEC O157, *Salmonella*, and STEC non-O157) presence and behavior in various environmental matrices (e.g., untreated BSAAO, amended soil) and on produce crops. Considering STEC O157 and STEC non-O157 in untreated bovine manure and *Salmonella* in untreated poultry manure as the contamination source, the preharvest modules simulated pathogen survival in amended soils, pathogen transfer to produce crops grown in amended soils via splash during irrigation and rainfall, pathogen population dynamics on produce surfaces, and then predicted the concentration of pathogens on produce crops at the time of harvest.

The postharvest modules of the BSAAO risk assessment model used lettuce as the primary commodity both sold as whole (RAC) or fresh-cut (processed) and modeling components included: pathogen survival, growth, and cross-contamination during processing (e.g., shredding); storage and transportation in the supply chain; consumption, and dose-response relationships for STEC and *Salmonella*. A conceptual framework of the BSAAO risk assessment model is provided in Fig. 1. Detailed descriptions of data, equations, and assumptions used in the model are provided in the following sections.

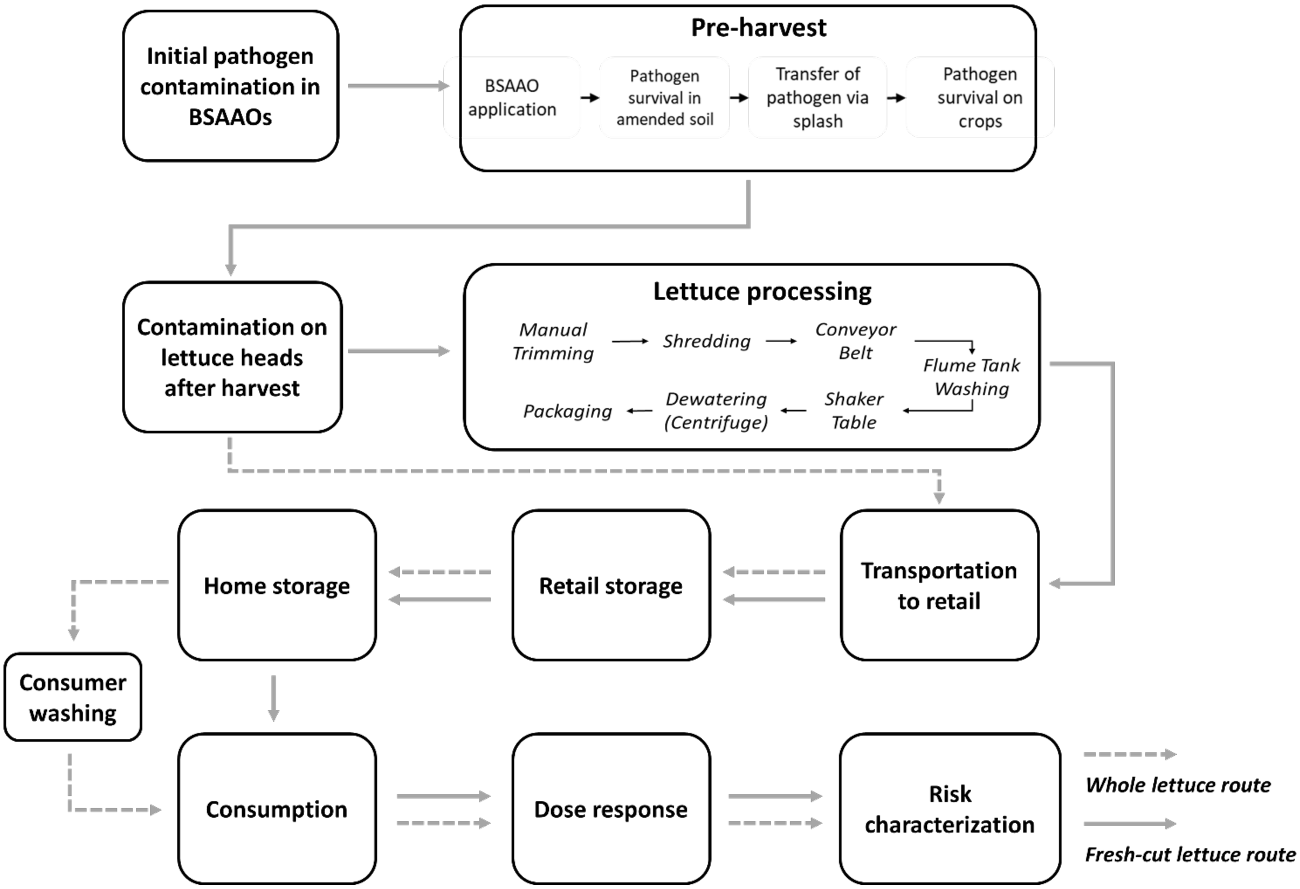

Fig. 1. Conceptual framework of the BSAAO risk assessment model for lettuce.

2.1 Processing

The processing module of the BSAAO risk assessment was developed following the framework presented in our previous studies (Mokhtari et al., 2018; Mokhtari et al., 2021; Pang et al., 2022). A summary of model parameters for the processing module is shown in Table 1. The processing module mathematically described pathogen cross-contamination dynamics during fresh-cut processing steps, including manual trimming, shredding, transfer on conveyor belts, flume tank washing, shaker table, dewatering (centrifuge), and packaging when pathogens are initially present on the incoming lettuce heads. Specifically, cross-contamination between processing equipment surfaces, lettuce, and food handlers were modelled following the approach discussed in Hoelzer et al. (2012) and Mokhtari and Van Doren (2019). Cross-contamination between any two objects (e.g., A and B) is described using the following equations:

$$C_A \sim Binomial\left(\sum_{i=1}^{n_A} C0_{Ai}, 1 - TR_{AB}\right) + Binomial\left(\sum_{j=1}^{n_B} C0_{Bj}, TR_{BA}\right) \tag{1}$$

$$C_B = \sum_{i=1}^{n_A} C0_{Ai} + \sum_{j=1}^{n_B} C0_{Bj} - C_A \tag{2}$$

where C_A and C_B are the updated levels of microbial contamination on object A and object B after a cross-contamination event, respectively; n_A and n_B are the number of grids on object A and object B, respectively, involved in the cross-contamination event; $C0_{Ai}$ is the initial level of microbial contamination on grid i on object A; $C0_{Bj}$ is the initial level of microbial contamination on grid j on object B; TR_{AB} and TR_{BA} are contamination transfer rates from object A to B and from object B to A, respectively. After draw of C_A and C_B, the bacteria were randomly distributed among the n_A and n_B grid cells involved in the cross-contamination event, respectively.

The processing module also adopted the flume tank washing module by Mokhtari et al. (2021) to describe the cross-contamination dynamics of pathogen transfer between wash water and cut lettuce using the following system of differential equations:

$$\frac{d(X_W)}{dt} = \beta_{WS} - \beta_{LW} \times X_W \times \frac{L}{V} - \alpha \times X_W \times FC_m \tag{3}$$

$$\frac{d(X_L)}{dt} = \beta_{LW} \times X_W - \alpha \times X_L \times FC_m - \frac{X_L}{WT} \tag{4}$$

$$\beta_{WS} = \frac{\sigma \times (1 - X_S)}{V \times WT} \tag{5}$$

where X_w is the concentration of pathogens in wash water (CFU/mL), β_{WS} is the effective rate of bacteria entering wash water (CFU/mL min), β_{LW} is the bacteria binding rate to pieces of shredded lettuce (mL/g min), L is the amount of shredded lettuce in the flume tank (g), V is the volume of water in the flume tank (ml), α is the bacteria inactivation rate *via* free chlorine (L/mg min), FC_m is the maintained free chlorine concentration in wash water (ppm), X_L (CFU/g) is the pathogen concentration on shredded lettuce inside the wash tank (CFU/g), σ is the number of pathogen cells on lettuce cuts inside the wash tank (CFU), X_S is the proportion of bacteria remaining on the lettuce pieces during washing, and WT is the washing time (min).

Table 1. Definition of input parameters for the processing module.

Model Input Descriptions	**Units**	**Value(s)**^{a}	**Reference**
Number of fresh-cut pieces per lettuce head	Unitless	150	Mokhtari et al., 2022
Number of fresh-cut lettuce pieces per package	Unitless	115	Mokhtari et al., 2022
Number of food handlers for manual trimming	Unitless	4	Mokhtari et al., 2018
Maintained free chlorine level during washing (FC_m)	ppm	10	Mokhtari et al., 2022
Inactivation rate via free chlorine (α)	L/(mg.min)	U(0.5, 0.75)	Mokhtari et al., 2021; Munther et al., 2015
Flume tank volume (V)	L	2500	Mokhtari et al., 2021
Pathogen binding rate: water to lettuce (β_{LW})	ml/(g min)	T(0.38, 0.75, 2.2)	Mokhtari et al., 2021; Munther et al., 2015
proportion of bacteria remaining on lettuce cuts during washing (X_S)	Unitless	T(0.003, 0.055, 0.149)	Smolinski et al., 2018
Produce-to-water ratio	kg/L	1/45	Mokhtari et al., 2022
Washing time (WT)	min	3/4	Mokhtari et al., 2022
Number of grids on conveyor belt surface	Unitless	288	Mokhtari et al., 2018
Number of grids on shaker table surface	Unitless	247	Mokhtari et al., 2018
Number of grids on centrifuge surface	Unitless	455	Mokhtari et al., 2018
Proportion of equipment surface area that are contamination niches	%	5	Mokhtari et al., 2022
Transfer coefficient from lettuce heads to food handlers	%	T(3, 10, 30)	Jensen et al., 2017
Transfer coefficient from food handlers to lettuce heads	%	T(0, 1, 3)	Zilelidou et al., 2015
Transfer coefficient from lettuce heads to knife	%	T(0, 2.5, 5.0)	Zilelidou et al., 2015
Transfer coefficient from knife to lettuce heads	%	T(0, 29.6, 59.2)	Zilelidou et al., 2015
Transfer coefficient from lettuce heads to shredder	%	T(0, 0.25, 0.53)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014
Transfer coefficient from shredder to lettuce heads	%	T(16, 20, 28)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014
Transfer coefficient from shredded lettuce to conveyor belt	%	T(0, 0.62, 1.39)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014

Transfer coefficient from conveyor belt to shredded lettuce	%	T(15, 18, 22)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014
Transfer coefficient from shredded lettuce to shaker table	%	T(0, 0.06, 0.38)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014
Transfer coefficient from shaker table to shredded lettuce	%	T(6, 28, 30)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014
Transfer coefficient from shredded lettuce to centrifuge	%	T(0, 0.35, 1.59)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014
Transfer coefficient from centrifuge to shredded lettuce	%	T(23, 27, 31)	Buchholz et al., 2012a; Buchholz et al., 2012b; Buchholz et al., 2014

[a]U: uniform distribution; T: triangular distribution.

2.2 Transportation, storage, and consumption

The BSAAO risk assessment adopted our previously published leafy green postharvest risk models (Mokhtari et al., 2018; Mokhtari et al., 2022; Pang et al., 2023) to describe potential growth of pathogens on lettuce during transportation and storage. A summary of parameters for transportation, storage, and consumption is provided in Table 2. Specifically, growth of STEC O157 was estimated using the following equations developed by Baranyi et al. (1995):

$$q_{t_i} = q_{t_{i-1}} + \Delta t \times \mu_{max}\left(T_{t_i}\right) \times q_{t_{i-1}}, \quad q_0 = \frac{\alpha_0}{1-\alpha_0} \tag{6}$$

$$X_{t_i} = X_{t_{i-1}} + \Delta t \times \mu_{max}\left(T_{t_i}\right) \times \frac{q}{(1+q)} \times \left(1 - \frac{X_{t_{i-1}}}{X_{max}}\right) \times X_{t_{i-1}} \tag{7}$$

where q_t is a parameter related to the physiological state of the pathogens at time 0 (α_0) at given time t, t_i denotes the time at time point i, and t_{i-1} denotes time point i-1, with $t_{i-1} - t_i = \Delta t$, μ_{max} is the maximum growth rate for pathogen as a function of storage temperature (T_{t_i} in °C), $X(t)$ represents the number of pathogens on lettuce at given time t, and X_{max} represents the maximum population density for pathogens in on lettuce. The relationship between temperature and maximum growth rate of pathogens (Eq. 13) was described using the square root model of Ratkowsky et al. (1982):

$$\sqrt{\mu_{max}(T_{t_i})} = b \times (T_{t_i} - T_{min}) \tag{8}$$

where b is a constant, and T_{min} is the theoretical minimum temperature for microbial growth.

