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# Risk Assessment of Salmonellosis from Consumption of Alfalfa Sprouts and Evaluation of the Public Health Impact of Sprout Seed Treatment and Spent Irrigation Water Testing

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We developed a risk assessment of human salmonellosis associated with consumption of alfalfa sprouts in the United States to evaluate the public health impact of applying treatments to seeds (0–5-$\log_{10}$ reduction in *Salmonella*) and testing spent irrigation water (SIW) during production. The risk model considered variability and uncertainty in *Salmonella* contamination in seeds, *Salmonella* growth and spread during sprout production, sprout consumption, and *Salmonella* dose response. Based on an estimated prevalence of 2.35% for 6.8 kg seed batches and without interventions, the model predicted 76,600 (95% confidence interval (CI) 15,400–248,000) cases/year. Risk reduction (by 5- to 7-fold) predicted from a 1-$\log_{10}$ seed treatment alone was comparable to SIW testing alone, and each additional 1-$\log_{10}$ seed treatment was predicted to provide a greater risk reduction than SIW testing. A 3-$\log_{10}$ or a 5-$\log_{10}$ seed treatment reduced the predicted cases/year to 139 (95% CI 33–448) or 1.4 (95% CI <1–4.5), respectively. Combined with SIW testing, a 3-$\log_{10}$ or 5-$\log_{10}$ seed treatment reduced the cases/year to 45 (95% CI 10–146) or <1 (95% CI <1–1.5), respectively. If the SIW coverage was less complete (i.e., less representative), a smaller risk reduction was predicted, e.g., a combined 3-$\log_{10}$ seed treatment and SIW testing with 20% coverage resulted in an estimated 92 (95% CI 22–298) cases/year. Analysis of alternative scenarios using different assumptions for key model inputs showed that the predicted relative risk reductions are robust. This risk assessment provides a comprehensive approach for evaluating the public health impact of various interventions in a sprout production system.

**KEY WORDS:** Interventions; pathogens in sprouts; risk assessment; seed treatment; spent irrigation water testing

## 1. INTRODUCTION

Outbreaks of foodborne illness related to the consumption of sprouts have been reported in the United States[1–4] and worldwide.[5–8] In the United States alone, there were approximately 46 outbreaks, accounting for 2,474 cases, attributed to sprouts between 1996 and 2016.[9,10] Those outbreaks were associated with various types of sprouts contaminated with a variety of pathogens, including *Salmonella*,

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*Escherichia coli* O157:H7, and other Shiga-toxin-producing *E. coli* and *Listeria monocytogenes*. Sprouts present a special food safety concern because the conditions under which they are produced (temperature, water activity, pH, and available nutrients) favor the growth of bacterial pathogens, if present.[2] In addition, sprouts are often consumed raw.[5,9]

Across the sprout industry, diverse food safety practices and interventions are implemented with various degrees of stringency, including the use of seed treatment, seed testing, sampling and testing of spent irrigation water (SIW) for certain pathogens (e.g., *Salmonella* and *E. coli* O157:H7), environmental monitoring for *Listeria*, and finished product testing, some of which are required by Food and Drug Administration (FDA) regulation.[2,4,9] European legislation[11] requires control for human pathogenic verotoxigenic *E. coli*, including five serogroups most commonly associated with sprouted seeds (O157, O26, O103, O111, and O145).[12] Many different seed treatments are used by sprouters (e.g., chemical treatment with chlorine compounds or physical treatments such as heat), each of varying degrees of efficacy.[1,13,14] Results from sprout research studies have been used to inform guidance for industry and to enhance the implementation of food safety best practices for sprout operations.[2,9,15]

Two microbial risk assessments have been published to evaluate contamination and risk associated with sprouts.[16,17] The model reported by Montville and Schaffner[16] predicts the behavior of bacterial pathogens (including *Salmonella* and *E. coli* O157:H7) during sprouting to determine the likelihood of sprout production batch contamination and the relative effect of preproduction sampling and postproduction sampling on the likelihood of detecting batch contamination. This model used hypothetical prevalence and levels for pathogens in seeds. It did not include consumption and dose–response elements and, thus, did not estimate the risk of illness to consumers. The second model, reported by Ding and Fu,[17] focused on potential public health impact from sprout consumption in the United States and estimated risk reduction from different mitigation approaches. It included pathogen contamination and growth during sprout production. However, variability in pathogen transfer during sprout production was not considered and systematic uncertainty characterization was not derived in this study.[17] Both previous models did not have access to data on the contamination of *Salmonella* for seeds used in sprout production in the United States.

In the notification of availability of the draft sprout guidance for industry in early 2017,[18] the U.S. FDA indicated the development of a risk assessment to evaluate the public health impact of seed treatment and SIW testing in a sprout production system. We present here FDA's risk assessment, which evaluates the risk of human salmonellosis associated with *Salmonella*-contaminated alfalfa sprout consumption by the U.S. population. The risk assessment provides a framework within which to understand the impact of seed treatment and SIW testing on reducing microorganisms of public health significance. *Salmonella* was the most common pathogen reported in outbreaks linked to sprouts and the majority of the outbreaks were attributed to alfalfa sprouts.[1,10] The risk assessment model we developed considers separately variability and uncertainty and uses updated data collected in the last decade. Sprout operations that are subject to subpart M of the produce safety rule[19] must use treated seeds and perform SIW testing. We used the risk assessment model to evaluate the reduction in risk resulting from the following interventions: (1) applying treatments to seeds intended for sprouting with different $\log_{10}$ reductions for *Salmonella*, (2) testing SIW for the presence of *Salmonella*, and (3) both applying a seed treatment and testing SIW for the presence of *Salmonella*.

## 2. MATERIALS AND METHODS

### 2.1. Typical Steps in Sprout Production and Overview of the Risk Assessment Model

The model is based on sprout production and other data relevant to the United States. Although sprout operations may vary depending on the types of sprouts, growing containers, sprouting practices, and other factors, typical steps include seed purchase from a supplier, pregermination soaking of seeds, seed germination and growth, sprout harvest, postharvest activities such as washing/handling/packaging, subsequent refrigerated storage, and distribution to the marketplace and consumers.[2,9,20] Seed treatment may occur by seed suppliers or after seed purchase by sprouters; SIW testing, when employed, occurs during the production. Fig. 1 illustrates the process contamination module. Table I provides model inputs and mathematical derivations used in the present risk assessment model. A web-based user interface that allows

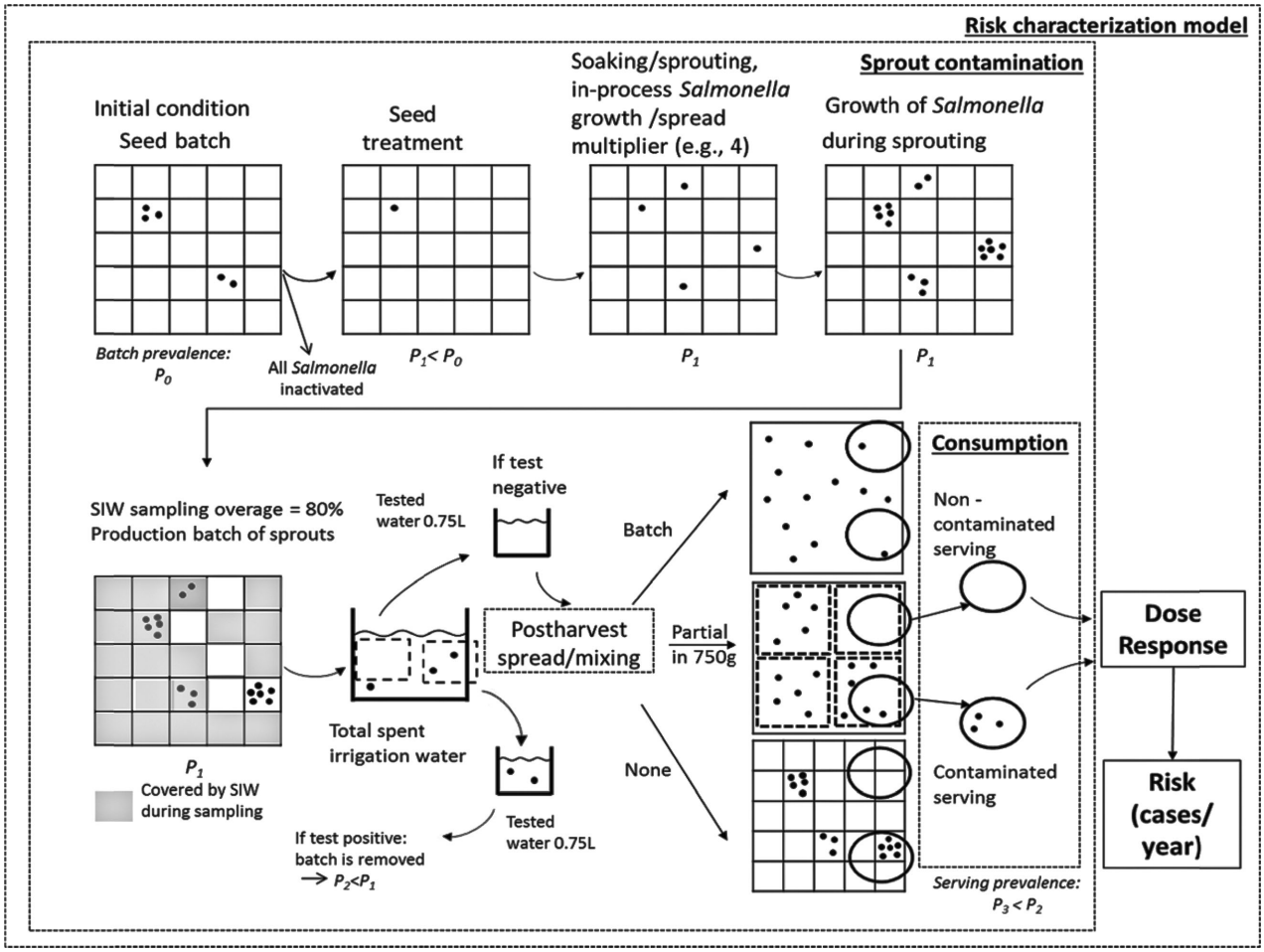


**Fig. 1.** Conceptual overview of the *Salmonella* in alfalfa sprouts risk assessment model.

users to enter different data sets and assumptions was developed. The code and the access to the web-based user interface are available on request to the corresponding author.

