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Research Paper

A Quantitative Risk Assessment of Human Salmonellosis from Consumption of Walnuts in the United States

SOFIA M. SANTILLANA FARAKOS,[1]* RÉGIS POUILLOT,[1] GORDON R. DAVIDSON,[1] RHOMA JOHNSON,[1] INSOOK SON,[1] NATHAN ANDERSON,[2] AND JANE M. VAN DOREN[1]

[1]*U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, College Park, Maryland 20740 (ORCID: http://orcid.org/0000-0002-6107-5212 [R.P.]); and* [2]*U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, Bedford Park, Illinois 60501, USA*

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ABSTRACT

We assessed the risk of human salmonellosis from consumption of shelled walnuts in the United States and the impact of 0- to 5-log reduction treatments for *Salmonella* during processing. We established a baseline model with *Salmonella* contamination data from 2010 to 2013 surveys of walnuts from California operations to estimate baseline prevalence and levels of *Salmonella* during preshelling storage and typical walnut processing stages, considered U.S. consumption data, and applied an adapted dose-response model from the Food and Agriculture Organization and the World Health Organization to evaluate risk of illness per serving and per year. Our baseline model predicted 1 case of salmonellosis per 100 million servings (95% confidence interval [CI], 1 case per 3 million to 1 case per 2 billion servings) of walnuts untreated during processing and uncooked by consumers, resulting in an estimated 6 cases of salmonellosis per year (95% CI, <1 to 278 cases) in the United States. A minimum 3-log reduction treatment for *Salmonella* during processing of walnuts eaten alone or as an uncooked ingredient resulted in a mean risk of <1 case per year. We modeled the impact on risk per serving of three atypical situations in which the *Salmonella* levels were increased by 0.5 to 1.5 log CFU per unit pretreatment during processing at the float tank or during preshelling storage or posttreatment during partitioning into consumer packages. No change in risk was associated with the small increase in levels of *Salmonella* at the float tank, whereas an increase in risk was estimated for each of the other two atypical events. In a fourth scenario, we estimated the risk per serving associated with consumption of walnuts with *Salmonella* prevalence and levels from a 2014 to 2015 U.S. retail survey. Risk per serving estimates were two orders of magnitude larger than those of the baseline model without treatment. Further research is needed to determine whether this finding reflects variability in *Salmonella* contamination across the supply or a rare event affecting a portion of the supply.

Key words: Low-moisture food; Recontamination; Retail; *Salmonella*; Shelled; Tree nuts

The United States is a leading exporter of English walnuts (75% of world trade), with 99% of its production (686,000 short tons in 2016 *(39)*) in California *(7)*. The U.S. harvest occurs once per year and begins with mature walnuts being mechanically shaken to the ground, swept into trailers, and transported to the huller-dryer. At the huller-dryer, walnuts are mechanically precleaned to remove sticks and leaves, passed through a float tank (which generally does not contain an antimicrobial agent) to remove rocks and other debris, passed through a mechanical huller where the hull (if still present) is removed, and then dried by forced heated air in bins to approximately 8% moisture. Following drying, walnuts are stored at 10 to 15°C for up to 1 year, sized, graded, and sold as inshell nuts or shelled and packaged as halves or pieces *(16)*. As reported by the California Walnut Board *(7),* in the 2015 to 2016 and 2016 to 2017 marketing years approximately 42 and 47%, respectively, of walnuts produced in California were inshell walnuts, and the majority of these (>95%) were exported. The remaining 58 and 53%, respectively, were sold as kernels (∼40% domestic and ∼60% exported). These percentages as reported for 2015 and 2016 are similar to those reported for the 2011 to 2012 marketing year by Blessington et al. *(5)*.