Time-temperature profiles during the transportation and storage steps were generated based on data reported by Zeng et al. (2014) and EcoSure (2007), respectively. Consumption of lettuce in the U.S. was estimated using data from What We Eat in America (WWEIA), the dietary survey portion of the National Health and Nutrition Examination Survey (NHANES), including all survey cycles from 2003 through 2010 (Centers for Disease Control and Prevention [CDC], 2013).

2.3 Dose-response and risk characterization

The dose-response models used in this risk assessment adapted the STEC O157 beta-Poisson model derived by Strachan et al. (2005) and the *Salmonella* beta-Poisson model derived by the Food and Agriculture Organization of the United Nations and World Health Organization (FAO/WHO, 2002). The beta-Poisson models were adapted to the discrete case (Haas, 2002) to estimate the risk of illness per serving (r_{serving}) with parameter values $\alpha = 0.0571$ and $\beta = 2.2183$ obtained from Strachan et al. (2005) for STEC O157, and $\alpha = 0.1324$ and $\beta = 54.45$ obtained from FAO/WHO (2002) for *Salmonella*:

$$r_{serving} = 1 - \frac{\Gamma(\alpha+\beta)\Gamma(\beta+d)}{\Gamma(\beta)\Gamma(\alpha+\beta+d)} \tag{9}$$

where $\Gamma(x)$ is the gamma function and d is the consumed integer dose of pathogens (CFU); and α, β are model parameters. Number of illnesses were then calculated as:

$$n_{illnessP} \sim \sum Bernoulli(\, r_{serving}) \tag{10}$$

Table 2. Definition of input parameters for lettuce transportation, storage, and consumption

Model Input Descriptions	**Units**	**Value(s)**	**Reference**
Growth model parameter: a_0	Unitless	STEC O157: 0.056 *Salmonella*: 0.097	Koseki & Isobe, 2005
Growth model parameter: b	Unitless	STEC O157: 0.033 *Salmonella*: 0.033	Koseki & Isobe, 2005
Theoretical minimum growth temperature (T_{min})	°C	STEC O157: 4.54 *Salmonella*: 4.96	Koseki & Isobe, 2005
Maximum population density	$\log_{10}$ CFU	7	Model assumption
Shelf life (lettuce)	day	15	Mokhtari et al., 2022
Transportation temperature	°C	Randomized profile from Zeng et al. (2014)	Zeng et al., 2014
Retail storage temperature	°C	Randomized profile from Zeng et al. (2014)	Zeng et al., 2014
Consumer storage temperature	°C	Random value from EcoSure (2007)	EcoSure, 2007
Transportation time	hr	T(30, 85, 64)	Zeng et al., 2014
Retail storage time	hr	T(12, 168, 96)	Zeng et al., 2014
Consumer storage time	hr	T(0, 0.3×[Shelf life - (Transportation time+Retail storage time)], Shelf life - (Transportation time+Retail storage time))	Mokhtari et al., 2022
Consumer storage time cut-off	hr	$\begin{cases} 168; T_c \leq 8^oC \\ 72; 8 \leq T_c \leq 10^oC \\ 24; 10 \leq T_c \leq 15^oC \end{cases}$	Mokhtari et al., 2022
Serving size	g/serving	Empirical distribution Mean value: 44.8	CDC, 2013

[a]T: Triangular distribution

2.4 Scenario analysis

2.4.1 Baseline model and evaluation of application interval impact on risk estimates

Following the approach described extensively in part 1 of the risk assessment, we developed two baseline models that estimate the risk of human illness from the consumption of lettuce grown in fields amended with composted manure (i.e., treated BSAAO per 21 CFR §112.54(b)): the zero-day baseline model that implements a 0-day application interval (i.e., BSAAO application occurs on the same day of harvest) and the 45-day baseline model that implements a 45-day interval between BSAAO application and harvest. We evaluated the impact of different application intervals at 0 day, 45 days, 60 days, 90 days, and 120 days on risk predictions across three regions (i.e., Mid-Atlantic, south, and west) and two growing seasons (summer-fall and winter-spring). The 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. The predicted number of illnesses per field from the untreated BSAAO scenarios with different application intervals was 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.

2.4.2 Potential impact of runoff on risk estimates

We evaluated the potential impact of runoff on the relative risk estimates between treated and untreated BSAAO scenarios at different application intervals. For runoff scenarios, it was assumed that runoff occurs immediately after BSAAO application and that runoff water evenly distributes pathogens in the amended soils to the entire field (i.e., all soil grids become contaminated at the same level after runoff event). Concentration of pathogens in soil grids after runoff was calculated as:

$$C_{grid} = (A_R \times P_{BSAAO} \times 10^{C_{BSAAO}})/n_{grid}, \qquad \text{Equation (11)}$$

where A_R is manure application rate (10,000 kg); P_{BSAAO} is the prevalence of pathogens in raw manure; C_{BSAAO} is the concentration of pathogens in raw manure ($\log_{10}$ CFU/g); and n_{grid} is the total number of grids within a field.

2.4.3 Risk associated with STEC non-O157 in untreated bovine manure

We also predicted the risk associated with consumption of lettuce from fields amended with untreated bovine manure contaminated with STEC non-O157. Modeling approaches for initial prevalence and concentration of STEC non-O157 in untreated BSAAO, STEC non-O157 survival in amended soil, STEC non-O157 transfer to crops, and subsequent survival on crops were described in part 1 of the risk assessment report. The dose-response relationship of 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 and used the beta-Poisson model described in section 2.3 for hazard and risk characterization of STEC non-O157.

2.4.4 Risk associated with consumption of produce that grows in the ground and on the ground

We estimated the risk associated with the consumption of onion (as an example of produce that grows in the ground) from fields amended with untreated bovine manure and consumption of fresh-cut cantaloupe (as an example of produce commodity that grow on the ground) from fields amended with untreated poultry manure. The preharvest modules for onion and cantaloupe scenarios are described in detail in the part 1 of the report. For onions, postharvest steps may include various processes such as packing and handling, storage, and processing (e.g., peeling and cutting). Given the lack of data to quantitatively assess the influence of postharvest processes on pathogen contamination on onions, the risk model assumed an 80% to 99% with a most likely value of 99% reduction of STEC O157 on onions during postharvest processes and estimated the number of illnesses based on onion consumption using the dose response model described in section 2.3. A conceptual framework of the onion risk assessment model is provided in Fig. 2.

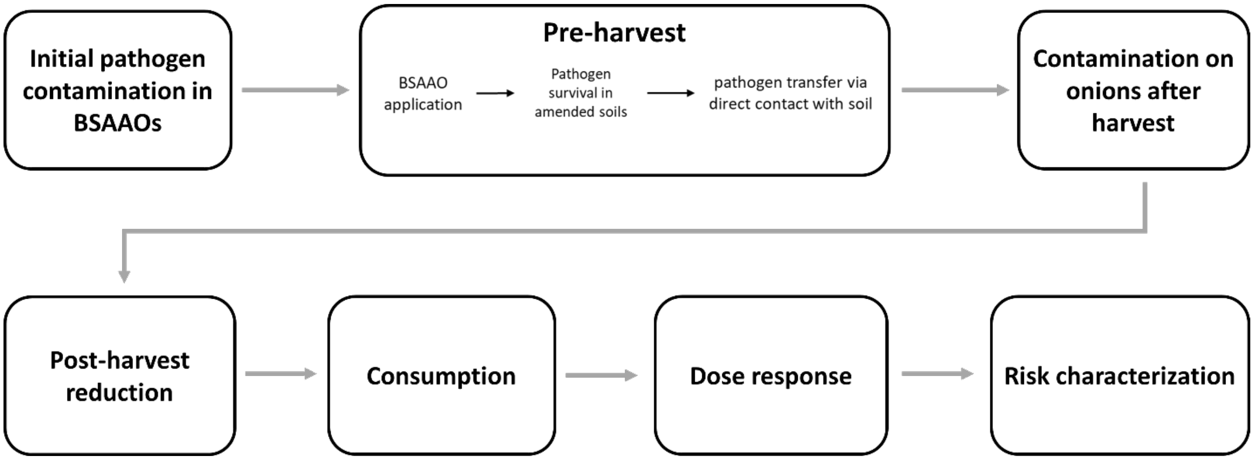

Fig. 2. Conceptual framework of the BSAAO risk assessment model for onions.

For cantaloupe scenarios, we adopted the postharvest risk assessment model from our previously published risk assessment (Mokhtari et al., 2023) and estimated the risk of illness associated with consumption of fresh-cut cantaloupes. A conceptual framework of the fresh-cut cantaloupe model is provided in Fig. 3.

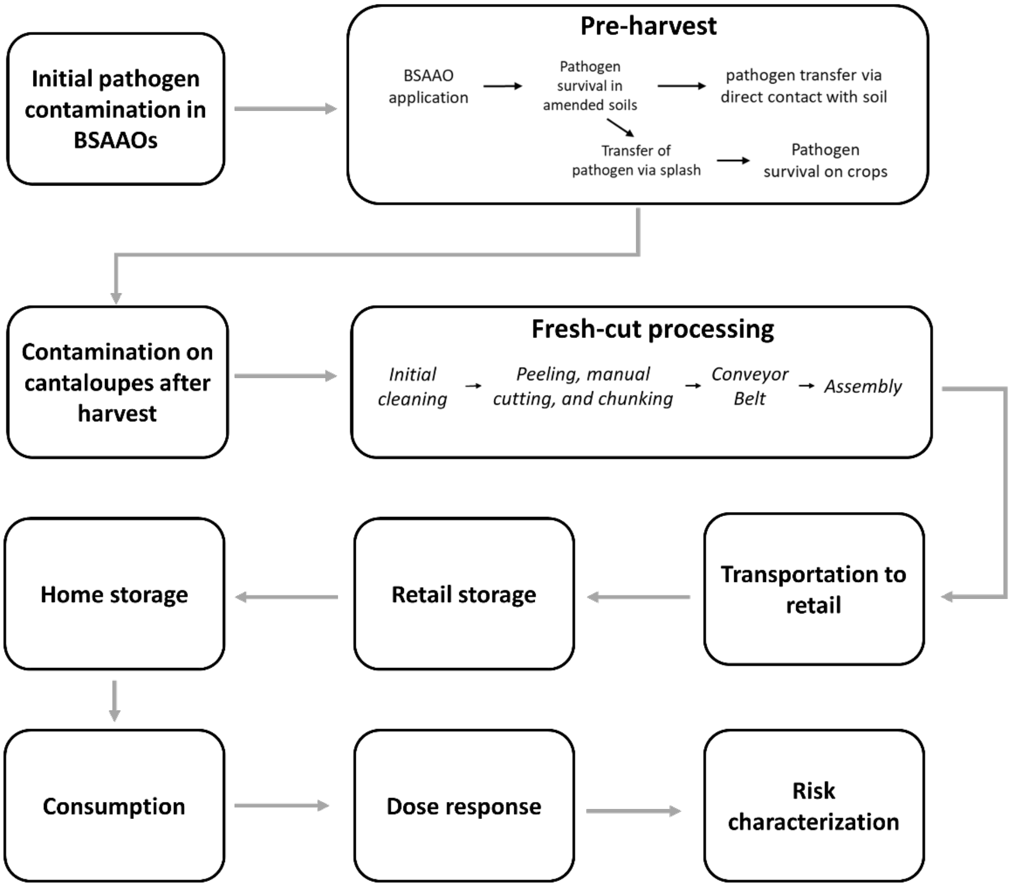

Fig. 3. Conceptual framework of the BSAAO risk assessment model for fresh-cut cantaloupes.