A second-order Monte Carlo simulation[21,22] was implemented to evaluate the expected number of salmonellosis cases per year from the consumption of alfalfa sprouts in the United States and the uncertainty surrounding this estimate. Within the simulation, the evolution of the prevalence of *Salmonella* in batches of seeds and production batches of sprouts, and the number of *Salmonella* cells in batches of seeds and production batches of sprouts were evaluated in parallel, using methodology previously described.[23–26] We considered the spatial distribution of the *Salmonella* cells in a contaminated batch at the scale of virtual units of 25 g of seed. By specifying the spatial distribution pattern that, in general, influences the estimated likelihood of a food to cause illness,[27] this model can predict the impact of cell spreading during production on the risk estimate (Fig. 1).

## 2.2. Prevalence and Initial Contamination of the Seed Batch

For the purpose of this model, a seed batch is defined as the amount of seeds sprouted at the same time, which yields one production batch of sprouts. A production batch of sprouts refers to the sprouts grown from one batch of seeds in one growing unit, e.g., a single drum or bin, or a single rack of trays that are connected to each other.[9] The types of growing units used may vary depending on the type of sprouts and sprouting equipment used.[2,20] Information from FDA inspections[28] showed that the average size is 15 lb (6.8 kg) for seed batch for rotary drums commonly used for growing alfalfa sprouts,

**Table I.** Overview of Input Variables, Mathematical Notations, and Equations for the *Salmonella*-Alfalfa Sprouts Risk Assessment Model

| Parameter | Symbol (Unit) | Definition[a] |
|---|---|---|
| **Exposure Module** | | |
| Initial conditions for seeds | | |
| Initial unit size | $M_u$ (g) | 25 g |
| Batch size | $M_b$ (g) | Uniform(6,810, 22,700) g, i.e., Uniform(15, 50) lb |
| Units per batches | $N_u$ | $M_b$ / $M_u$ |
| Units per 6.8 kg batches | $N_v$ | 6,810 / $M_u$ |
| Initial batch prevalence | $P_0$ | 2.35% (i.e., 4/170 per 6.8 kg batches) [Beta(4+0.5,170–4+0.5)][b] |
| Prevalence of contaminated units | $P_u$ | $1-(1-P_0)^{N_v^{-1}}$ |
| Initial number of positive units per contaminated batch | $N_0$ | ~ BinomialPos($N_u$, $P_u$) |
| Initial levels in positive unit | $C_{0,u}$ (CFU) | UniformDiscrete (1, $C_{max}$), [$C_{max}$ ~ Uniform(10,12)][b] |
| Total number of cells in positive batch | $C_{0b}$ (CFU) | $\sum_{u=1}^{N_u} C_{0,u}$ |
| **Treatment** | | |
| $Log_{10}$ reduction from seed treatment | $L$ | Choice: 0 $log_{10}$, 1 $log_{10}$, 2 $log_{10}$, 3 $log_{10}$, 4 $log_{10}$, or 5 $log_{10}$ |
| Probability of survival | $\rho_s$ | $10^{(-L)}$ |
| Number of cells in positive batch post seed treatment disinfection | $C_{1b}$ (CFU) | ~ BinomialPos($C_{0b}$, $\rho_s$) |
| Number of cells in positive units | $C_{1,u}$ (CFU) | ~ Multinomial($C_{1b}$, $C_{0,u}$ / $C_{0b}$) (the remaining cells are dispatched in positive units, with a probability proportional to the origin contamination) |
| Number of positive units per contaminated batch post seed treatment disinfection | $N_1$ | Number of units with $C_{1,u} > 0$ |
| Prevalence of contaminated batches post seed treatment disinfection | $P_1$ | $P_0 \times (1-(1-_s)^{C_{0b}})$ |
| **Multiplier** | | |
| In-process pathogen spread multiplier | $M$ | UniformDiscrete(1,5) |
| Number of positive units | $N_2$ | $N_1 \times M$ |
| Number of cells in positive units post spread | $C_{2,u}$ (CFU) | $C_{1,u}$: The number of cells in each of the new positive units is set to the number of cells in the original positive units, assuming simultaneous growth and spread of cells in the original positive units |
| **Growth** | | |
| Pathogen growth: growth rate | $\mu$ (log/hour) | BetaPert(0.03, 0.11, 0.54) × log(10) |
| Maximum growth: number of generations | $G$ (log) | Uniform($G_m$, 16) × log(2) [$G_m$ : Pr($G_m = 0$)=20%, Pr($G_m = 3$)=80%][b] |
| Growth at time $t$ | $g(t)$ (log) | minute($\mu \times t$, $G$) |
| Maximum population density | MPD (CFU/g) | 4 $log_{10}$ |
| Time of SIW sampling | $t_1$ (hour) | 48 hours |
| Duration of cell growth during production | $t_2$ (hour) | 120 hours |
| Number of cells in each unit at the time of SIW sampling | $C_{3,u}$ (CFU) | ~ min($C_{2,u}$ + NegativeBinomial($C_{2,u}$, $e^{-g(t_1)}$), $10^{MPD} \times M_u$) |
| Number of cells in each unit at the end of cell growth during production | $C_{4,u}$ (CFU) | ~ min($C_{3,u}$ + NegativeBinomial($C_{3,u}$, $e^{-(g(t_2)-g(t_1))}$), $10^{MPD} \times M_u$) |

(*Continued*)

**Table I** (Continued)

| Parameter | Symbol (Unit) | Definition[a] |
|---|---|---|
| **Irrigation** | | |
| Coverage of production batch by irrigation water during sampling | $\delta$ | Choice: 0, 20, 40, 60, 80, or 100 (%) |
| Number of positive units touched by spent irrigation water | $N_3$ | $\sim$ HypergeometricPos($N_2$, $N_u - N_2$, [$N_u \times \delta$]) |
| Probability to have a positive unit touched by spent irrigation water | $\rho_1$ | $P_1 \times (1 - \frac{(N_u-N_2)!(N_u-[N_u\times\delta])!}{N_u!((N_u-N_2)-[N_u\times\delta])!})$ (The fraction corresponds to the probability to observe 0 success in the hypergeometric process) |
| Corresponding number of cells in sampled in-process sprouts | $C_s$ (CFU) | Sum of $C_{3,u}$ cells (at the time of SIW sampling) present in $N_3$ positive units randomly sampled among the $N_2$ positive units |
| Yield of sprouts | $W$ | Uniform(6, 7) times |
| Ratio of volume of water to seeds/in-process sprouts per irrigation cycle | $V_m$ | [volume Uniform(1, 5):1][b] |
| Differences in pathogen $\log_{10}$ concentrations between spent irrigation water and sprouts | $A$ | Empirical distribution (Fig. 2) |
| Proportion of cells transferring from in-process sprouts to water | $B$ | $1/(1 + (W/V) \times 10^A)$, see Appendix |
| **SIW testing** | | |
| Number of cells in the positive SIW | $C_w$ (CFU) | $\sim$ BinomialPos($C_s$, $B$) |
| Probability to have a contaminated SIW | $\rho_2$ | $\rho_1 \times (1 - (1 - P_t)^{C_s})$ |
| Volume water used for irrigation during sampling | $V_w$ (L) | $M_u \times V_m \times \delta$ / 1,000 |
| Volume of SIW tested | $V_t$ (L) | Choice: 0.75 L, 0.20 L, and 1.5 L |
| Number of cells in the positive SIW sample | $C_s$ (CFU) | $\sim$ BinomialPos($C_w$, $V_t$ / $V_w$) |
| Probability to have a contaminated sample | $\rho_3$ | $\rho_2 \times (1 - (1 - V_t/V_w)^{C_w})$ |
| Probability to detect one cell | $\rho_d$ | Choice: 1 and 0.8 |
| Probability to obtain a positive sample | $\rho_4$ | $\rho_3 \times (1 - (1 - \rho_d)^{C_s})$ |
| Testing efficiency | $T$ | $\rho_4$ / $P_1$ |
| Probability of a batch of sprouts being positive post SIW test | $\rho_5$ | $P_1 \times (1 - T)$ |
| Batch kept following SIW testing | | Sample with replacement over iteration, each batch having a probability $(1/\rho_{4,b})/\sum_i^N (1/\rho_{4,i})$ of being in the post SIW test process, where $N$ is the number of iterations |
| Prevalence post-test and discard of positive batches | $P_2$ | Mean over the iterations of $\rho_5$ |
| Size of a batch of sprouts | $M_{bs}$ (g) | $M_b \times W$ |
| Size of a unit of sprouts | $M_{us}$ (g) | $M_u \times W$ |