The presence of *Salmonella* on walnuts has led to recalls in 2010 (halves and pieces), 2012 (inshell), 2014 (pieces), and 2015 (two separate recalls of chopped and of halves and pieces) *(28)*. However, no human salmonellosis outbreaks have been linked to walnuts *(22)*. A multiyear (2010 to 2013) survey (3,838 samples) of *Salmonella* prevalence and contamination levels on inshell walnuts during preprocess storage revealed prevalences of <0.11% (2010; 100-g samples; 95% confidence interval [CI], 0 to 0.41%) and 0.14% (2011 to 2013; 375-g samples; 95% CI, 0.054 to 0.35%), with contamination in positive samples of 0.32 to 0.42 most probable number (MPN)/100 g *(12)*. A 2014 to 2015 survey of the prevalence and levels of *Salmonella* on shelled walnuts at retail in the United States (658 samples) conducted by the U.S. Food and Drug

* Author for correspondence. Tel: 240-402-2816; Fax: 301-436-2633; E-mail: sofia.santillanafarakos@fda.hhs.gov.

Administration (FDA) revealed a higher *Salmonella* prevalence of 1.22% (375-g samples; 95% CI, 0.53 to 2.40%) and contamination of <0.30 to 0.36 MPN/100 g *(43)*. In a subsequent 2015 to 2016 FDA retail survey in the United States, no *Salmonella*-positive samples were found among the 498 retail samples examined *(19)*. Inshell walnuts can become contaminated in the orchard through application of foliar sprays mixed with contaminated water or by animal intrusion, during harvest through direct contact with contaminated soil, during handling at the huller-dryer, and during storage. Contaminants on the shell can further transfer to the kernel during cracking and shelling or during further processing *(5)*. The marketing order issued by the U.S. Department of Agriculture, Agricultural Marketing Service *(38)* does not require handlers to subject their walnuts to a treatment process to reduce *Salmonella*. The proportion of walnuts sold in the United States that have been treated by one or more processes is not known. Processes that may be used by walnut processors include propylene oxide and steam treatments *(23)*.

The objective of this study was to conduct a quantitative risk assessment of human salmonellosis arising from the consumption of walnut kernels in the United States and to evaluate the impact of *Salmonella* reduction treatments on that risk to inform risk management decisions. We used the 2010 to 2013 survey contamination data on inshell walnuts during preprocess storage published by Davidson et al. *(12)* as the starting point of the quantitative model and examined six levels of *Salmonella* reduction treatments: no treatment and reduction by 1, 2, 3, 4, or 5 log CFU). We also estimated the impact on public health risks of atypical *Salmonella* recontamination events that can occur during walnut processing, either pre- or posttreatment. We then evaluated risk estimates using the *Salmonella* contamination levels found in the 2014 to 2015 retail samples from U.S. retail markets published by Zhang et al. *(43)* and compared these values to those obtained with the baseline model. To our knowledge, this is the first published quantitative microbiological risk assessment for *Salmonella* on walnuts.