2.5 Uncertainty analysis

In this risk assessment, uncertainty analyses were conducted in which selected input variables were assigned an upper-bound value or a lower-bound value (or both), and the model was rerun to evaluate the impact of such change on risk estimates for untreated BSAAO and the comparison with results from the treated BSAAO baseline models following the approach described by Pérez-Rodríguez et al. (2017). Sources of uncertainties and assumptions in the risk assessment model are summarized in Table 3. Field specification variables and postharvest model parameters have minimal impact when comparing the risk estimates associated with untreated BSAAO versus treated BSAAO (as each scenario uses the exact same field specification settings and the exact same postharvest modules) and were therefore excluded from uncertainty analysis. Variables evaluated in the uncertainty analysis included initial contamination conditions (prevalence and concentration) in untreated BSAAO, survival models for pathogens in amended soils, pathogen transfer models, splash radius, and pathogen survival rates on crops grown in the field. Fresh-cut lettuce and whole lettuce use the exact same preharvest modules and have the exact same sources of uncertainty of interest (as uncertainties from postharvest modules were excluded). Therefore, uncertainty analysis was performed and results were presented using lettuce sold as fresh-cut as the primary commodity. We followed the same approach described in part 1 of the risk assessment report and estimated the overall model uncertainty range of risk estimates by rerunning the model using the upper-bound or the lower-bound values for all abovementioned variables (based on their expected impact on risk estimates) simultaneously. Analysis of uncertainty for each individual variable was also conducted by rerunning model using the upper or lower-bound value for the tested variable while keeping the original values for other variables. A summary of model uncertainty scenarios is provided in Table 4 and additional detailed descriptions can be found in part 1 of the risk assessment report.

Table 3. Description of sources of uncertainties in the BSAAO risk assessment model.

Variables	**Description**	**Model assumptions**	**Expected impact on relative risk**
Field specifications	Properties of the virtual produce field (e.g., length, width, spacing of crops, BSAAO application rate)	Field specifications are representative of typical produce growing operations in the U.S.	Minimal
Initial concentration	Initial concentration of STEC and *Salmonella* in untreated BSAAOs	Data from commissioned studies are representative for each region	+
Initial prevalence	Initial prevalence of STEC and *Salmonella* in untreated BSAAOs	Data from commissioned studies are representative for each region	+
Survival model - amended soils	Survival of STEC O157 and *Salmonella* in soils amended with untreated BSAAO	Models (Pang et al., 2020; Bardsley et al., 2021; Murphy et al. 2024) are valid to evaluate pathogen survival in soils amended with untreated BSAAO under field conditions	+
Transfer model	Pathogen transfer from soil to crops grown in the field during irrigation or rain events	Pathogen transfer is described using data for animal feces assuming the same mechanisms for amended soils. Pathogen transfer is influenced by irrigation/rain intensity and distance to contamination source. Predictive models are valid to evaluate pathogen transfer from amended soils to crops due to splash.	+
Splash radius	Maximum distance pathogen can transfer from soil to crops due to splash	Maximum splash radius was assumed to be 3 meters based on Jeamsripong et al. (2019)	+
Survival rates on crops	Survival of pathogens on crops grown in the field	Inclusion and exclusion criteria for literature search are appropriate and collected data are representative to estimate decline rates of pathogens on crops under field conditions	+
Postharvest model	Parameters describing microbial behavior during postharvest steps (e.g., time-temperature profile, serving size, dose-response model)	Parameter values and models are valid in describe pathogen behavior during postharvest processes	Minimal

*"+" indicates a positive correlation with model results, e.g., upper-bound value leads to higher risk estimates. "-" indicates a negative relationship between variable and model output, i.e., lower-bound value leads to higher risk estimates.

Table 4. Description of uncertainty scenarios.

Variables	**Values or distributions for uncertainty scenarios**
Initial concentration in untreated BSAAOs	Beta-binomial distribution parameter α for each region was assigned an upper-bound value equals to 200% of its original value and a lower-bound value equals to 50% of its original value for west and south region; And 200% or 50% of the original contamination prevalence value for Mid-Atlantic region
Initial contamination prevalence in untreated BSAAOs	Slope of the prevalence/concentration function was assigned an upper-bound value equals to the mean estimated slope plus standard error and a lower-bound value equals to the mean minus standard error. For pathogen/region combinations described by empirical distributions in the original model simulations, upper-bound and lower-bound values were described by empirical distributions derived using values equal to 200% and 50% of the original values.
Pathogen survival in amended soils	• STEC O157: using the upper-bound and lower-bound models described in Pang et al. (2020) for upper-bound and lower-bound risk estimates; Additionally, using alternative Weibull survival models derived from Murphy et al. (2024) and Franz et al. (2011). • *Salmonella*: using original Weibull survival model parameter D values equal to original value plus or minus the standard error for upper-bound and lower-bound risk estimates; Additionally, using Weibull models derived from survival data from Bardsley et al. (2021) adjusted for strain variability and irrigation regimen.
Pathogen transfer from amended soils to crops during irrigation or rainfall	Using the upper and lower-bound predictions from the transfer coefficient model for upper-bound and lower-bound risk estimates.
Maximum distance pathogen can transfer from soil to crops due to splash	Increased the splash radius to 4 m.
Survival of pathogens on crops grown in the field	Use survival rate on crops based on new inclusion criteria that included survival data from growth chamber/laboratory studies (Stine et al., 2005; Harapas et al., 2015; Lopez-Velasco et al., 2015; López-Gálvez et al., 2018; Erickson et al., 2019) and field studies with less than 10-day observations (Erickson et al., 2019; Belias et al., 2020)

3. RESULTS and DISCUSSION

Predicted results from the risk assessment models across different scenarios are provided in the following sections. Key results presented in the report are also available in the interactive FDA BSAAO-Produce Risk Assessment Output Explorer (https://pub-connect.foodsafetyrisk.org/content/6851ecb5-0122-45b7-af14-bd326ee07e6f/).

3.1 Impact of application intervals on risk estimates

3.1.1 Leafy greens

3.1.1.1 Predicted risk associated with STEC O157:H7 and *Salmonella* in untreated BSAAOs at different application intervals

The mean predicted number of illnesses from consumption of fresh-cut lettuce grown in fields amended with STEC O157 contaminated bovine manure varied by region and by growing season (Table 5 & Fig. 4). Despite regional and seasonal differences, application intervals of 45, 60, 90, and 120 days drastically reduced the predicted number of illnesses from consumption of STEC O157 contaminated fresh-cut lettuce (Table 5 & Fig. 4) compared to the predictions from 0-day application intervals. Compared to the mean estimated risk with a 0-day application interval, the risk estimates decreased by 62- to 72-fold for the Mid-Atlantic region, 21 to 45-fold for the south region, and 463 to 1243-fold for the west region, with a 120-day application interval for the two growing seasons (in general lower risk reduction in the summer-fall season), highlighting substantial differences across the three regions (Table 5).

Risk estimates from consumption of fresh-cut lettuce associated with *Salmonella* in untreated poultry manure also varied between regions with the highest predicted number of illnesses observed from the Mid-Atlantic region (Table 5 & Fig. 4). Risk estimates for *Salmonella* were generally higher for summer-fall season especially for Mid-Atlantic and south region where difference in *Salmonella* prevalence between growing seasons in untreated BSAAO was significant (Table 5); there is a 29- to 59-fold risk reduction across the seasons and regions except for a 210-fold risk reduction for the south region in the winter-fall season. Overall, larger application intervals greatly reduced the predicted number of illnesses for *Salmonella* across different regions and growing seasons (Table 5 & Fig. 4). Risk estimates for *Salmonella* in untreated poultry manure were generally higher than the risk estimates for STEC O157 in untreated bovine manure.

Table 5. Mean predicted number of illnesses associated with *E. coli* O157:H7 and *Salmonella* from consumption of fresh-cut lettuce grown in fields (20,000 lettuce heads) amended with untreated BSAAO by region and growing season.

Growing season	**Region**	**Application interval (days)**	**Predicted average number of illnesses associated with STEC O157 per lettuce field**	**Predicted average number of illnesses associated with *Salmonella* per lettuce field**
Summer-fall	Mid-Atlantic	0	0.016	1251
Summer-fall	Mid-Atlantic	45	0.002	514
Summer-fall	Mid-Atlantic	60	0.002	210
Summer-fall	Mid-Atlantic	90	3.51×10^{-4}	63.0
Summer-fall	Mid-Atlantic	120	2.10×10^{-5}	25.8
Summer-fall	South	0	0.226	434
Summer-fall	South	45	0.097	242
Summer-fall	South	60	0.037	112
Summer-fall	South	90	0.016	42.3
Summer-fall	South	120	0.011	12.6
Summer-fall	West	0	4.63	61.5
Summer-fall	West	45	0.391	38.3
Summer-fall	West	60	0.045	24.6
Summer-fall	West	90	0.014	6.94
Summer-fall	West	120	0.010	2.09
Winter-spring	Mid-Atlantic	0	0.016	521
Winter-spring	Mid-Atlantic	45	0.024	114
Winter-spring	Mid-Atlantic	60	0.005	70.3
Winter-spring	Mid-Atlantic	90	4.02×10^{-4}	16.8
Winter-spring	Mid-Atlantic	120	2.26×10^{-4}	8.82
Winter-spring	South	0	0.226	269
Winter-spring	South	45	0.058	41.6
Winter-spring	South	60	0.038	12.4
Winter-spring	South	90	0.012	2.85
Winter-spring	South	120	0.005	1.28
Winter-spring	West	0	1.06	61.5
Winter-spring	West	45	0.059	29.9
Winter-spring	West	60	0.005	11.5
Winter-spring	West	90	0.001	3.75
Winter-spring	West	120	8.53×10^{-4}	1.41

The mean predicted number of illnesses from consumption of whole lettuce grown in fields amended with treated or untreated BSAAO at different application intervals by region and growing region are shown in Table 6. Similar to fresh-cut lettuce scenarios, highest risk estimates for whole lettuce are from scenarios with a zero-day application interval. As application interval increases to 45, 60, 90, and 120 days, the mean estimated number of illnesses decreases for both STEC O157 and *Salmonella*. Compared to fresh-cut lettuce (Table 5), risk estimates for STEC O157 contaminated whole lettuce (Table 6) 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).

Table 6. Mean predicted number of illnesses associated with *E. coli* O157:H7 and *Salmonella* from consumption of whole lettuce grown in fields (20,000 lettuce heads) amended with untreated BSAAO by region and growing season.