(*Continued*)

**Table I** (Continued)

| Parameter | Symbol (Unit) | Definition[a] |
|---|---|---|
| **Postharvest** | | |
| Extent of mixing/pathogen spread | $M_p$ | [Ranging from no spread, to partial spread, to complete spread from one or more contaminated units [$M_u$] to the entire production batch ($M_b$)][b] |
| Size of partial mixing of sprouts | $M_{ps}$ | $M_p \times W$ |
| Number of contaminated units in the partial mixing | $N_4$ | ~HyperGeometricPos($N_2$, $N_u - N_2$, [$M_p$/ $M_u$]) |
| Corresponding number of cells in partial mix | $C_p$ | Sum of cells $C_{4,u}$ (end of production) present in $N_4$ positive units randomly sampled among the $N_2$ positive units |
| Probability to have a contaminated partial mix | $\rho_6$ | $P_2 \times (1 - \frac{(N_u - N_2)!(N_u - [M_p/M_u])!}{N_u!((N_u - N_2) - [M_p/M_u])!})$ |
| **Consumption Module** | | |
| Grams of sprouts per eating occasion (i.e., per serving) | $E$ (g) | Empirical distribution |
| Number of cells in a contaminated serving | $C_s$ | ~BinomialPos($C_p$, $E$ / $M_{ps}$) |
| Prevalence in servings | $P_3$ | $\rho_6 \times (1 - (1 - E/M_{ps})^{C_p})$ |
| **Risk Prediction** | | |
| Parameter of the beta-Poisson dose–response model | $\alpha$, $\beta$ | Median estimates evaluated using $\alpha = 0.1{,}324$, $\beta = 51.45$, [lower CI bound (2.5th) evaluated using $\alpha = 0.094$, $\beta = 43.75$; upper CI bound (97.5th) evaluated using $\alpha = 0.1{,}817$, $\beta = 56.35$][b] |
| Risk per contaminated serving | $R$ | 1 – BetaFunction($\alpha$, $\beta + C_s$) / BetaFunction($\alpha$, $\beta$) |
| Eating occasions per year | $N_s$ | 8.52E+07 |
| Expected number of cases | | $N_s \times$ mean($P_3 \times R$) |

[a][$x$]: integer part of $x$. $x$!: factorial $x$. BinomialPos($n$, $p$): binomial distribution restricted on the positive domain. HypergeometricPos($m$, $n$, $k$): hypergeometric distribution with $m$, the number of successes, $n$, the number of failures, and $k$ the number of samples, restricted on the positive domain. Beta($a$, $b$): $\beta$ distribution. BetaFunction($x$, $y$): $\beta$ function.
[b]Uncertainty for the model input is shown within the bracket for: initial seed batch prevalence, initial levels per positive seed unit, vol/wt ratio of volume of water to seeds/in-process sprouts per irrigation cycle; pathogen growth maximum number of generations, postharvest extent of mixing/pathogen spread, and dose response.

and the seed quantity in one growing unit might be as high as 50 lb (22.7 kg). We chose a range of 15–50 lb (6.8–22.7 kg) to represent the size (weight) of seed batch (uniform distribution).

The prevalence of *Salmonella* contamination in seeds was based on FDA data for a variety of seeds and beans (mung, alfalfa, clover, soybean, and other sprout varieties) reported in the FDA "FY 2014–2016 microbiological sampling assignment summary report: sprouts" (referred to henceforth as "FDA FY14–16 Sprouts Assignment").[28] Overall, 170 samples were taken from various seed lots and four samples were positive for *Salmonella*. The analytical sample size was equivalent to 750 g. The four seed samples positive for *Salmonella* were obtained from four different lots from three sprouters. A literature search for peer-reviewed studies through searches in the National Center for Biotechnology Information PubMed database and the Google Scholar search engine was unfruitful to find any other studies on *Salmonella* prevalence in seeds for sprouting not involved in an outbreak, except a study from the United Kingdom.[29] Based on the available data from the FDA FY14–16 Sprouts Assignment,[28] we estimated the prevalence of *Salmonella* in 6.8 kg seed batches as $P_0 = 2.35\%$ (four of 170), and represented the uncertainty surrounding the estimate in a Binomial process by using a Bayesian approach with the Jeffreys prior[30] Beta(0.5,0.5). The prevalence of *Salmonella* in 25 g seed units, $P_u$, was derived from the prevalence in 6.8 kg seed batches. The probability $P_0$ for a 6.8 kg seed batch of being contaminated is equal to the probability to have at least one contaminated 25 g unit among the $N_v$ units it contains. Assuming independence in the probability of the 25 g units to be contaminated in the 6.8 kg seed batch,

$$P_0 = 1 - (1 - P_u)^{N_v},$$

which solves in $P_u$ as

$$P_u = 1 - (1 - P_0)^{N_v^{-1}},$$

with $N_v$ the number of units per 6.8 kg batch, i.e., $N_v = 6{,}810/25 = 272.4$.

A review of studies in the scientific literature shows that when seed samples are positive, the pathogen contamination levels in seeds are low,[2,16,31] as is typical for *Salmonella* in low water activity foods.[32] Montville and Schaffner used a "low" contamination level of 1–5 cells per 25 g units, and a "high" contamination level of 100–5,000 CFU/25 g[16] as assumptions, but they did not cite any studies for their choice of the "high" contamination level (i.e., the high levels were hypothetical). Reported *Salmonella* levels for seeds in two different seed lots associated with outbreaks were 1.3 ± 0.4–1.6 ± 0.2 cells per 100 g.[31] In this model, the levels of *Salmonella* in positive 25 g seed units were represented by a uniform distribution, with a minimum of one cell (by definition). The maximum number of cells per contaminated 25 g unit was modeled using an uncertainty distribution Uniform(10, 12). The maximum levels were derived based on two assumptions: (1) levels in seeds implicated in outbreaks represent upper bounds of baseline contamination, where 1.3 ± 0.4–1.6 ± 0.2 cells per 100 g correspond to 10–12 cells per 750 g; (2) the 10–12 cells in 750 g were clustered within a single 25 g unit.

### 2.3. Seed Treatment with Various Log Reductions

In the model, each *Salmonella* cell had an identical and independent probability of inactivation by the seed treatment, equal to $1–10^{-L}$, where $L$ is the $\log_{10}$ reduction (Table I). The model estimated the likelihood of finding a positive batch of seeds and also estimated the number of cells in the positive batch of seeds with or without seed treatment. When seed treatment was applied, the model evaluated the effects from seed treatment that reduced the *Salmonella* population by 1 $\log_{10}$ CFU, 2 $\log_{10}$ CFU, 3 $\log_{10}$ CFU, 4 $\log_{10}$ CFU, or 5 $\log_{10}$ CFU, as a fixed log reduction (Table I). These target reduction values were considered as a potential performance criterion, as might be considered in a guidance document, and as was considered in a previous risk assessment on salmonellosis in almonds in the United States.[33]

### 2.4. *Salmonella* Growth and Spread During Seed Soaking and Sprouting

During the process of seed soaking and sprouting with multiple irrigation cycles,[2] pathogens can grow and spread from contaminated seeds/sprouts to noncontaminated seeds/sprouts.[34,35] The degree of *Salmonella* spreading can be influenced by factors such as the types of growing units (e.g., rotary drum, bins, or trays) and irrigation practices (e.g., overhead). The model includes a spread multiplier described with a discrete distribution, UniformDiscrete(1, 5). It was assumed that growth of cells in the contaminated seed/sprout unit(s) and spread of the resultant cells to initially noncontaminated units take place simultaneously. For example, a multiplier of four represents certain growing units and sprouting

practices where, in a production batch of sprouts contaminated with *Salmonella*, the cells in a seed unit positive for *Salmonella* grow two generations and spread to three previously negative units within the batch (Fig. 1) during the early stage of production.

### 2.5. *Salmonella* Growth During Sprouting

Once spreading to the new unit(s), cell growth takes place in parallel among all units. *Salmonella* growth data on naturally contaminated[31,36] and artificially inoculated[35,37–39] alfalfa seeds during sprouting were collected from the peer-reviewed literature. The Baranyi growth model[40] was fit on the curves including more than five points using DMFit v2.0.[41] Estimated specific growth rates ($\log_{10}$CFU/hour) were 0.19 for growth on naturally contaminated seeds,[31] 0.028–0.20 for artificially inoculated seeds with background microflora,[38,39] and 0.26–0.54 for artificially inoculated seeds with little background microflora.[35] The growth rates of *Salmonella* on both naturally contaminated and artificially inoculated seeds were eventually combined and considered in the model by using a BetaPert[42] distribution of variability with minimum 0.03 $\log_{10}$CFU/hour, mode 0.11 $\log_{10}$CFU/hour, and maximum 0.54 $\log_{10}$CFU/hour.