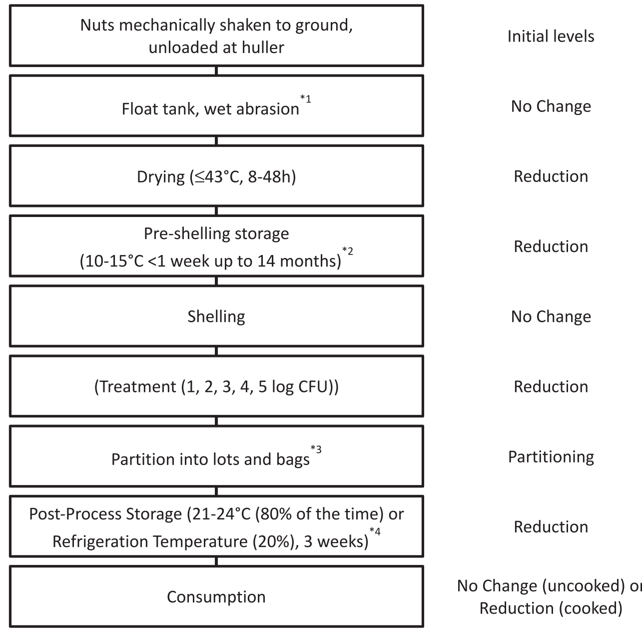

FIGURE 1. *Walnut production steps (left) and expected change in* Salmonella *levels (right) as a result of the corresponding production step.* Salmonella *prevalence and levels of contamination as reported by Davidson et al. (12) were used as initial levels in baseline risk assessment model. Preshelling storage is the point in production where the baseline model begins. Asterisks indicate points in production associated with alternative scenarios modeling atypical events or other situations: *1, atypical situation 1: cross-contamination with* Salmonella *at the float tank; *2, atypical situation 2: contamination with* Salmonella *during preshelling storage; *3, atypical situation 3: posttreatment recontamination with* Salmonella *before partitioning into lots and bags; *4, retail risk assessment model with* Salmonella *prevalence and levels of contamination at consumption as reported by Zhang et al. (43) from the 2014 to 2015 U.S. retail survey.*

MATERIALS AND METHODS

Overview of the exposure assessment model for *Salmonella* on walnuts. We assessed prevalence and levels of *Salmonella* on walnuts starting from storage at the sheller up to the point of consumption (Fig. 1). The assessment includes the major steps in a production process for walnuts to be sold shelled. These steps include preshelling storage (at ∼10 to 15°C for <1 week to 14 months), shelling, potential *Salmonella* reduction treatment, partitioning (into smaller units and consumer-size packages and bags), and postprocess storage (at ∼20 to 24°C for <1 to 9 weeks). A treatment to reduce *Salmonella* levels by 1, 2, 3, 4, or 5 log CFU was included to evaluate the impact of a treatment on the risk of salmonellosis. Minor variations to this production process scheme could exist, depending on the operation. In the absence of more detailed information, we assumed that the minor variations for individual shellers would not significantly change the prevalence or levels of *Salmonella* on walnut kernels and thus would not impact the estimated risk obtained in this assessment. Consumer home storage was not included in the exposure assessment model because consumer storage practices at home were considered beyond the scope of this risk assessment (i.e., not part of risk mitigation for regulatory purposes) *(34–36)*. The exposure assessment model thus assumes that walnuts are consumed after purchase with no further storage. However, if the consumer stored walnuts at room temperature (20 to 25°C) or in the refrigerator or freezer after purchase, *Salmonella* levels would be maintained (refrigeration or freezing) or would decrease (ambient temperature), depending on the time-temperature characteristics of storage *(4)*. The model does consider whether the product would be consumed as purchased or would be used in a product further cooked by the consumer (e.g., as an ingredient in a cooked food).

Certain exposure assessment process steps are expected to change the *Salmonella* prevalence and/or levels on inshell walnuts and/or walnut kernels (Fig. 1). For instance, a decrease in *Salmonella* (both prevalence and levels) is expected as a result of dry storage at ambient temperature (∼20 to 25°C) *(4, 5),* hot air drying *(20),* a *Salmonella* reduction step (e.g., propylene oxide gas or steam treatment), or cooking in the home. No change in *Salmonella* level is expected as a result of partitioning of walnut units (*Salmonella* cells would be only redistributed). *Salmonella* levels would not be expected to change postpurchase (in the home) when walnuts are consumed without further cooking. As in previous FDA tree nut risk assessment models *(34–36),* this model considers variability and uncertainty of parameters separately to accurately estimate risk *(14, 17, 27)* and to provide a measure of the uncertainty of the estimated number of salmonellosis cases per year. We also evaluated the probability of contamination and *Salmonella* levels separately for each step throughout the production process for better accuracy *(10, 30)*. The model includes the survival parameters for *Salmonella* on tree nuts developed previously by our research group *(33),* which included quantified survival parameter variability and uncertainty.