Growing season	Region	Application interval (days)	Predicted average number of illnesses associated with STEC O157 per lettuce field	Predicted average number of illnesses associated with *Salmonella* per lettuce field
Summer-fall	Mid-Atlantic	0	0.280	5414
Summer-fall	Mid-Atlantic	45	0.025	3097
Summer-fall	Mid-Atlantic	60	0.021	1403
Summer-fall	Mid-Atlantic	90	0.006	575
Summer-fall	Mid-Atlantic	120	0.003	297
Summer-fall	South	0	3.52	3598
Summer-fall	South	45	1.57	1991
Summer-fall	South	60	0.666	873
Summer-fall	South	90	0.270	413
Summer-fall	South	120	0.145	159
Summer-fall	West	0	35.6	460
Summer-fall	West	45	6.07	13.6
Summer-fall	West	60	0.729	5.07
Summer-fall	West	90	0.219	1.42
Summer-fall	West	120	0.116	0.682
Winter-spring	Mid-Atlantic	0	0.280	2241
Winter-spring	Mid-Atlantic	45	0.664	729
Winter-spring	Mid-Atlantic	60	0.141	438
Winter-spring	Mid-Atlantic	90	0.007	171
Winter-spring	Mid-Atlantic	120	0.004	96.1
Winter-spring	South	0	3.52	1038
Winter-spring	South	45	0.846	423
Winter-spring	South	60	0.583	142
Winter-spring	South	90	0.204	42.2
Winter-spring	South	120	0.046	20.1
Winter-spring	West	0	11.5	460
Winter-spring	West	45	0.915	14.5
Winter-spring	West	60	0.083	5.19
Winter-spring	West	90	0.026	1.54
Winter-spring	West	120	0.012	0.541

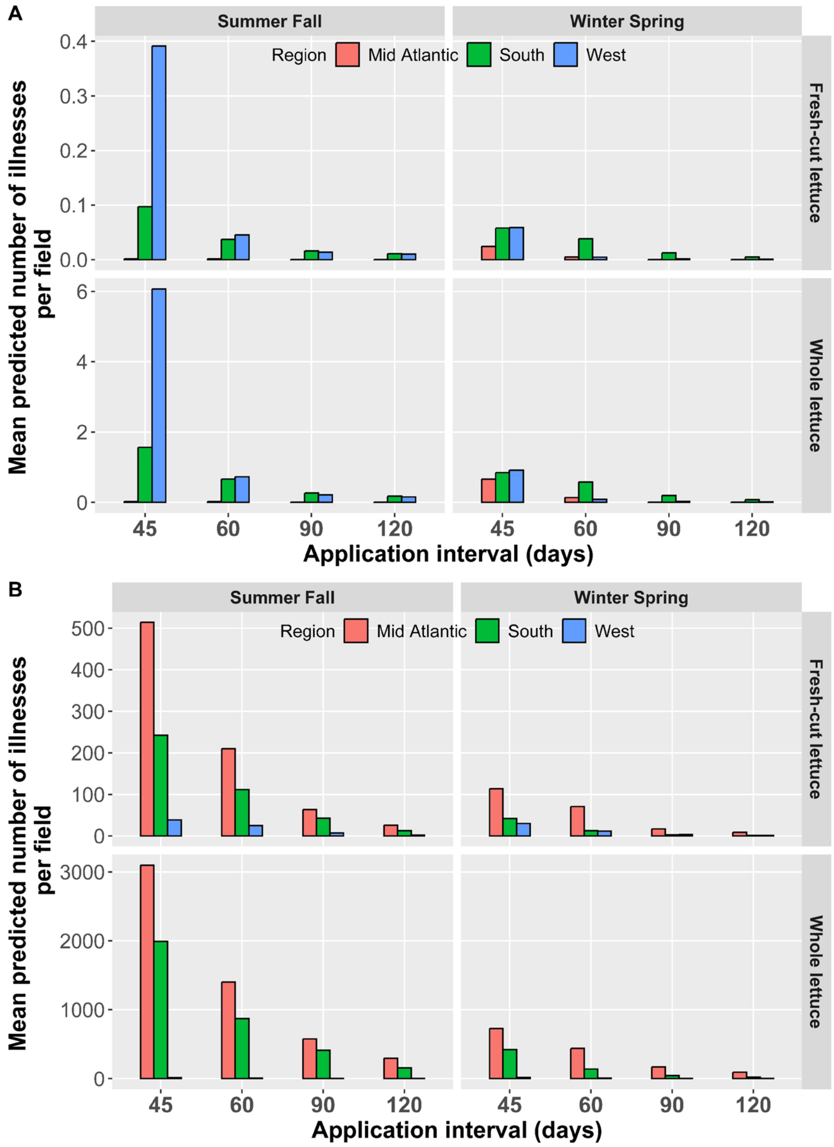

Fig. 4. Mean predicted number of illnesses from consumption of (A) STEC O157 or (B) *Salmonella* contaminated lettuce grown in fields amended with untreated BSAAO.

3.1.1.2 Relative risks of untreated BSAAO compared to the baseline model

Predicted number of illnesses from untreated BSAAO scenarios were compared to the results from baseline models for treated BSAAO (summarized in Table 7). Risk associated with STEC O157 and *Salmonella* from consumption of lettuce grown in fields amended with untreated BSAAO relative to the risk estimates from the baseline models (relative risk) with treated BSAAO using a zero-day and a 45-day interval is shown in Fig. 5–6. Relative risk values were calculated using the mean predicted number of illnesses from the three regions (i.e., Mid-Atlantic, south, and west) across the two growing seasons (i.e., summer-fall and winter-spring). Y-axis values indicate the change of risk estimates in orders of magnitude compared to the baseline model and values less than 1 indicate risk estimates that are less than baseline model predictions while values greater than 1 indicate risk estimates that are higher. As seen in Fig. 5, predicted relative risk of illness associated with STEC O157 in untreated bovine manure varied by growing seasons where risk estimates from the same region were generally higher for the summer-fall season. Relative risk estimates for STEC O157 also varied by region within the same growing season. For the summer-fall season, west and south regions showed higher risk estimates than zero-day baseline across all intervals (45-120 days) while Mid-Atlantic region remained below baseline regardless of application interval (Fig. 5A). Only 120-day intervals for STEC O157 reduced risk estimates to below 45-day baseline model prediction across all regions (Fig 5B). For the winter-spring season, risk estimates from all regions exceeded zero-day baseline at 45- to 60-day interval but a 90- to 120-day interval reduced risk below zero-day baseline for Mid-Atlantic and west regions. Both 90- and 120-day intervals reduced STEC O157 risk below 45-day baseline across all regions (Fig. 5B). For *Salmonella*, similar regional and seasonal patterns were observed. Despite the reduction in order of magnitude as application interval increases, predicted number of illness associated with fresh-cut and whole lettuce from fields amended with *Salmonella* contaminated untreated BSAAO remained above both zero-day and 45-day baseline results at all intervals (45 to 120 days), which is due in part to the relatively low risk estimates for the baselines for *Salmonella*. We performed additional simulations to estimate the risk associated with *Salmonella* in untreated poultry manure with application intervals greater than 120 days. Results (Appendix C) show that the mean predicted number of illnesses associated with consumption of *Salmonella* contaminated fresh-cut and whole lettuce from fields amended with untreated poultry manure is lower than the zero-day baseline model predictions with an application interval of 600 days.

Table 7. Predicted average number of illnesses from baseline models with treated BSAAOs.

Lettuce type	**Pathogen**	**Predicted average illnesses per field for the zero-day baseline model**[1]	**Predicted average illnesses per field for the 45-day baseline model**
Fresh-cut lettuce	*E. coli* O157:H7	0.004	0.013
Fresh-cut lettuce	*Salmonella*	3.23×10^{-4}	3.69×10^{-5}
Whole lettuce	*E. coli* O157:H7	0.060	0.225
Whole lettuce	*Salmonella*	0.006	7.65×10^{-4}

[1] 20,000 lettuce heads per field.

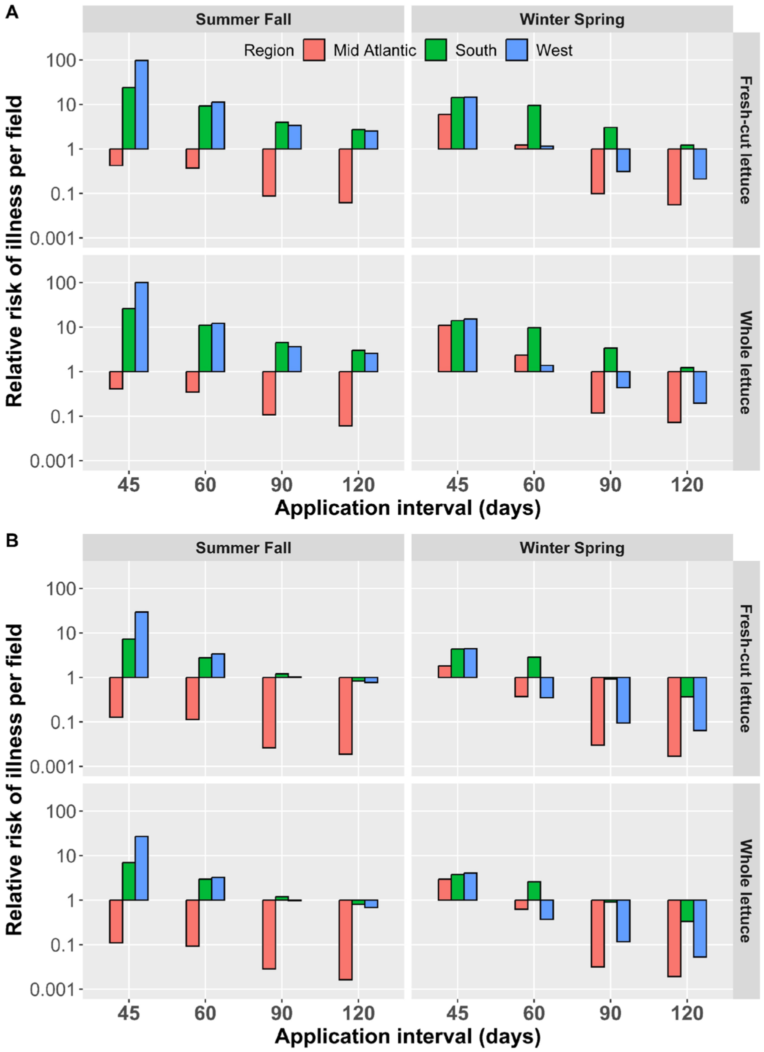

Fig. 5. Mean predicted number of illnesses from consumption of STEC O157 contaminated lettuce grown in fields amended with untreated BSAAO relative to the results from the baseline model with treated BSAAO (compost) using (A) a zero-day interval and (B) a 45-day interval across region and growing season.

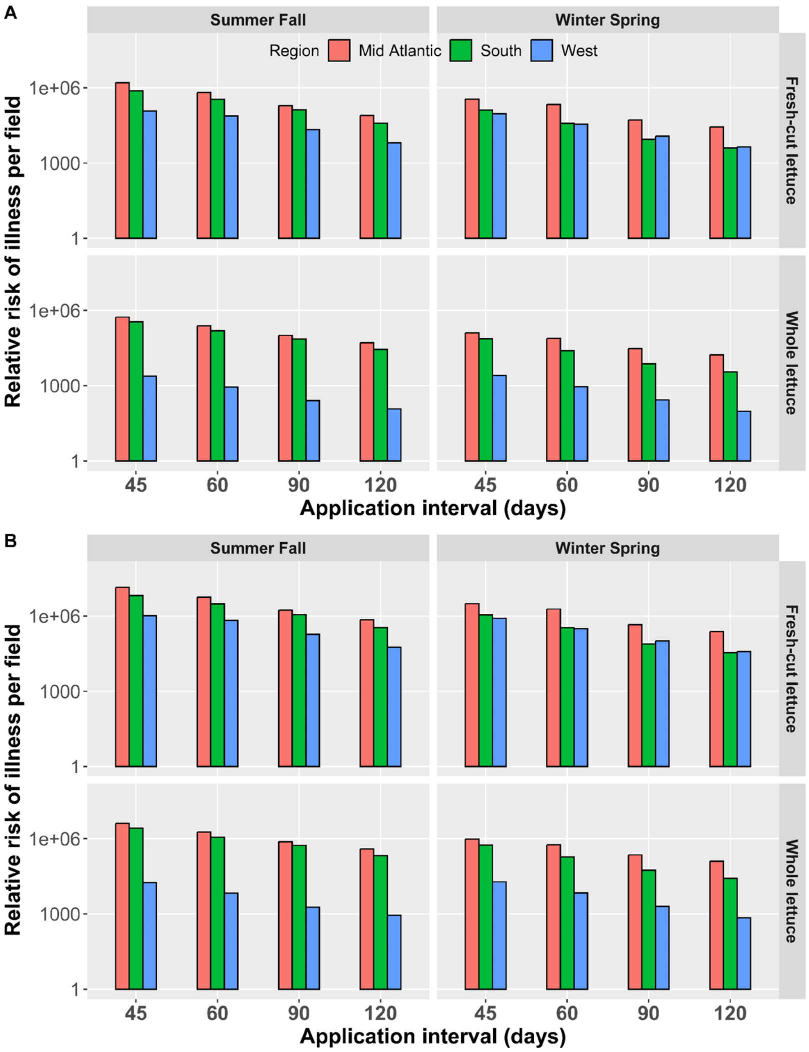

Fig. 6. Mean predicted number of illnesses from consumption of *Salmonella* contaminated lettuce grown in fields amended with untreated BSAAO relative to the results from the baseline model with treated BSAAO (compost) using (A) a zero-day interval and (B) a 45-day interval across region and growing season.