Literature data suggest that, beyond the varying growth rate, the maximum growth reached by *Salmonella* when grown on naturally contaminated alfalfa seeds is usually lower than that from inoculated seeds.[31,35,36,38,39] Conditions for maximum growth of *Salmonella* on artificially inoculated seeds are generally more optimal (e.g., no cell injury, sometimes no background flora, and higher starting number of cells) and usually show significantly higher maximum growth (6–7 $\log_{10}$ CFU, i.e., 20–24 generations) than what is observed on naturally contaminated seed. The observed maximum growths among naturally contaminated seeds ranged from 3 generations (i.e., 0.90 $\log_{10}$) at 20 °C to 16 generations (i.e., 4.8 $\log_{10}$) at 25–28.5 °C in 48 hours.[31,36] Variability in maximum growth in the model was represented by Uniform(3, 16) generations, i.e., Uniform(0.90, 4.8) $\log_{10}$ CFU. An uncertainty distribution was derived based on data obtained from the peer-reviewed literature,[31,36] with a probability of having a minimum of zero generations (no growth) of 0.2, and a probability of having a minimum of three generations of 0.8. There were insufficient data to develop a secondary model to predict growth as a function of sprouting temperature.

A Spearman rank correlation factor of 0.7 was built between the growth rate and the maximum population level using the Iman and Conover method[43] in order to consider the positive correlation between growth rate and maximum population growth. Growth was eventually modeled using a stochastic process based on the Yule process,[42,44] as:

$$N_t = N_0 + \text{Negative Binomial}\,(N_0, \exp(-g)),$$

where $N_t$ is the number of bacteria after growth, $N_0$ is the initial number of bacteria, NegativeBinomial($s$, $p$) is the negative binomial distribution with $s$ the size parameter and $p$ the probability parameter, and $g$ is the overall expected growth, in $\log_{10}$ (Table I). After the amount of growth was simulated and the total number of cells was determined per 25 g unit, the final maximum population density was set to be 4 $\log_{10}$ CFU/g based on the data from the naturally contaminated seeds. We defined the yield of alfalfa sprouts as 6–7 times the mass of seeds;[45] this was used to determine the *Salmonella* level (CFU/g) of sprouts at the end of growth simulation.

### 2.6. Data and Assumptions for Testing of Spent Irrigation Water

During irrigation, *Salmonella* cells present on the seeds/sprouts can be washed off and transferred to the SIW. A portion of SIW can be sampled for microbiological testing.[9] The model evaluated the effect of SIW testing when the water sample was taken after 48 hours of sprouting (and at other time intervals in alternative scenarios; see below). If SIW testing is positive for *Salmonella*, the associated production batch is not distributed for consumption, i.e., only the fraction of production batches contaminated with *Salmonella* that were not detected by the SIW testing regime contribute to predicted cases in the model.

In an ideal situation, the sampled SIW would have traveled across and covered all the sprouts in the production batch (complete coverage, i.e., representative of the entire production batch of sprouts). Depending on factors such as irrigation practices, how the SIW is sampled, and how representative the water sample is of the batch, the coverage might represent only a proportion of the sprout production batch (Fig. 1). For example, if the growing unit included a rack of five trays of equal size, and SIW was taken from four trays, then the coverage would be 80%. The model includes an input representing the proportion (which can vary) of a production batch covered during sampling. Available guidelines[46–49]

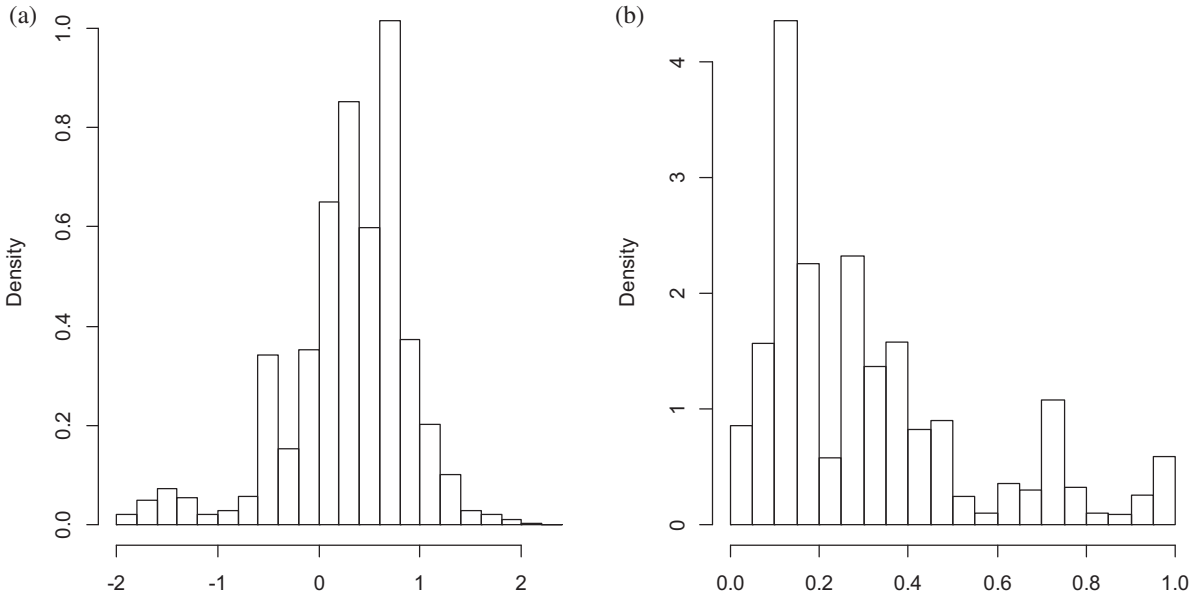


**Fig. 2.** Histogram for parameter *A,* the differences in pathogen concentrations ($\log_{10}$ CFU/g) between in-process sprouts and spent irrigation water (data extracted from Refs. 31, 35, 36, 50, and 51), and parameter *B*, the proportion of pathogen cells transferred from the sprouts to the spent irrigation water.

and research findings[50] highlighted that sampling SIW (that has flowed over and through the production batch of sprouts) is a good indicator of microbial conditions and an efficient method to detect *Salmonella* contamination in sprouts, when using an appropriate sampling plan and testing protocol.[9] Bacteria are distributed more uniformly in water than in seeds or sprouts, and it is easier to collect a representative sample of SIW from the growing units.

The proportion of the *Salmonella* cells that transfers to the SIW may vary substantially, by as much as three orders of magnitude, as reported in a comprehensive review of published data by Montville and Schaffner in 2005.[16] In this study, we included additional data on the subject published since 2005 and, adapting the Montville and Schaffner approach, we explicitly included the mass of in-process sprouts and the SIW volume in modeling this transfer. The mathematical derivation of the number of bacteria in SIW as a function of the number of bacteria in the sprouts and the difference in $\log_{10}$ concentration of bacteria in sprouts and water is provided in the Appendix. Five studies[31,35,36,50,51] regarding the transfer of *Salmonella* and *E. coli* O157:H7 from sprouts to SIW reported the pathogen concentrations in sprouts and corresponding concentrations in SIW immediately after irrigation or rinsing of the sprouts. We derived an empirical distribution of the difference in concentration in sprouts and water (parameter *A*; see Appendix), based on data points extracted from the studies on enumeration of pathogens (*Salmonella* and *E. coli* O157:H7).[31,35,36,50,51] Fig. 2 shows the histogram for parameter *A*, and the proportion of pathogen cells transferred from the in-process sprouts to the SIW (parameter *B*).

During SIW sampling, the volume of water used to irrigate the seeds or in-process sprouts per irrigation cycle was uncertain; we assumed a ratio ranging from 1 mL/1 g of seeds/in-process sprouts to 5 mL/1 g of seeds/in-process sprouts, i.e., a vol/wt ratio of 1:1–5:1, based on information from the transfer studies.[31,35,36,50,51] The analytical unit tested for *Salmonella* was 0.75 L.[52] Homogeneous distribution of the pathogens in the SIW was assumed. Based on the FDA analytical method,[52] we assumed 100% probability of detecting one cell if present in the 0.75 L sampled water. We also evaluated alternative scenarios with 80% probability of detecting one cell.

### 2.7. *Salmonella* Spread Post Harvesting

Postharvest washing and handling of sprouts at the packaging step may lead to further spreading of *Salmonella* cells, when present, from one part of the sprout production batch to another part. We

considered a range of postharvest spread potential, from no spread, to partial spread, and complete spread across the sprout production batch (Fig. 1). The degree of postharvest spread was represented as a range of uncertainty in the model (Table I).