Estimating prevalence and level of *Salmonella* on walnuts during storage. We used data from the 2010, 2011, 2012, and 2013 surveys published by Davidson et al. *(12)* in this assessment. These data are the only prevalence data at the processor known to be available. The 3,838 inshell walnut samples were collected from 15 walnut operations located throughout five walnut growing regions in California, which process approximately half the total production volume harvested in the state. Samples were shipped to DFA of California (Safe Food Alliance, https://dfaofcalifornia.com/), stored at 4°C, and subjected to microbiological analysis within 3 months of collection. Subsamples of 100 g in 2010 and 375 g in 2011, 2012, and 2013 were analyzed for *Salmonella* by AOAC official method 2001.09 (mini VIDAS assay system) *(1)*. Positive results were confirmed using standard culture methods, and *Salmonella* levels were determined using the FDA *Bacteriological Analytical Manual (42)*. We determined a rarity index for each MPN pattern as described in Blodgett *(6)*. The rarity index is defined as the probability of observing a given pattern for the MPN divided by the probability of observing the most probable pattern for that MPN. A pattern was defined as rare when the rarity index was <0.05 *(6)*. None of the patterns as analyzed were defined as rare, indicating a homogeneous distribution of the pathogen within the unit of product (i.e., the unit size in grams) and no error or issue in the protocol.

We fit the observed MPN patterns to a lognormal distribution of sample *Salmonella* levels *(32)* and estimated uncertainty around the mean and standard deviation (SD) using a parametric bootstrap procedure *(13)*.

A unit was defined as an independent unit quantity of walnuts (inshell or kernels) in mass (measured in grams). For each iteration of the simulation, one mean and SD of the *Salmonella* level per unit was sampled from a coupled mean-SD (μ_u, σ_u; to keep the correlation structure) of the bootstrap samples to represent uncertainty. The size of a unit at the sheller step of the walnut production process was estimated to follow a triangular distribution with a minimum of 1,000 kg, a mode of 11,350 kg, and a maximum of 11,350 kg *(20)*. This unit size changes as a result of partitioning posttreatment (see "Partitioning"). Throughout the risk assessment model, the minimum level in *Salmonella*-positive units was 1 CFU, and all *Salmonella* levels are whole numbers.

Estimates for the prevalence (probability of having at least one *Salmonella* cell in the given food unit) and level of contamination (modeled as a discrete CFU per positive unit, i.e., a unit containing >0 *Salmonella* cells) were tracked separately throughout the simulation. We assumed that the *Salmonella* cells were Poisson distributed in a given unit (homogeneous distribution). The initial prevalence was then defined as

$$P_0 = 1 - \exp(-\lambda \times s)$$

where $\log(\lambda) \sim \text{Normal}(\mu_u, \sigma_u)$ is the *Salmonella* level per gram in the unit and s is the size of the unit (in grams)

***Salmonella* survival during storage.** Storage of walnuts in silos occurs at 10 to 15°C, and storage times (in weeks) vary. We modeled storage time as 5% of storage times follow a triangular distribution (minimum = 0, mode = 2, maximum = 2 weeks), 90% of storage times follow a uniform distribution (minimum = 2, maximum = 49 weeks), and 5% of occurrences follow a triangular distribution (minimum = 49, mode = 49, maximum = 73 weeks) *(20)*. Storage of walnuts postprocessing (posttreatment and retail) was modeled to occur 80% of the time at ~23°C and 20% of the time at refrigeration temperatures, all for a period of 3 weeks *(20)*.

Santillana Farakos et al. *(33)* developed a Weibull model that considers variability and uncertainty separately to predict survival of *Salmonella* on almonds, pecans, pistachios, and walnuts at ambient storage temperature (21 to 24°C). This model was developed using survival data on both inshell and shelled walnuts collected at a relative humidity below 50% *(4, 5)*. Frelka et al. *(16)* collected *Salmonella* survival data at ~10°C on inshell walnuts stored at ~65% relative humidity (commercial operation) and obtained a reduction rate of 0.33 log CFU per nut per month. When we fit a Weibull model to the ~10°C survival data of Frelka et al. and compared the results to the ~23°C model of Santillana Farakos et al. *(36),* we found the time to the first log reduction was shorter at ~10°C than at 23°C, which was unexpected. This results was presumed to be due to the higher humidity in the experiments of Frelka et al. at ~10°C; survival of *Salmonella* in low-water-activity foods increases with decreasing temperature and water activity *(2, 29, 37)*. The model of Santillana Farakos et al. *(33)* specific to walnuts obtained at 21 to 24°C was used in this risk assessment for both preshelling storage at ~10 to 15°C and postprocess storage at 23°C. Comparing the results we obtained with the result we would have obtained using the reduction rate of Frelka et al., no difference in the estimated risk per year was found.