The mean predicted number of illnesses per lettuce field using different application intervals across all regions and seasons are provided in Table 8 and Table 9 for STEC O157 and *Salmonella* respectively. For STEC O157, a 120-day application interval reduced mean risk estimates from untreated BSAAO scenarios (both fresh-cut and whole lettuce) below zero-day baseline model estimates and both 90-day and 120-day intervals reduced mean risk estimates from untreated BSAAO scenarios below the 45-day baseline model predictions (Table 8). For *Salmonella,* mean predicted number of illnesses from fresh-cut and whole lettuce grown in fields amended with untreated BSAAO exceeded both baseline models at all tested application intervals (45-120 days) (Table 9).

We also compared the predicted density distributions of the number of illnesses from untreated BSAAO scenarios at different application intervals across different regions and seasons to those from the baseline model scenarios with treated BSAAO (Fig. 7). Predicted distributions of number of illnesses were highly skewed with high frequency of zero illnesses. Therefore, in Fig. 7, the predicted number of illnesses was transformed using a logw0 transformation that preserves relationships within the data that includes zeros [(McCune and Grace, 2002; Weller et al., 2017, see additional details in Appendix A]. For both STEC O157 and *Salmonella*, the predicted distributions shifted left when application interval increased from 45 to 120 days, indicating a decrease in the number of predicted illnesses as seen in Fig. 7A and Fig. 7B respectively. Compared to the results from the zero-day baseline model, the predicted distributions for STEC O157 from untreated BSAAO scenarios shifted to a lower average number of illnesses only when application interval increased to 120 days (Fig. 7A). When compared to the 45-day baseline model, predicted distributions from untreated BSAAO scenarios for STEC O157 shifted to a lower average number of illnesses when application interval was either 90 or 120 days (Fig. 7A). For *Salmonella,* an increase in the average number of illnesses was predicted from all untreated BSAAO scenarios across all application intervals when compared to the results from both baseline models (Fig 7B).

Table 8. Mean predicted number of illnesses associated with STEC O157 from consumption of fresh-cut or whole lettuce grown in fields (20,000 lettuce heads) amended with untreated BSAAO.

Lettuce type	**Application interval (days)**	**Predicted average number of illnesses per lettuce field**	**Comparison with the zero-day baseline model**[1]	**Comparison with the 45-day baseline model**
Fresh-cut lettuce	45	0.089	+	+
Fresh-cut lettuce	60	0.022	+	+
Fresh-cut lettuce	90	0.007	+	-
Fresh-cut lettuce	120	0.004	-	-
Whole lettuce	45	1.723	+	+
Whole lettuce	60	0.394	+	+
Whole lettuce	90	0.133	+	-
Whole lettuce	120	0.057	-	-

[1] "+"Indicates risk estimates that are greater than baseline model predictions; "-"Indicates risk estimates that are less than baseline model predictions.

Table 9. Mean predicted number of illnesses associated with *Salmonella* from consumption of fresh-cut or whole lettuce grown in fields (20,000 lettuce heads) amended with untreated BSAAO.

Lettuce type	**Application interval (days)**	**Predicted average number of illnesses per lettuce field**	**Comparison with the zero-day baseline model**[1]	**Comparison with the 45-day baseline model**
Fresh-cut lettuce	45	144	+	+
Fresh-cut lettuce	60	64.9	+	+
Fresh-cut lettuce	90	19.4	+	+
Fresh-cut lettuce	120	7.64	+	+
Whole lettuce	45	787	+	+
Whole lettuce	60	359	+	+
Whole lettuce	90	146	+	+
Whole lettuce	120	71.8	+	+

[1] "+"Indicates risk estimates that are greater than baseline model predictions; "-"Indicates risk estimates that are less than baseline model predictions.

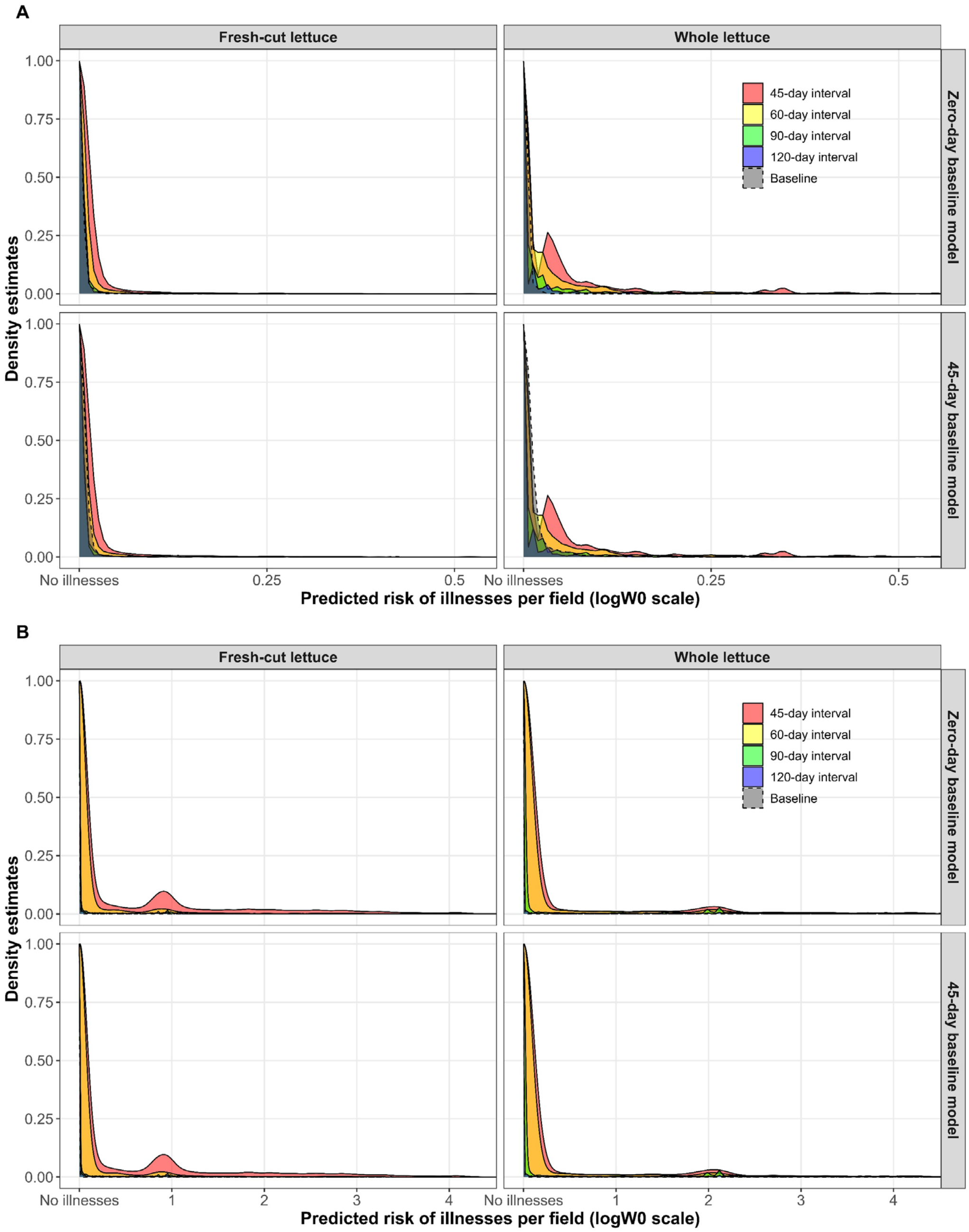

Fig. 7. Predicted density distributions of number of illnesses (logw0 scale) from consumption of (A) STEC O157- and (B) *Salmonella*-contaminated lettuce grown in fields amended with untreated or treated BSAAO (compost).

3.1.1.3 Impact of runoff on risk estimates

Risk estimates from runoff scenarios for each region and each season at different application intervals are summarized in Table 10. Runoff impact on risk estimates varied by region and season compared to non-runoff scenarios (Table 5). For example, runoff increased risk estimates associated with STEC O157 in Mid-Atlantic and west regions for both growing seasons, but decreased the risk estimates in south region for both growing seasons. These regional differences likely resulted from varying initial contamination prevalence and concentration in untreated BSAAO, as runoff spreads pathogens across fields while lowering concentrations on initially contaminated soil grids. Table 11 presents the mean predicted number of illnesses from runoff scenarios across all regions and seasons. Compared to non-runoff scenarios (Tables 7), runoff generally increased average risk estimates across all application intervals, but the trends compared to baseline models remained consistent with original non-runoff scenarios for both pathogens.

Table 10 Mean predicted number of illnesses from runoff scenarios by region and season at different application intervals.

Growing season	Region	Application interval (days)	Predicted average number of illnesses associated with STEC O157 per lettuce field	Predicted average number of illnesses associated with *Salmonella* per lettuce field
Summer-fall	Mid-Atlantic	45	0.040	474
Summer-fall	Mid-Atlantic	60	0.035	268
Summer-fall	Mid-Atlantic	90	0.011	75.8
Summer-fall	Mid-Atlantic	120	0.005	22.1
Summer-fall	South	45	0.066	242
Summer-fall	South	60	0.010	113
Summer-fall	South	90	0.006	34.6
Summer-fall	South	120	0.002	13.0
Summer-fall	West	45	0.471	23.7
Summer-fall	West	60	0.086	15.8
Summer-fall	West	90	0.025	4.76
Summer-fall	West	120	0.006	2.69
Winter-spring	Mid-Atlantic	45	1.04	222
Winter-spring	Mid-Atlantic	60	0.423	156
Winter-spring	Mid-Atlantic	90	0.011	48.1
Winter-spring	Mid-Atlantic	120	0.006	4.80
Winter-spring	South	45	0.055	40.2
Winter-spring	South	60	0.013	11.4
Winter-spring	South	90	0.005	2.80
Winter-spring	South	120	0.001	0.974
Winter-spring	West	45	0.462	26.0
Winter-spring	West	60	0.068	15.1
Winter-spring	West	90	0.016	8.26
Winter-spring	West	120	0.003	1.77

Table 11. Mean predicted number of illnesses from runoff scenarios across region and seasons at different time intervals.

Pathogen	Application interval (days)	Predicted average number of illnesses per lettuce field	Comparison with the zero-day baseline model[1]	Comparison with the 45-day baseline model
STEC O157	45	0.352	+	+
STEC O157	60	0.104	+	+
STEC O157	90	0.012	+	-
STEC O157	120	0.004	-	-
Salmonella	45	171	+	+
Salmonella	60	96.6	+	+
Salmonella	90	29.0	+	+
Salmonella	120	7.56	+	+

[1] "+"Indicates risk estimates that are greater than baseline model predictions; "-"Indicates risk estimates that are less than baseline model predictions.

3.1.1.4 Risk estimates associated with STEC non-O157

The mean predicted illnesses associated with consumption of STEC non-O157 contaminated fresh-cut and whole lettuce from fields amended with untreated bovine manure were similar across two growing seasons (Table 12) as none of the sampling data from the three regions suggested significant impact of season (summer-fall vs. winter-spring) on the presence of STEC non-O157 in bovine manure. For both fresh-cut and whole lettuce, the application interval that resulted in the highest risk estimates is zero-day and larger application intervals at 45 to 120 days resulted in decreased predicted illnesses across all three regions (Table 12). Fig. 8 presents the mean relative risk of the STEC non-O157 scenarios across three regions when compared to the results from the baseline models. As seen in Fig. 8, predicted number of illnesses from untreated BSAAO scenarios remained higher than the risk estimates obtained from both baseline models across all application intervals up to 120 days. 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). It was also worth noting that the results and interpretations for STEC non-O157 were obtained under the assumption that STEC non-O157 strains follow the same dose-response relationship as STEC O157 strains. Based on evidence from investigation of STEC outbreaks, Koutsoumanis et al. (2020) suggested that the probability of infection upon exposure to other

STEC strains may approach that of O157:H7. Future epidemiology data and studies on STEC non-O157 strains can be helpful to better characterize the dose-response relationship.