### 2.8. Consumption Patterns, Dose–Response Relationship, and Risk Characterization

Consumption of alfalfa sprouts in the total U.S. population per year was determined by using data from What We Eat in America (WWEIA), the food consumption data collected as part of the National Health and Nutrition Examination Survey, for survey cycles 2003–2012.[53] For alfalfa sprouts, the estimated total number of servings per year was $8.52 \times 10^7$ servings using the WWEIA data and the U.S. total population (census data as of 06/01/2016 from http://www.census.gov/popclock/). The serving size (amount per eating occasion), also from this source, was represented by an empirical distribution with a median and maximum of 8.7 g and 49.5 g, respectively. The *Salmonella* dose–response model used in the present risk assessment was adapted from the one published in the FAO/WHO 2002 risk assessment of *Salmonella* in eggs and broiler chickens,[54] which was based in part on outbreak data from a variety of foods. It is a beta-Poisson model characterized by two parameters $\alpha$ and $\beta$. The adaptation of the beta-Poisson model to the discrete case, i.e., the beta-binomial dose–response model,[55, Equation 11] was used in this risk assessment. Three sets of $\alpha$ and $\beta$ value from the FAO/WHO risk assessment[54] were used to consider the uncertainty of the dose–response relationship (Table I). In addition, we used a reported disability-adjusted life year (DALY) template, 0.0188 DALYs per case of *Salmonella* infection from the literature,[56] to estimate the DALYs per year in risk characterization.

### 2.9. Considering Variability, Uncertainty, and Alternative Scenarios

The model integrated variability of approximately 20 model inputs. Additionally, uncertainty in the risk estimate was characterized by integrating uncertainty distributions for key model parameters through a second-order Monte Carlo simulation. Uncertainty in six model inputs was considered using probability distribution: the batch prevalence (a $\beta$ distribution), the initial level of contamination per positive unit (a triangular distribution with uncertainty defined for the maximum), the ratio of volume of water to seeds/in-process sprouts per irrigation cycle (a uniform distribution for the volume of water), the pathogen growth (a uniform distribution with a different minimum number of generations), the postharvest mixing/spreading of *Salmonella* (from no spread, to spread to the entire production batch of sprouts), and the dose response (Table I).

Data with direct biological relevance to alfalfa sprouts (e.g., *Salmonella* growth rate and maximum population density, consumption patterns) were represented in the model by variability distributions/parameters. Where data specific to alfalfa sprouts were not available, data on sprouts in general (e.g., prevalence and levels in seeds intended for sprouting) and sprout production practices (e.g., ratio of volume of water to seeds/in-process sprouts per irrigation cycle) were used to develop plausible alternative scenarios; these and the uncertainty in dose–response relationship were represented by uncertainty distribution/parameters in the model. For several of the model inputs where data are lacking (e.g., in-process pathogen spread multiplier), assumptions were made based on knowledge about sprouts production to allow evaluation of more than one scenario, and these were represented by uncertainty parameters in the model. By separately characterizing variability and uncertainty, the risk assessment model provides a means to integrate data with different weight of evidence separately. Furthermore, risk estimates were generated for a number of alternative scenarios to evaluate the potential impact of alternative assumptions.

Model convergence was tested in both variability and uncertainty dimensions. Risk estimates were generated by using 30,000 iterations in the variability dimension and 1,000 iterations in the uncertainty dimension. Risk and prevalence reduction compared to a baseline (without treatment and no SIW test) were evaluated within each of the uncertainty iterations. The model was developed using the R software.[57]

## 3. RESULTS

### 3.1. Seed Treatment: Estimated Fraction of Contaminated Batches, Number of Illnesses, and Risk Reduction

Based on the available data (Table I), the model estimates that 5.22% of the sprout batches were predicted to be contaminated with *Salmonella* (Table II)

**Table II.** Predicted Fraction of Sprout Batches Contaminated, Number of Cases per Year, and Risk Reduction, with Uncertainty Range, Before and After Seed Treatment Alone or Combined with SIW Testing

| Scenario, Seed Treatment with or without SIW Testing[a] | Percent of Sprout Batches Contaminated[a,b] | Number of Cases/Year[a] | Risk Reduction[a,c] | |
|---|---|---|---|---|
| | | | Percent Reduction in Cases | $Log_{10}$ Change in Cases |
| No treatment | 5.22 [1.84, 12.0] | 76,600 [15,400, 248,000] | | |
| 1 log | 2.32 [0.81, 5.46] | 12,100 [2,900, 39,300] | 84.4 [79.7, 84.7] | –0.807 [–0.693, –0.817] |
| 2 log | 0.310 [0.107, 0.743] | 1,360 [327, 4,390] | 98.2 [97.6, 98.3] | –1.76 [–1.62, –1.77] |
| 3 log | 0.0320 [0.0111, 0.0768] | 139 [33.1, 448] | 99.8 [99.76, 99.83] | –2.75 [–2.62, –2.76] |
| 4 log | 0.00321 [0.00111, 0.00771] | 13.9 [3.29, 44.9] | 99.98 [99.976, 99.983] | –3.75 [–3.61, –3.76] |
| 5 log | 0.000321 [0.000111, 0.000771] | 1.39 [0.329, 4.48] | 99.998 [99.9976, 99.9983] | –4.75 [–4.61, –4.76] |
| 0-log, SIW test | 0.811 [0.260, 2.34] | 12,100 [2,400, 41,200] | 85.9 [72.5, 87.2] | –0.851 [–0.561, –0.892] |
| 1 log, SIW test | 0.688 [0.220, 1.68] | 3,560 [821, 11,400] | 95.6 [93.1, 96.0] | –1.36 [–1.16, –1.40] |
| 2 log, SIW test | 0.100 [0.0321, 0.248] | 441 [100, 1,420] | 99.5 [99.1, 99.5] | –2.27 [–2.07, –2.30] |
| 3 log, SIW test | 0.0104 [0.00332, 0.0259] | 44.9 [10.2, 146] | 99.94 [99.91, 99.949] | –3.25 [–3.06, –3.29] |
| 4 log, SIW test | 0.00105 [0.000334, 0.00260] | 4.50 [1.02, 14.8] | 99.994 [99.991, 99.995] | –4.25 [–4.06, –4.29] |
| 5 log, SIW test | 0.000105 [0.0000334, 0.000260] | 0.449 [0.103, 1.47] | 99.9994 [99.9991, 99.9995] | –5.26 [–5.06, –5.29] |

[a]Spent irrigation water (SIW) testing (when applied) based on 100% coverage. Median estimate is shown with the 95% confidence interval in the bracket.

[b]Percent of sprout batches (15–50 lb or 6.8–22.7 kg, finished product) contaminated before or after the intervention(s) and sold to the market.

[c]Risk reduction calculated in two ways based on $R$ = [cases with treatment/cases without treatment]: the percent risk reduction defined as $(1–R)\times100$; and the $\log_{10}$ risk reduction defined as $\log_{10}R$.

in the absence of seed treatment or SIW testing. The predicted fraction of contaminated sprout batches following seed treatment was reduced to, for example, 2.32%, 0.0320%, and 0.000320% when 1-$\log_{10}$, 3-$\log_{10}$, or 5-$\log_{10}$ seed treatment was applied, respectively (Table II). With combined SIW testing and seed treatment, the fraction of positive sprout batches was reduced further (Table II).

The expected number of cases per year in the U.S. population for the scenario with no interventions is 76,600 cases (95% CI 15,400–248,000) per year. The estimated risk from the consumption of sprouts from untreated seeds was approximately 5- to 7-fold higher than that for sprouts from seeds that receive a 1-$\log_{10}$ seed treatment. For each additional $\log_{10}$ reduction in the treatment, the risk reduction after seed treatment was ~10-fold. When seed treatment is applied, the model predicted fewer cases, e.g., 139 or 1.39 cases for a 3-$\log_{10}$ or 5-$\log_{10}$ treatment, respectively. The predicted risk reduction from 1-$\log_{10}$ seed treatment alone is comparable to that from SIW testing alone at 100% coverage (Table II).

While the uncertainty in model inputs resulted in substantial uncertainty in the predicted number of cases, it had little effect on the predicted relative risk reduction (Table II). For example, after a 3-$\log_{10}$ seed treatment, the estimated 95% CI cases were 33–448 (indicating an uncertainty range of approximately an order-of magnitude difference between the lower and the upper confidence limits of the predicted cases), but the risk reduction was 99.76–99.83%, or –2.62 $\log_{10}$ to –2.76 $\log_{10}$ (indicating a much narrower range of uncertainty in the predicted risk reduction, <0.1% or <0.2 $\log_{10}$, respectively). Similar trends were obtained for 2-$\log_{10}$ or greater pathogen reduction from seed treament, alone or in combination with SIW testing (Table II), which suggests that the predicted relative risk reduction from the interventions is not sensitive to the uncertainty in the predicted number of cases.

### 3.2. Spent Irrigation Water Testing: Risk Estimates and Fraction of Contaminated Batches

The predicted effectiveness of testing was highly influenced by the degree to which the sampled SIW covered the production batch (Table III). If the coverage was complete (100% representative of the production batch), with no seed treatment, the model predicted that SIW testing alone would detect approximately 86% (95% CI 72–87%) of contaminated batches, assuming that SIW from every production batch was subject to testing. Because only a proportion of the cells present in a contaminated production

**Table III.** Predicted Number of Cases per Year and Production Batch Contamination After Spent Irrigation Water Testing, But without Seed Treatment

| Scenario, Irrigation Coverage | Number of Cases/Year[a] | Risk Reduction[a,b] | Percent of Sprout Batches Contaminated, Remained[a,c] | Percent Reduction in Sprout Batches Contaminated[a] |
|---|---|---|---|---|
| 0% (no testing) | 76,600 [15,400, 248,000] | | 5.22 [1.84, 12.0] | |
| 20% | 44,200 [9,000, 141,000] | 42.5 [36.9, 45.0] | 3.05 [1.06, 7.03] | 42.5 [36.9, 43.8] |
| 40% | 28,400 [5,700, 89,900] | 64.5 [54.3, 66.1] | 1.95 [0.662, 4.50] | 64.5 [54.3, 65.3] |
| 60% | 19,800 [3,910, 61,900] | 76.0 [64.5 ,77.1] | 1.36 [0.448, 3.25] | 75.5 [63.7, 76.6] |
| 80% | 15,100 [3,010, 50,600] | 82.2 [69.8, 83.4] | 1.02 [0.333, 2.71] | 81.8 [69.1, 83.0] |
| 100% | 12,100 [2,400, 41,200] | 85.9 [72.5, 87.1] | 0.811 [0.260 ,2.34] | 85.9 [72.5, 87.1] |

[a]Median estimate is shown with the 95% confidence interval in the bracket.
[b]Percent reduction in cases, defined as (1–[cases with SIW testing]/[cases without SIW testing])×100. SIW, spent irrigation water.
[c]Percent of sprout batches (15–50 lb or 6.8–22.7 kg, finished product) contaminated sold to the market.

batch of sprouts are transferred to irrigation water during sampling (Fig. 2) and only 0.75 L was tested, SIW testing did not detect all the contaminated production batches. Reducing the test volume (e.g., from 0.75 L to 0.20 L) resulted logically in a smaller fraction of contaminated batches being detected, and thus a lower degree of risk reduction (e.g., a 10–12% smaller reduction in risk when testing 0.20 L than would be achieved when testing 0.75 L); conversely, increasing the test volume to 1.5 L resulted in a larger fraction of contaminated batches being detected, and a 4–6% greater reduction in risk than would be achieved by testing 0.75 L (data not shown).

As the coverage of the irrigation water over the production batch decreased, e.g., to 80% and 60%, the fraction of positive batches that would be detected also decreased, e.g., an estimated 82% (95% CI 70–83%) and 76% (95% CI 64–77%) of the contaminated batches would be detected (Table III), which corresponds to an estimated 82% and 76% reduction in cases/year, respectively. The effectiveness of the SIW testing intervention is strongly dependent on whether the water sample is taken from a pool of SIW that together has touched all parts of the production batch (Table III), or otherwise is representative of all parts of the growing unit.

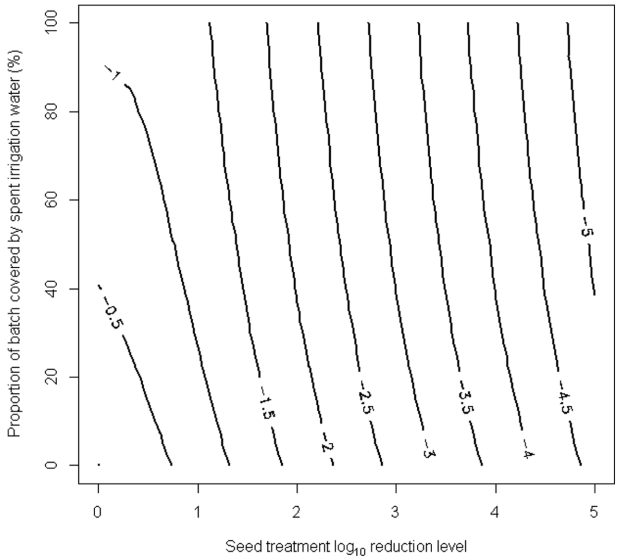


**Fig. 3.** Contour plot of the $\log_{10}$ reduction in the number of cases/year after combined seed treatment and SIW testing (from simulations without considering uncertainty). The log reduction was defined as the $\log_{10}$ ([cases with treatment and/or testing]/[cases without treatment or testing]) as a function of the irrigation water coverage (the *y*-axis, from 0% to 100%) and the $\log_{10}$ reduction in the seed treatment disinfection step (the *x*-axis, from 0 $\log_{10}$ to 5 $\log_{10}$).

### 3.3. Combined Seed Treatment and Spent Irrigation Water Testing: Risk Estimates

When SIW water testing was implemented in combination with seed treatment, the model predicted a greater degree of risk reduction than that from using either intervention alone (Table II). A 5-$\log_{10}$ seed treatment combined with SIW testing with 100% coverage would reduce the number of illnesses per year to <1 (95% CI 0.10–1.5) (Table II). The contour plot (Fig. 3) illustrates the $\log_{10}$ reduction in the number of cases/year as a function of the combined seed treatment (with different $\log_{10}$ reduction) and SIW testing (with different irrigation water coverage) interventions, based on model simulations without considering uncertainty. When the SIW sampling coverage was decreased from 100% to

**Table IV.** Predicted Batch Contamination and the Number of Cases per Year Given Alternative Assumptions

| Scenario, Alternative Assumptions[a] | Percent of Sprout Batches Contaminated[b] | Number of Cases/Year[b] | $Log_{10}$ Change in Cases[c] |
|---|---|---|---|
| **Reference scenario without SIW test** | **0.0320 [0.0111, 0.0768]** | **139 [33.1, 448]** | **–2.75 [–2.62, –2.76]** |
| MPD 7 $log_{10}$CFU/g | 0.0320 [0.0111, 0.0768] | 139 [33.1, 449] | –2.75 [–2.62, –2.76] |
| Seed treatment variability (3 ± 0.5) $log_{10}$ | 0.0380 [0.0131, 0.0903] | 166 [40.0, 531] | –2.66 [–2.54, –2.68] |
| Growth rate and MPD correlation factor = 0 | 0.0320 [0.0111, 0.0768] | 138 [32.9, 449] | –2.74 [–2.62, –2.76] |
| Prevalence in seeds 0.235% | 0.00457 [0.000161, 0.0290] | 19.7 [0.647, 161] | –2.76 [–2.62, –2.76] |
| Prevalence in seeds 23.5% | 0.355 [0.262, 0.487] | 1,620 [617, 2910] | –2.70 [–2.59, –2.71] |
| **Reference scenario with SIW test** | **0.0104 [0.00332, 0.0259]** | **44.9 [10.2, 146]** | **–3.25 [–3.06, –3.29]** |
| MPD 7 $log_{10}$CFU/g | 0.0104 [0.00332, 0.0259] | 45.0 [10.2, 147] | –3.25 [–3.06, –3.29] |
| Seed treatment variability (3 ± 0.5) $log_{10}$ | 0.0123 [0.00393, 0.0311] | 53.4 [12.3, 171] | –3.18 [–2.98, –3.22] |
| Growth rate and MPD correlation factor = 0 | 0.0109 [0.00347, 0.0269] | 47.0 [10.7, 152] | –3.24 [–3.05, –3.27] |
| Ratio of water to in-process sprouts 1:1 | 0.00979 [0.00317, 0.0245] | 42.8 [9.92, 139] | –3.29 [–3.07, –3.31] |
| Ratio of water to in-process sprouts 5:1 | 0.0108 [0.00352, 0.0268] | 47.3 [10.9, 153] | –3.24 [–3.04, –3.26] |
| Probability to detect one cell: 0.8 | 0.0112 [0.00358, 0.0275] | 48.3 [11.0, 158] | –3.22 [–3.03, –3.26] |
| Time to SIW sampling 12 hours | 0.0178 [0.00602, 0.0437] | 77.2 [18.5, 257] | –3.00 [–2.86, –3.03] |
| Time to SIW sampling 24 hours | 0.0117 [0.00380, 0.0286] | 50.4 [11.6, 164] | –3.20 [–3.03, –3.24] |
| Time to SIW sampling 36 hours | 0.0107 [0.00340, 0.0263] | 46.5 [10.6, 149] | –3.24 [–3.05, –3.28] |
| Prevalence in seeds 0.235% | 0.00147 [0.0000485, 0.00995] | 6.29 [0.193, 52.8] | –3.26 [–3.07, –3.30] |
| Prevalence in seeds 23.5% | 0.114 [0.0782, 0.181] | 517 [198, 956] | –3.21 [–3.03, –3.24] |

[a]Predicted based on 3-$log_{10}$ seed treatment and the specified alternative assumption replacing the corresponding input in the reference scenario. Reference scenario inputs: maximum population density (MPD) 4 $log_{10}$CFU/g, growth rate and MPD correlation factor = 0.7, time to SIW sampling 48 hours, ratio of water to in-process sprouts Uniform(1,5):1, probability to detect one cell = 1, prevalence in seeds 2.35%, and 100% coverage if SIW test is performed. The 0.235% and 23.5% prevalence was defined based on a total number of 170 samples to be consistent with how the reference prevalence of 2.35% was defined. SIW, spent irrigation water.

[b]Median estimate is shown with the 95% confidence interval in the bracket.