Table 12. Mean predicted number of illnesses associated with STEC non-O157 from consumption of fresh-cut or whole lettuce grown in fields (20,000 lettuce heads) amended with untreated BSAAO by region and growing season.

Growing season	Region	Application interval (days)	Predicted average number of illnesses per field for fresh-cut lettuce	Predicted average number of illnesses per field for whole lettuce
Summer-fall	Mid-Atlantic	0	4650	11476
Summer-fall	Mid-Atlantic	45	289	1370
Summer-fall	Mid-Atlantic	60	80.1	673
Summer-fall	Mid-Atlantic	90	9.85	121
Summer-fall	Mid-Atlantic	120	1.87	29.3
Summer-fall	South	0	23.6	228
Summer-fall	South	45	0.142	2.30
Summer-fall	South	60	0.114	1.80
Summer-fall	South	90	0.019	0.326
Summer-fall	South	120	0.003	0.050
Summer-fall	West	0	419	1941
Summer-fall	West	45	28.4	148
Summer-fall	West	60	7.98	88.2
Summer-fall	West	90	0.829	12.9
Summer-fall	West	120	0.264	4.01
Winter-spring	Mid-Atlantic	0	4650	11476
Winter-spring	Mid-Atlantic	45	280	1290
Winter-spring	Mid-Atlantic	60	59.5	505
Winter-spring	Mid-Atlantic	90	7.90	104
Winter-spring	Mid-Atlantic	120	1.85	28.8
Winter-spring	South	0	23.6	228
Winter-spring	South	45	0.151	2.39
Winter-spring	South	60	0.102	1.37
Winter-spring	South	90	0.019	0.274
Winter-spring	South	120	0.002	0.027
Winter-spring	West	0	419	1941
Winter-spring	West	45	27.9	146
Winter-spring	West	60	6.63	78.8
Winter-spring	West	90	1.20	18.2
Winter-spring	West	120	0.290	4.59

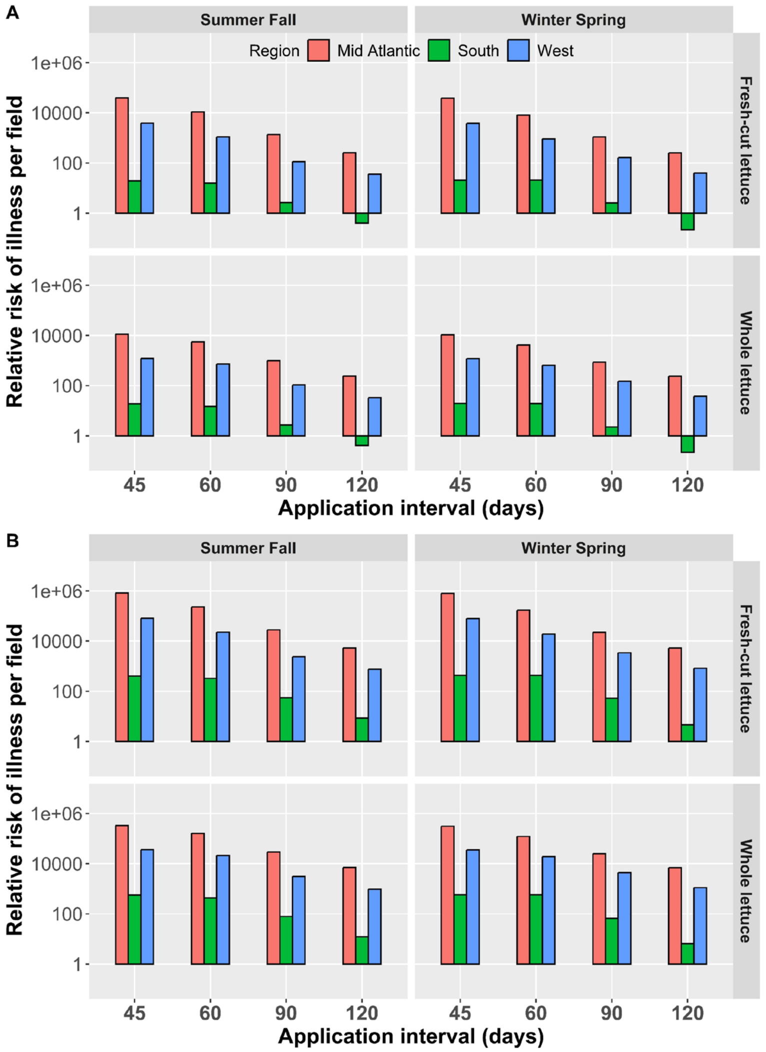

Fig. 8. Mean predicted number of illnesses associated with STEC non-O157 contaminated lettuce grown in fields amended with untreated BSAAO relative to the results from the baseline model with treated BSAAO (compost) using (A) a zero-day interval and (B) a 45-day interval across region and growing season.

3.1.2 Produce that grows in the ground (onion) and on the ground (cantaloupe)

The predicted number of illnesses from consumption of onions from fields amended with STEC O157 contaminated bovine manure and fresh-cut cantaloupes from fields amended with *Salmonella* contaminated poultry manure at different application intervals are summarized in Table 13. For both onions and fresh-cut cantaloupe, the highest mean predicted number of illnesses was observed from scenarios with a zero-day application interval and predicted illnesses decreased with increasing application intervals. The baseline models for onion and STEC O157 predicted an average of 0.21 illnesses per field with a zero-day interval and an average of 3.9×10^{-4} illnesses per field with a 45-day interval. For fresh-cut cantaloupe and *Salmonella*, predicted illnesses per field from the baseline models were 2.4×10^{-4} and 2.5×10^{-6} for zero-day interval and 45-day interval respectively. Risk and relative risk estimates varied by region and were generally higher in summer-fall growing season compared to winter-spring growing season (Table 13 & Fig. 9). The mean predicted risk across all regions and growing seasons are summarized in Table 14. For onion and STEC O157, predicted illnesses from 45- to 120-day interval scenarios were lower than zero-day baseline predictions but higher than 45-day baseline predictions (Table 14). For cantaloupe and *Salmonella*, all untreated BSAAO scenarios predicted higher number of illnesses than both baseline models' predictions (Table 14).

Table 13. Mean predicted number of illnesses from consumption of STEC O157 contaminated onions and *Salmonella* contaminated fresh-cut cantaloupes grown in fields amended with untreated BSAAO by region and by growing season.

Growing season	Region	Application interval (days)	Predicted average number of illnesses per field for onion and STEC O157	Predicted average number of illnesses per field for cantaloupe and *Salmonella*
Summer-fall	Mid-Atlantic	0	1.33	524
Summer-fall	Mid-Atlantic	45	0.002	69.5
Summer-fall	Mid-Atlantic	60	0.001	44.1
Summer-fall	Mid-Atlantic	90	2.00×10^{-4}	15.8
Summer-fall	Mid-Atlantic	120	6.67×10^{-5}	4.70
Summer-fall	South	0	10.8	253
Summer-fall	South	45	0.023	31.6
Summer-fall	South	60	0.012	20.1
Summer-fall	South	90	0.003	6.82
Summer-fall	South	120	0.001	2.18
Summer-fall	West	0	167	57.1
Summer-fall	West	45	0.345	6.13
Summer-fall	West	60	0.223	3.99
Summer-fall	West	90	0.055	0.873
Summer-fall	West	120	0.014	0.513
Winter-spring	Mid-Atlantic	0	1.33	364
Winter-spring	Mid-Atlantic	45	0.002	22.4
Winter-spring	Mid-Atlantic	60	0.001	14.8
Winter-spring	Mid-Atlantic	90	1.52×10^{-4}	4.64
Winter-spring	Mid-Atlantic	120	6.94×10^{-5}	1.72
Winter-spring	South	0	10.8	141
Winter-spring	South	45	0.020	8.00
Winter-spring	South	60	0.007	4.26
Winter-spring	South	90	0.002	1.51
Winter-spring	South	120	0.001	0.451
Winter-spring	West	0	74.6	57.1
Winter-spring	West	45	0.007	2.989
Winter-spring	West	60	0.002	1.880
Winter-spring	West	90	0.001	0.612
Winter-spring	West	120	1.54×10^{-4}	0.277

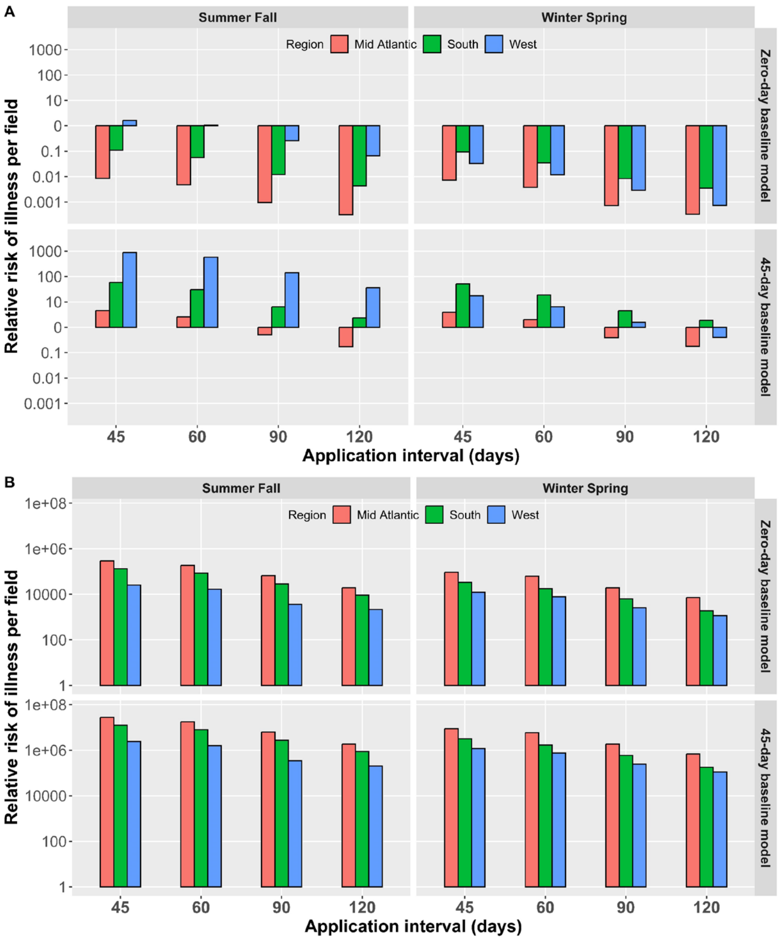

Fig. 9. Mean predicted number of illnesses associated with consumption of (A) onions contaminated with STEC O157 and (B) fresh-cut cantaloupes contaminated with *Salmonella* from fields amended with untreated BSAAO relative to the results from the baseline model with treated BSAAO (compost).

Table 14. Mean predicted number of illnesses associated with consumption of onions contaminated with STEC O157 and fresh-cut cantaloupes contaminated with *Salmonella* from fields amended with untreated BSAAO.

Scenario	Application interval (days)	Predicted average number of illnesses per field	Comparison with the zero-day baseline model[1]	Comparison with the 45-day baseline model
Onion/STEC O157	45	0.041	-	+
Onion/STEC O157	60	0.032	-	+
Onion/STEC O157	90	0.008	-	+
Onion/STEC O157	120	0.002	-	+
Fresh-cut cantaloupe/*Salmonella*	45	19.3	+	+
Fresh-cut cantaloupe/*Salmonella*	60	12.2	+	+
Fresh-cut cantaloupe/*Salmonella*	90	4.09	+	+
Fresh-cut cantaloupe/*Salmonella*	120	1.36	+	+

[1] “+”Indicates risk estimates that are greater than baseline model predictions; “-”Indicates risk estimates that are less than baseline model predictions.