[c]Risk reduction $log_{10}$(cases with treatment/cases without treatment). Cases without treatment are 76,600 [15,400, 248,000], 10,600 [320, 91,200], and 798,000 [243,000, 1,390,000] based on prevalence in seeds for the reference (2.35%), the lower assumption (0.235%), and the higher assumption (23.5%), respectively.

80%, 60%, 40%, and 20%, the model predicted an increasing number of cases and thus a lower degree of risk reduction, i.e., a higher degree of the risk remaining. For example, combined with a 3-$log_{10}$ seed treatment, SIW testing with 20% coverage resulted in an estimated 92.2 (95% CI 21.9–298) cases/year, corresponding to a $log_{10}$ reduction in the number of cases/year to between –2.5 and –3.0. In comparison, SIW testing with coverage of 100% was predicted to result in a greater $log_{10}$ reduction in the number of cases/year, to between –3.0 and –3.5 (Fig. 3). Thus, depending on the degree to which the SIW has covered the entire production batch, the impact of SIW testing may vary.

### 3.4. Alternative Assumptions

The influence of alternative assumptions on the risk estimate is shown in Table IV. Among the range of assumptions evaluated, the two that had the largest impact were assuming a lower (0.235%) and a higher prevalence (23.5%) of *Salmonella* in seeds (10-fold difference from the reference), where the model predicted a 7-fold lower and 11-fold higher number of cases/year, respectively, after a 3-$log_{10}$ seed treatment. Assuming 0.235% prevalence in seeds, the 95% CI of the predicted cases is an order of magnitude wider than that for the reference (2.35% prevalence). The uncertainty around 0.235% was evaluated using a Beta(0.4 + 0.5, 170–0.4 + 0.5) to preserve the sample size (a total of 170 seed samples from available U.S. data[28]); this led to a wider uncertainty range in prevalence and a wider uncertainty range in the risk estimate. Sampling SIW earlier than after 48 hours of sprouting, as recommended in the FDA draft sprout guidance,[9] resulted in a higher fraction of contaminated sprout batches not being detected and remaining after testing, especially if the sample is taken at 12 hours or 24 hours, and thus a higher predicted cases/year (Table IV). Given 2.35% prevalence of *Salmonella* in seeds, the predicted number of cases changed depending on the assumptions made; however, the $log_{10}$ change in cases differs

by <0.25 among the various scenarios evaluated (Table IV).

## 4. DISCUSSION

The risk assessment model considered here expands on the sprout production process model by Montville and Schaffner[16] by adding a sprout consumption module and a *Salmonella* dose–response relationship to predict the risk of illness associated with consumption of alfalfa sprouts by the U.S. population. Our model introduces several new parameters and refinements, including the use of *Salmonella* in seeds prevalence data relevant to U.S. sprout production operations, the vol/wt ratio of water to seed/in-process sprouts per irrigation cycle, in-process pathogen spread multiplier, and the extent of postharvest pathogen spread, to enable the evaluation of the impact of these key factors on sprouts contamination and subsequent risk to the consumer. In addition, this risk assessment uses updated data to predict public health risk and the changes in public health risk by the various interventions of interest to risk managers.[9] The results presented in this study were based on data and sprout practices relevant to the United States. However, the model is applicable to address sprout food safety issues in other countries and regions with region-specific modifications, such as prevalence and levels of *Salmonella* in alfalfa seeds, sprout consumption, and any unique aspects in those sprout production operations (e.g., the size of seed batch and control measures other than seed treatment and SIW testing).

### 4.1. Considering Variability and Uncertainty, and Model Complexity

Ideally, risk assessment models separately consider variability and uncertainty[25,58] but it can be challenging to do so for food-safety risk assessment models. In this risk assessment model, we incorporated and systematically characterized the variability and uncertainty in the data and assumptions for *Salmonella* contamination in alfalfa seeds, *Salmonella* growth and spread during sprout production, alfalfa sprout consumption patterns, and *Salmonella* dose–response relationship through a second-order Monte Carlo simulation. Results from this study suggest that the predicted relative risk reduction is robust. The relative impact of seed treatment ($\log_{10}$ reduction) and SIW testing, implemented alone or in combination, is essentially the same regardless of uncertainty in key parameters, as illustrated by the narrow range of predicted 95% CI reduction in the number of cases for the reference scenario (Table II) and alternative scenarios based on alternative assumptions (Table IV). Collecting additional data would not likely increase the certainty of the estimates of relative risk reduction; however, it would narrow down the uncertainty range of the predicted number of cases per year.

In this risk model, we simulated the (discrete) number of bacteria (CFU per 25 g seed units, CFU per batch of seeds or per production batch sprouts) rather than the (continuous) concentration (log CFU/g of seeds or sprouts). Results from this risk assessment suggest that, for treatment at $\geq 2$ $\log_{10}$, an additional $\log_{10}$ in the treatment would lead to an additional $\log_{10}$ reduction in the expected number of cases. However, this proportionality is not true for application of the first $\log_{10}$ reduction. Actually, this simple proportionality, even for the higher log reduction, should not be assumed without the systematic evaluation by modeling of the dynamics of *Salmonella* inactivation, transfer, and growth during sprout production. A previous theoretical study[24] illustrates that modeling pathogens as discrete entities is especially important in a process including an inactivation step followed by a growth step, such as the case in this model. Indeed, in this risk model, the seed treatment/inactivation step results in the complete elimination of cells from some of a contaminated unit/batch, in particular when the seed contamination level is low. The subsequent "growth" step has then no impact in such a noncontaminated batch, but does have an impact in any batch where at least one *Salmonella* cell survives the seed treatment. We chose not to model *Salmonella* levels in the batch as concentration (log CFU/g) with a continuous parameter because doing so would not adequately capture the effect of eliminating cells from some units after seed treatment, and therefore could underestimate the risk reduction from interventions.[24] After a 2-$\log_{10}$ reduction, the seed treatment inactivation has a linear impact on risk reduction because, if a seed unit is still contaminated, it is predicted that it would have no more than one cell. Therefore, the predicted prevalence of contaminated sprout batches, the predicted number of cases/year, and the overall risk reduction are proportional to the seed treatment log reduction. This proportionality was illustrated by Williams *et al.*[59] in a situation where risk estimation can be simplified after a linear relationship between

product contamination and human illnesses has been demonstrated.

### 4.2. Comparison with Surveillance Data and Previous Risk Assessments

A typical challenge in conducting microbial risk assessments has been the lack of baseline prevalence and enumeration data for food–hazard combinations of interest. Over the years, this situation has prompted data calls by regulatory agencies[60–62] and targeted data collection by collaborative government, academia, and industry efforts.[63–66] Because of the lack of data on the contamination of *Salmonella* in seeds, previously published models either did not include prevalence data and treated prevalence as a user-input parameter[16] or used data from the United Kingdom.[17] The prevalence data for seed samples used in this risk assessment model were not known to be associated with reported outbreaks or sporadic cases of salmonellosis. The risk estimates based on the prevalence estimate of 2.35% in 6.8 kg seed batches may be representative of usual situations leading to sporadic cases, while the risk estimates based on an assumption of a higher prevalence (e.g., 23.5% in 6.8 kg seed batches) may be representative of an extreme event. In an outbreak situation, *Salmonella* prevalence has been reported to be as high as 30–90% in 100 g seed samples,[16,67–69] which would correspond to 8.5–44% in 25 g seed units. In this risk assessment, data from the FDA FY14–16 Sprouts Assignment[28] that included sampling of seeds intended for sprouting anchor the model to the U.S. sprout production. However, more data would be needed to refine this prevalence and to reduce the uncertainty in the estimated number of salmonellosis cases (Table II).

Available sampling data for sprouts provide insights into the plausibility of the prevalence estimate. In this risk assessment, we used a prevalence of 2.35% in seed batches, which resulted in an estimated ~5% sprout batches contaminated (or a predicted average of 0.003% contamination rate for 50 g sprout samples, given no interventions). As part of the USDA/AMS Microbiological Data Program in which samples were collected from distribution centers or at retail from more than 10 states in the United States, [70] a large number of alfalfa or alfalfa-containing sprout samples were analyzed, and the results show a *Salmonella* positive rate of 0.26% (six of 2,277 samples, ~50 g analytical sample size) in 2009, 0.22% (five of 2,313 samples) in 2010, and 0.34% (seven of 2,082 samples) in 2011. In an FDA survey study of RTE foods collected from retail (T. Hammack, personal communication, December 2016), a total of 2,688 sprout samples (622 alfalfa sprouts and alfalfa-sprout-containing samples with mixed types of sprout) were collected from retail stores in four states in the United States between 2012 and 2014, and none of the samples were positive for *Salmonella* (analytical unit size 50 g); thus, the positive rate was estimated to be <0.16% (based on 50 g analytical sample size). Future research that may reduce the uncertainty in risk estimates, particularly the predicted number of cases, includes an enhanced baseline study of the prevalence of *Salmonella* in seeds and further investigation of the degree of spread of *Salmonella* during pilot-scale sprouts production (in-process spread) and the degree of postharvest pathogen spread, for different types of sprouts and growing containers.