3.2 Uncertainty analysis

The overall model uncertainty range of risk estimates considering model uncertainty sources is provided in Table 15 and uncertainty analysis results for individual variables are summarized in Table 16 for STEC O157 and Table 17 for *Salmonella*, where the predicted number of illnesses was calculated as the average of the unweighted mean predicted number of illnesses across the three regions and two growing seasons. Detailed results for each region are provided in Appendix C. Despite the differences in risk estimates from the uncertainty analysis, the impact of application intervals on predicted risk was generally consistent with the original model simulations where increasing time intervals reduced the predicted illnesses. When compared to results from the baseline model with treated BSAAO, most of the tested variables have a low impact on the relative risk and the overall comparison trend is consistent with the observations from the original model simulations. For STEC O157 in untreated bovine manure, predicted illnesses generally remained higher than the results from treated BSAAO baseline models until application interval increased to 120 days. For *Salmonella* in untreated poultry manure, predicted illnesses were higher than treated BSAAO baseline model results across all time intervals.

Among tested variables, the model is more sensitive to changes in initial contamination prevalence and initial contamination level in untreated bovine manure when predicting the risk associated with STEC O157. Using upper-bound values for initial contamination prevalence and levels, the predicted illness numbers exceeded the 0-day interval baseline model predictions even with a 120-day application interval, though the difference was minimal. However, considering the uncertainty range in prevalence, the upper-bound risk estimates for 90- or 120-day intervals fell below the 45-day baseline risk estimate (Table 16). When lower-bound initial contamination prevalence values were applied, a 90-day interval reduced predicted illness numbers below the risk estimates from the treated BSAAO baseline model. As a comparison, a 120-day interval was required to achieve similar reductions based on the original model simulations (Table 8). These findings demonstrate that initial contamination conditions can influence the predicted risk associated with application of untreated BSAAO in produce field. The BSAAO risk assessment model accounts for regional variations in pathogen contamination by modeling sampling data from three distinct regions separately. Future incorporation of additional manure survey studies and quantitative pathogen prevalence and level data, as they become available, could help reduce uncertainty in initial contamination conditions for untreated BSAAO. The longitudinal study conducted by Ramos et al. (2021) evaluated pathogen prevalence and persistence, including STEC and *Salmonella*, in manure-amended soils across multiple U.S. regions. While this study did not provide pathogen enumeration data from positive manure samples for direct use in risk assessment calculations, it supplied valuable multi-regional prevalence data that informed our baseline prevalence estimates for STEC non-O157 in the current risk assessment.

The risk assessment model is also sensitive to *Salmonella* survival rate on crops grown in the field, as the predicted number of illnesses was reduced drastically when alternative criteria were used (Table 17). In the original model simulations, only one study satisfied the inclusion criteria, yielding an average decline rate of 0.03 $\log_{10}$ CFU day^{-1} for *Salmonella*, which is substantially slower than the rates observed for STEC O157. During uncertainty analysis, the inclusion of additional data from laboratory/greenhouse studies and field trials with observation periods under 10 days increased the average daily die-off rate for *Salmonella* on lettuce to 0.28 $\log_{10}$ CFU day^{-1}. This represents approximately a 10-fold increase compared to the value used in the original model scenarios and resulted in a dramatic reduction in *Salmonella* risk estimates.

Considering the differences between field and laboratory/greenhouse survival data, additional field studies may improve the understanding of *Salmonella* survival on crops under field conditions over the long term. When comparing the relative risks estimates from uncertainty analysis with the baseline models, the trend remains consistent with the original model simulations as *Salmonella* risk estimates remained higher than baseline model predictions across all time intervals, indicating that the risk estimates are robust to the uncertainties in *Salmonella* survival rate on crops.

Table 15. Estimated overall model uncertainty range.

Interval (Day)	**Range of predicted risk for STEC O157 (min, max)[1]**	**Range of predicted risk for *Salmonella* (min, max)[1]**
45	(0.003, 8.59)	(2.02, 3932)
60	(0.001, 4.34)	(0.972, 1970)
90	(4.18×10^{-4}, 0.754)	(0.113, 997)
120	(3.35×10^{-4}, 0.027)	(0.027, 530)

[1] Calculated as number of illnesses from fields consist of 20,000 lettuce heads consumed as fresh cut.

Table 16. Summary of uncertainty analysis results for STEC O157.

Tested parameters	Test scenario[1]	Interval (Day)	Predicted risk[2]	Zero-day baseline results	45-day baseline risk estimates	Comparison to zero-day baseline[3]	Comparison to 45-day baseline[3]
Original model scenarios	Model default	45	0.089	0.004	0.013	1.34	0.83
Original model scenarios	Model default	60	0.022	0.004	0.013	0.73	0.22
Original model scenarios	Model default	90	0.007	0.004	0.013	0.25	-0.27
Original model scenarios	Model default	120	0.004	0.004	0.013	-0.003	-0.51
Initial prevalence	UB	45	0.308	0.004	0.013	1.88	1.37
Initial prevalence	UB	60	0.049	0.004	0.013	1.08	0.58
Initial prevalence	UB	90	0.010	0.004	0.013	0.39	-0.11
Initial prevalence	UB	120	0.005	0.004	0.013	0.09	-0.41
Initial prevalence	LB	45	0.022	0.004	0.013	0.74	0.23
Initial prevalence	LB	60	0.011	0.004	0.013	0.44	-0.07
Initial prevalence	LB	90	0.003	0.004	0.013	-0.13	-0.64
Initial prevalence	LB	120	0.001	0.004	0.013	-0.61	-1.11
Initial level	UB	45	0.788	0.004	0.013	2.29	1.78
Initial level	UB	60	0.094	0.004	0.013	1.37	0.86
Initial level	UB	90	0.028	0.004	0.013	0.84	0.33
Initial level	UB	120	0.016	0.004	0.013	0.60	0.09
Initial level	LB	45	0.186	0.004	0.013	1.66	1.16
Initial level	LB	60	0.023	0.004	0.013	0.76	0.25
Initial level	LB	90	0.006	0.004	0.013	0.17	-0.34
Initial level	LB	120	0.003	0.004	0.013	-0.13	-0.64
Soil survival model	UB	45	0.563	0.004	0.025	2.15	1.35
Soil survival model	UB	60	0.206	0.004	0.025	1.71	0.92
Soil survival model	UB	90	0.022	0.004	0.025	0.73	-0.06
Soil survival model	UB	120	0.008	0.004	0.025	0.32	-0.48
Soil survival model	LB	45	0.055	0.004	0.008	1.14	0.84
Soil survival model	LB	60	0.018	0.004	0.008	0.65	0.35
Soil survival model	LB	90	0.005	0.004	0.008	0.05	-0.43
Soil survival model	LB	120	0.002	0.004	0.008	-0.30	-0.60
Soil survival model	Alternative[4]	45	0.246	0.004	0.001	1.76	2.39
Soil survival model	Alternative	60	0.029	0.004	0.001	0.83	1.46
Soil survival model	Alternative	90	0.004	0.004	0.001	0.02	0.65
Soil survival model	Alternative	120	8.48×10^{-4}	0.004	0.001	-0.70	-0.07
Maximum transfer radius	UB	45	0.613	0.004	0.014	2.16	1.64
Maximum transfer radius	UB	60	0.054	0.004	0.014	1.10	0.59
Maximum transfer radius	UB	90	0.015	0.004	0.014	0.54	0.03
Maximum transfer radius	UB	120	0.004	0.004	0.014	-0.03	-0.54
Transfer model	UB	45	0.862	0.009	0.029	1.94	1.47
Transfer model	UB	60	0.176	0.009	0.029	1.25	0.78
Transfer model	UB	90	0.028	0.009	0.029	0.45	-0.02
Transfer model	UB	120	0.009	0.009	0.029	-0.04	-0.51
Transfer model	LB	45	0.045	0.003	0.07	1.17	0.80
Transfer model	LB	60	0.014	0.003	0.07	0.66	0.29
Transfer model	LB	90	0.007	0.003	0.07	0.36	-0.01
Transfer model	LB	120	0.003	0.003	0.07	-0.01	-0.38

[1]UB: upper-bound parameter values; LB: lower-bound parameter values. [2]Calculated as number of illnesses from fields consist of 20,000 lettuce heads consumed as fresh cut. [3]Calculated as the ratio of results from untreated BSAAO scenarios to the baseline models' results in $\log_{10}$scale. [4]Alternative Weibull survival model for survival of STEC O157 in amended soils.

Table 17. Summary of uncertainty analysis results for *Salmonella*.

Tested parameters	Test scenario[1]	Interval (Day)	Predicted risk[2]	Zero-day baseline results	45-day baseline risk estimates	Comparison to zero-day baseline[3]	Comparison to 45-day baseline[3]
Original model scenarios	Model default	45	144	3.23×10^{-4}	3.69×10^{-5}	5.65	6.59
Original model scenarios	Model default	60	64.6	3.23×10^{-4}	3.69×10^{-5}	5.30	6.24
Original model scenarios	Model default	90	19.4	3.23×10^{-4}	3.69×10^{-5}	4.78	5.72
Original model scenarios	Model default	120	7.64	3.23×10^{-4}	3.69×10^{-5}	4.37	5.32
Initial prevalence	UB	45	153	3.23×10^{-4}	3.69×10^{-5}	5.68	6.62
Initial prevalence	UB	60	93.5	3.23×10^{-4}	3.69×10^{-5}	5.46	6.40
Initial prevalence	UB	90	30.1	3.23×10^{-4}	3.69×10^{-5}	4.97	5.91
Initial prevalence	UB	120	9.30	3.23×10^{-4}	3.69×10^{-5}	4.46	5.40
Initial prevalence	LB	45	29.6	3.23×10^{-4}	3.69×10^{-5}	4.96	5.90
Initial prevalence	LB	60	5.37	3.23×10^{-4}	3.69×10^{-5}	4.22	5.16
Initial prevalence	LB	90	2.12	3.23×10^{-4}	3.69×10^{-5}	3.82	4.76
Initial prevalence	LB	120	0.486	3.23×10^{-4}	3.69×10^{-5}	3.18	4.12
Initial level	UB	45	402	3.23×10^{-4}	3.69×10^{-5}	6.10	7.04
Initial level	UB	60	151	3.23×10^{-4}	3.69×10^{-5}	5.67	6.61
Initial level	UB	90	56.1	3.23×10^{-4}	3.69×10^{-5}	5.24	6.18
Initial level	UB	120	15.6	3.23×10^{-4}	3.69×10^{-5}	4.68	5.63
Initial level	LB	45	71.9	3.23×10^{-4}	3.69×10^{-5}	5.35	6.29
Initial level	LB	60	24.8	3.23×10^{-4}	3.69×10^{-5}	4.89	5.83
Initial level	LB	90	9.76	3.23×10^{-4}	3.69×10^{-5}	4.48	5.42
Initial level	LB	120	3.62	3.23×10^{-4}	3.69×10^{-5}	4.05	4.99
Soil survival model	UB	45	134	3.23×10^{-4}	1.46×10^{-4}	5.62	5.96
Soil survival model	UB	60	49.2	3.23×10^{-4}	1.46×10^{-4}	5.18	5.53
Soil survival model	UB	90	17.9	3.23×10^{-4}	1.46×10^{-4}	4.74	5.09
Soil survival model	UB	120	7.00	3.23×10^{-4}	1.46×10^{-4}	4.34	4.68
Soil survival model	LB	45	89.9	3.23×10^{-4}	9.75×10^{-6}	5.44	6.96
Soil survival model	LB	60	29.1	3.23×10^{-4}	9.75×10^{-6}	4.95	6.47
Soil survival model	LB	90	8.54	3.23×10^{-4}	9.75×10^{-6}	4.42	5.94
Soil survival model	LB	120	2.60	3.23×10^{-4}	9.75×10^{-6}	3.91	5.43
Soil survival model	Alternative[4]	45	164	3.23×10^{-4}	1.64×10^{-6}	5.68	5.99
Soil survival model	Alternative	60	73.7	3.23×10^{-4}	1.64×10^{-6}	5.33	5.65
Soil survival model	Alternative	90	19.0	3.23×10^{-4}	1.64×10^{-6}	4.74	5.06
Soil survival model	Alternative	120	6.23	3.23×10^{-4}	1.64×10^{-6}	4.26	4.58
Maximum transfer radius	UB	45	115	3.42×10^{-4}	3.76×10^{-5}	5.53	6.49
Maximum transfer radius	UB	60	51.9	3.42×10^{-4}	3.76×10^{-5}	5.18	6.14
Maximum transfer radius	UB	90	16.4	3.42×10^{-4}	3.76×10^{-5}	4.68	5.64
Maximum transfer radius	UB	120	5.30	3.42×10^{-4}	3.76×10^{-5}	4.19	5.15
Transfer model	UB	45	306	4.10×10^{-4}	6.14×10^{-5}	5.87	6.92
Transfer model	UB	60	126	4.10×10^{-4}	6.14×10^{-5}	5.49	6.53
Transfer model	UB	90	17.1	4.10×10^{-4}	6.14×10^{-5}	4.62	5.58
Transfer model	UB	120	9.17	4.10×10^{-4}	6.14×10^{-5}	4.35	5.40
Transfer model	LB	45	55	2.53×10^{-4}	1.31×10^{-5}	5.34	6.62
Transfer model	LB	60	18.4	2.53×10^{-4}	1.31×10^{-5}	4.86	6.15
Transfer model	LB	90	4.36	2.53×10^{-4}	1.31×10^{-5}	4.24	5.52
Transfer model	LB	120	0.92	2.53×10^{-4}	1.31×10^{-5}	3.56	4.85
Survival model - crops	Alternative[5]	45	3.87	3.23×10^{-4}	1.01×10^{-6}	4.08	6.58
Survival model - crops	Alternative	60	0.809	3.23×10^{-4}	1.01×10^{-6}	3.40	5.90
Survival model - crops	Alternative	90	0.134	3.23×10^{-4}	1.01×10^{-6}	2.62	5.12
Survival model - crops	Alternative	120	0.005	3.23×10^{-4}	1.01×10^{-6}	1.19	3.69

[1] UB: upper-bound parameter values; LB: lower-bound parameter values. [2] Calculated as number of illnesses from fields consist of 20,000 lettuce heads consumed as fresh-cut. [3] Calculated as the ratio of results from untreated BSAAO scenarios to the baseline models' results in $\log_{10}$ scale. [4] Alternative Weibull survival model for *Salmonella* survival in amended soils; [5] Alternative inclusion criteria for *Salmonella* survival rate on crops.

3.3 Summary and conclusions

In summary, we developed a risk assessment model to estimate the risk of human illness associated with the consumption of produce grown in fields amended with treated and untreated BSAAO. Using data specific for three different regions (Mid-Atlantic, south, and west) and two growing seasons (summer-fall and winter-spring), we assessed the impact of various application intervals on the estimated risk associated with different produce commodities, pathogens, and type of BSAAO. In general, application intervals (from 45 days to 120 days) greatly reduced the mean predicted number of illnesses associated with pathogens from produce grown in fields amended with untreated BSAAO; however, the mean risk estimates varied greatly by region and by growing season. For both fresh-cut and whole lettuce, when compared to the baseline model with treated BSAAO, the average relative risk estimates across regions and growing seasons for STEC O157 in untreated bovine manure were reduced to below the risk estimates from the baseline models using treated BSAAO when a 120-day interval between application and harvest was used. However, the predicted number of illnesses associated with *Salmonella* in untreated poultry manure remained above the risk estimates from treated BSAAO even at intervals of up to 120 days for lettuce. For onions harvested from fields after a time interval of 45 to 120 days post application of untreated bovine manure, predicted illnesses associated with STEC O157 were lower than the risk estimates from treated BSAAO with a zero-day interval but higher than those from treated BSAAO with a 45-day interval. For fresh-cut cantaloupes, predicted illnesses associated with *Salmonella* in untreated poultry manure were higher than the risk estimates from treated BSAAO even when an interval of up to 120 days between application and harvest was used.

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. The virtual production systems incorporated into the model reflect specifications and agricultural practices representative of certain commercial produce operations in the U.S. While the overall trends identified in the results are expected to remain consistent, specific quantitative risk estimates will likely vary for farms operating under different conditions or employing different agricultural practices (e.g., types of irrigation). The model represents the most accurate estimates possible given currently available data and information, and uncertainty analysis indicates that the findings and conclusions remain reliable despite inherent uncertainties in the data. Future model refinements could incorporate additional data as they become available, including pathogen contamination data from untreated BSAAO in other geographic regions, pathogen survival data in amended soils, pathogen transfer and subsequent survival on produce crops, and STEC non-O157 dose-response relationships. These additions can potentially extend and improve the BSAAO risk assessment model predictions.

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Appendix A: R script for the logw0 data transformation.

The logw0 data transformation uses the R function below described in Weller et al. (2017). The function is an implementation of the data transformation method described in McCune and Grace (2002) that preserves the original order of magnitude and maps zero to zero.

R function script:

```
logw0 <- function (myzerodata){
         c <- floor(log(min(myzerodata[myzerodata!=0],na.rm = T)))
         d <- exp(c)
         xt <- log10(myzerodata+d) - c
         return(list(xt= xt , c=c, d=d))
        }
```

Reference:

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Appendix B: Additional results for risk associated with *Salmonella* in untreated poultry manure.

Table B1. Mean predicted number of illnesses associated with *Salmonella* from consumption of fresh-cut lettuce grown in fields (20,000 lettuce heads) amended with untreated poultry manure at application intervals from 180 to 600 days.

Application interval (days)	Mean predicted number of illnesses associated with *Salmonella*[*]
180	0.838
240	0.153
360	0.002
480	4.09×10^{-4}
600	1.76×10^{-4}

[*] Calculated as number of illnesses from fields consisting of 20,000 lettuce heads consumed as fresh cut.

Table B2. Mean predicted number of illnesses associated with *Salmonella* from consumption of whole lettuce grown in fields (20,000 lettuce heads) amended with untreated poultry manure at application intervals from 180 to 600 days.

Application interval (days)	Mean predicted number of illnesses associated with *Salmonella*[*]
180	13.6
240	2.53
360	0.038
480	0.007
600	0.003

[*] Calculated as number of illnesses from fields consisting of 20,000 lettuce heads consumed as whole.

Appendix C: Results for uncertainty analysis.

Detailed results from uncertainty analysis are shown in the tables below.

Table C1. Uncertainty analysis – impact of initial contamination prevalence in untreated BSAAOs on the predicted number of illnesses[1].

Region/ scenario	Interval (day)	Results for STEC O157 using upper-bound values	Results for STEC O157 using lower-bound values	Results for *Salmonella* using upper-bound values	Results for *Salmonella* using lower-bound values
Mid Atlantic	45	0.075	0.015	341	39.4
Mid Atlantic	60	0.017	0.003	192	3.63
Mid Atlantic	90	0.001	1.13×10^{-4}	62.1	1.02
Mid Atlantic	120	8.36×10^{-4}	3.15×10^{-5}	16.7	0.414
South	45	0.068	0.022	74.2	43.7
South	60	0.053	0.017	70.2	10.3
South	90	0.016	0.006	22.8	4.91
South	120	0.006	0.002	9.4	0.827
West	45	0.781	0.027	43.1	5.95
West	60	0.078	0.012	18.2	2.14
West	90	0.019	0.003	5.33	0.415
West	120	0.008	0.001	1.81	0.217

[1] Calculated as number of illnesses from fields consisting of 20,000 lettuce heads consumed as fresh cut.

Table C2. Uncertainty analysis – impact of initial contamination level in untreated BSAAOs on the predicted number of illnesses[1].

Region/ scenario	**Interval (day)**	**Results for STEC O157 using upper-bound values**	**Results for STEC O157 using lower-bound values**	**Results for *Salmonella* using upper-bound values**	**Results for *Salmonella* using lower-bound values**
Mid Atlantic	45	0.039	0.014	433	210
Mid Atlantic	60	0.008	0.004	121	72.5
Mid Atlantic	90	7.30×10^{-4}	1.91×10^{-4}	56.9	28.8
Mid Atlantic	120	3.34×10^{-4}	1.67×10^{-4}	18.1	10.7
South	45	0.251	0.045	74.2	3.91
South	60	0.117	0.036	70.2	0.900
South	90	0.050	0.007	22.8	0.337
South	120	0.033	0.005	9.4	0.102
West	45	2.075	0.498	43.1	1.75
West	60	0.158	0.028	18.2	0.944
West	90	0.033	0.010	5.33	0.166
West	120	0.015	0.004	1.81	0.083

[1] Calculated as number of illnesses from fields consisting of 20,000 lettuce heads consumed as fresh cut.

Table C3. Uncertainty analysis – impact of soil survival model on the predicted number of illnesses[1].

Region/ scenario	Interval (day)	Results for STEC O157 using upper-bound values	Results for STEC O157 using lower-bound values	Results for *Salmonella* using upper-bound values	Results for *Salmonella* using lower-bound values
Mid Atlantic	45	0.090	8.03×10^{-4}	292	200
Mid Atlantic	60	0.029	7.05×10^{-4}	100	72.1
Mid Atlantic	90	0.003	2.01×10^{-4}	39.2	20.8
Mid Atlantic	120	0.001	5.08×10^{-5}	15.0	5.97
South	45	0.521	0.039	69.9	55.9
South	60	0.352	0.020	34.6	10.9
South	90	0.039	0.005	9.66	3.26
South	120	0.014	0.004	3.70	0.972
West	45	1.078	0.127	39.1	13.6
West	60	0.238	0.034	12.7	4.18
West	90	0.023	0.003	4.99	1.53
West	120	0.010	0.002	2.35	0.861

[1] Calculated as number of illnesses from fields consisting of 20,000 lettuce heads consumed as fresh cut.

Table C4. Uncertainty analysis – impact of splash radius on the predicted number of illnesses[1].

Region/ scenario	Interval (day)	Results for STEC O157 using upper-bound values	Results for *Salmonella* using upper-bound values
Mid Atlantic	45	0.039	242
Mid Atlantic	60	0.008	108
Mid Atlantic	90	4.13×10^{-4}	34.6
Mid Atlantic	120	2.24×10^{-5}	11.8
South	45	0.507	68.4
South	60	0.215	31.2
South	90	0.008	10.1
South	120	0.003	2.62
West	45	1.39	33.2
West	60	0.721	16.0
West	90	0.036	4.42
West	120	0.005	1.49

[1] Calculated as number of illnesses from fields consisting of 20,000 lettuce heads consumed as fresh cut.

Table C5. Uncertainty analysis – impact of pathogen transfer models on the predicted number of illnesses[1].

Region/ scenario	Interval (day)	Results for STEC O157 using upper-bound values	Results for STEC O157 using lower-bound values	Results for *Salmonella* using upper-bound values	Results for *Salmonella* using lower-bound values
Mid Atlantic	45	0.155	0.008	639	115
Mid Atlantic	60	0.032	0.003	269	39.3
Mid Atlantic	90	0.005	0.001	37.3	9.39
Mid Atlantic	120	0.002	0.001	19.9	1.96
South	45	0.233	0.012	182	33.4
South	60	0.048	0.004	73	10.8
South	90	0.008	0.002	8.94	2.50
South	120	0.002	0.001	4.57	0.544
West	45	2.20	0.115	95.8	14.9
West	60	0.449	0.036	34.5	4.97
West	90	0.071	0.018	4.99	1.19
West	120	0.023	0.008	2.98	0.266

[1] Calculated as number of illnesses from fields consist of 20,000 lettuce heads consumed as fresh cut.

Table C6. Uncertainty analysis – impact of *Salmonella* survival rate on crops on the predicted number of illnesses[1].

Region/ scenario	Interval (day)	Results for STEC O157 using alternative survival rate values
Mid Atlantic	45	9.43
Mid Atlantic	60	1.61
Mid Atlantic	90	0.326
Mid Atlantic	120	0.130
South	45	1.75
South	60	0.698
South	90	0.061
South	120	0.024
West	45	0.430
West	60	0.119
West	90	0.015
West	120	0.004

[1] Number of illnesses associated with consumption of fresh-cut lettuce from fields consisting of 20,000 heads.