Based on epidemiological data from 1998 to 2008, Painter *et al.*[71] estimated that 32,703 cases/year of *Salmonella* and other bacterial pathogens in the United States (the most probable estimate) were attributed to bacterial contamination in sprouts, and that there was uncertainty in this estimate. The estimate reported by Painters *et al.*[71] is within the 95% CI of the predicted cases/year (15,400–248,000) for *Salmonella* in alfalfa sprouts predicted in this risk model for the scenario with no interventions and the estimates for sprout production with seed treatment of 1-log or SIW testing only. One challenge in comparing epidemiological estimates with the present risk predictions is actually a lack of knowledge regarding the full picture of current industry practices regarding the extent of seed treatment and SIW testing. Available information from the FDA FY14–16 Sprouts Assignment[28] shows that approximately half of 69 domestic sprout operations used seed treatments cited as examples by FDA or other international sprout guidelines, or used a treatment shown to be comparable in the literature. More than half of the firms inspected conducted microbiological testing of SIW. In this study, by modeling pathogen behavior during sprout production, the risk model provides a means to quantify the public health impact of different interventions.

The risk estimates from this study with no interventions are more than two orders of magnitude smaller than that reported by Ding and Fu.[17] Based on 0.0188 DALYs per case for salmonellosis,[56] the risk estimate from this study is 1,440 (95% CI 289–4,660) DALYs, compared to 690,000 DALYs

reported in the previous study.[17] The difference in estimates between the earlier study[17] and the present study can be traced in large part to the value assumed for *Salmonella* prevalence in seeds. Ding and Fu did not have access to the recent U.S. data and used a much larger value of 2% (25 g analytical sample size) based on data from a survey in the United Kingdom,[17,29] compared to the 0.0087% prevalence for 25 g units of seeds (2.35% prevalence for 6.8 kg batches) in this study.

### 4.3. Impact of Interventions: Seed Treatment and SIW Testing

Application of seed treatment and/or SIW testing reduces the number of cases of salmonellosis from the consumption of sprouts. The risk reduction (by 5- to 7-fold) predicted from a 1-$\log_{10}$ seed treatment alone was comparable to that from SIW testing alone, and each additional 1-$\log_{10}$ seed treatment was predicted to provide a greater risk reduction than SIW testing. Combining SIW testing with seed treatment resulted in approximately three times greater risk reduction compared to seed treatment alone at $\geq$2 $\log_{10}$ reduction seed treatment. As the target $\log_{10}$ seed treatment increased to 2 and greater, the predicted risk reduction was approximately 10-fold from each additional 1-$\log_{10}$ seed treatment versus by approximately threefold by SIW testing (100% coverage). For seed treatment, in practice, there is variability in pathogen reduction, sometimes broad variability around an average $\log_{10}$ reduction of 3, 4, or greater,[13,14] achieved by chemical treatments (e.g., chlorine compounds and sanitizers with active ingredient peroxyacetic acid), physical treatments (e.g., ionizing radiation, high hydrostatic pressure, and heat), and other interventions.[4,72] Any control measure used to deliver a target inactivation level should be validated.[9] Otherwise, the predicted reduction in the number of cases would be lower when variability in log reduction occurs.

SIW testing is predicted to be less efficient in detecting a contaminated sprout production batch in this study than that reported in Montville and Schaffner.[16] The differences may be explained by the assumption on the vol/wt ratio of water to seed/in-process sprouts used in the Montville and Schaffner[16] model (a ratio equivalent to 1:1) to evaluate the impact of SIW testing. In this study, from available data, we considered a wider range of ratio, from 1:1 to 5:1. Actually, increasing the vol/wt ratio of water to seed/in-process sprouts per irrigation cycle from 1:1 (e.g., to a ratio of 5:1) decreases the probability of detecting a contaminated batch because the cells would be distributed among a larger volume of SIW from which a sample is taken. Other assumptions/choices made, such as the sample size, growth potential, and the probability of detecting one cell, also affect the estimated risk reduction by SIW testing. In Montville and Schaffner,[16] a higher growth potential (up to 21 generations) was used as input based on evaluation of growth data from naturally contaminated as well as inoculated seeds. The present study only uses data from naturally contaminated seeds, which appear to have a smaller growth potential. Evaluation of alternative scenarios with different assumptions for growth potential using this model suggests that growth potential is a key factor influencing the predicted number of cases, but has little effect on the predicted relative risk reduction (data not shown). Given no seed treatment, we found that the likelihood of detecting a contaminated batch would increase with increasing initial *Salmonella* prevalence in seeds, as was reported in Montville and Schaffner.[16] If initial seed contamination is relatively high, e.g., if the contamination rate is 23.5% (instead of 2.35%) in the 6.8 kg seed batches, then SIW testing is relatively effective in detecting the majority of the contaminated batches but would also lead to the removal of a large fraction (43.3%) of the sprout batches. When seed treatment is applied to reduce contamination before SIW testing is undertaken, a larger fraction of the sprout production batches would test negative (i.e., would be free from pathogen contamination).

Seed treatment is predicted to be much more effective than SIW testing alone in reducing risk to the consumer when the treatment applied achieves $>$1 log reduction in pathogens present. The effectiveness (level of reduction) of the seed treatment is thus a key to the overall risk reduction. We targeted a point value for each $\log_{10}$ reduction level for seed treatment to evaluate the impact such a performance criterion would have on the public health risk.[73–75] Comprehensive reviews previously published on various seed treatments show that available chemical, physical, and other types of treatments are highly variable in their effectiveness in reducing *Salmonella* and other pathogens.[13,14] The results presented here demonstrate that such variability in the efficacy of seed treatment would affect the public health impact realized because that impact is strongly dependent on the seed treatment $\log_{10}$ reduction level. With

regard to the impact of seed treatment, the findings from this study, which are based on reduction in predicted cases, are consistent with those in Montville and Schaffner,[16] which are based on reduction in the contaminated production batches. Furthermore, we not only confirm the findings from sprout contamination assessment by Montville and Schaffner,[16] but also provide a risk model to predict changes in public health burden, besides predicting changes in *Salmonella* contamination in the sprout supply, from different levels of intervention.

In conclusion, this risk assessment provides a comprehensive approach to evaluating the public health impact of seed treatment and SIW testing in a complex sprout production system. The model we developed to inform the assessment integrates data and assumptions specific to *Salmonella* in alfalfa sprouts on contamination in seeds, growth and spread of *Salmonella* in sprouts, consumption patterns, and dose–response relationships, all of which influence the risk to the consumer. The model provides a means to quantitatively understand the magnitude of the relative risk reduction from seed treatment and SIW testing interventions. Expansion of the model to consider the impact on risk from other interventions (such as seed testing), other sprout varieties, or from other pathogen contamination is possible, given sufficient data to inform the model inputs.

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## APPENDIX

### Partition of Pathogen Cells between Sprouts and Water During Sprout Irrigation

Assuming no loss of cells, mass balance gives: $S_0 = S_1 + W_1$, with $S_0$, the number of *Salmonella* in sprouts before the transfer, $S_1$, the number of *Salmonella* in sprouts after the transfer, and $W_1$, the number of *Salmonella* in the water. We have $g_0[S_0] = g_1[S_1] + w_1[W_1]$ with $g_0$ (g), $g_1$ (g), and $w_1$ (mL) the corresponding mass or volume for an irrigation cycle during sprouts production (assuming 1 mL of SIW weighs 1 g). Because the mass of in-process sprouts is the same before and after the transfer, $g_1 = g_0$ at the time of each irrigation cycle. Available published studies regarding the transfer of *Salmonella* and *E. coli* O157:H7 from sprouts to irrigation water[31,35,36,50,51] reported the pathogen concentration ($\log_{10}$ CFU/g) in sprouts, $\log_{10}[S_1]$, and the corresponding concentration in water, $\log_{10}[W_1]$, right after irrigation or rinsing of the sprouts. The differences in $\log_{10}$ concentration between in-process sprouts and water, designated as $A$, can be obtained from the experiments in the published laboratory studies:

$$A = \log[S_1] - \log[W_1] = \log\frac{[S_1]}{[W_1]} \Rightarrow [S_1] = 10^A[W_1],$$

where $A$ is on a $\log_{10}$ CFU/g scale. Assuming that the (distribution of) concentration ratios (i.e., data for the parameter $A$) derived from the data reported in the five laboratory studies represent the partition of pathogen cells during sprouts production, we have:

$$g_0[S_0] = g_1 10^A[W_1] + w_1[W_1] = [W_1]\left(g_1 10^A + w_1\right),$$

$$[W_1] = \frac{g_0[S_0]}{g_1 10^A + w_1},$$

$$W_1 = \frac{w_1 g_0[S_0]}{g_1 10^A + w_1} = \frac{w_1 S_0}{g_1 10^A + w_1} = \frac{S_0}{\frac{g_1}{w_1}10^A + 1} = B\,S_0,$$

where

$$B = \left(\frac{g_1}{w_1}10^A + 1\right)^{-1}.$$

This relationship allows deriving the number of bacteria in SIW as a function of the number of bacteria in the sprouts, the difference in $\log_{10}$

concentration, as reported in the literature, and the ratio of volume of water to mass of in-process sprouts per irrigation cycle.

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