We assumed that the decrease in *Salmonella* levels was negligible when the 3-week postprocess storage period occurred at refrigeration temperature based on the reduction of 0.1 log CFU per nut per month at 4°C reported by Blessington et al. *(3, 5)*.

In the Weibull model, the survival rate depends on time; therefore, it is necessary to consider the survival curve at the start of survival. The probability that a *Salmonella* cell selected at random will survive from time t_1 to time t_2 (a specified storage time) is defined as

$$P_{\text{surv}} = 10^{-\left(\frac{{t_2}^{\rho} - {t_1}^{\rho}}{\delta^{\rho}}\right)}$$

where δ is the time it takes to reduce the population by 1 log, ρ is a parameter that defines the shape of the curve, and t_1 and t_2 are times since the beginning of the survival step (t_0). In the present assessment, t_0 is defined as the start of the storage step for walnuts at the silo. We used a binomial process restricted to positive values to evaluate the level of *Salmonella* in positive units at the end of each stage of the exposure assessment model:

$$N_2 \sim \text{Binomial}(N_1, P_{\text{surv}}), \text{ with } N_2 > 0$$

where N_2 is the level of *Salmonella* in the contaminated unit at the end of survival (t_2) and N_1 is the level of *Salmonella* in the contaminated unit at the beginning of survival (t_1). The binomial model assumes that each *Salmonella* cell has an independent probability of survival. The probability of contamination is accordingly adjusted to

$$P_2 = P_1 \left[1 - (1 - P_{\text{surv}})^{N_1}\right]$$

accounting for units in which there are no *Salmonella* remaining, where P_{surv} and N_1 are defined as above, P_2 is the probability of contamination at the end of survival (t_2), and P_1 is the probability of contamination at the beginning of survival (t_1).

Shelling. After storage, inshell walnuts to be sold shelled are cracked to remove the shell. In the absence of available data on *Salmonella* transfer rates from shell to kernel, we assumed all *Salmonella* present inshell would transfer to the shelled product (worst case scenario). Thus, no change in prevalence and levels of contamination were modeled.

***Salmonella* reduction treatment.** We modeled six reduction treatment scenarios: no *Salmonella* reduction and reduction of *Salmonella* levels by 1 to 5 log CFU. The treatment levels were defined per unit of product being treated. Variability in treatment reduction was not considered because data were unavailable. The impact of a specific treatment level on the risk of salmonellosis can be derived from the results provided in this risk assessment when the reduction value or range of values is known. For the *Salmonella* reduction treatment step, we assumed that each *Salmonella* cell had an identical and independent probability of inactivation. We used a binomial process restricted to positive values to evaluate the level of *Salmonella* at the end of this stage:

$$P_{\mathrm{surv}T} = 10^{-L}$$

$$N_{2T} \sim \mathrm{Binomial}(N_{1T}, P_{\mathrm{surv}T}), \text{ with } N_{2T} > 0$$

$$P_{2T} = P_{1T}\left[1 - (1 - P_{\mathrm{surv}T})^{N_{1T}}\right]$$

where L is the log reduction (1, 2, 3, 4, or 5 log CFU), $P_{\mathrm{surv}T}$ is the probability of survival at the *Salmonella* reduction treatment level, P_{2T} is the probability of contamination posttreatment, P_{1T} is the probability of contamination pretreatment, N_{2T} is the level of *Salmonella* in the contaminated unit posttreatment, and N_{1T} is the level of *Salmonella* in the contaminated unit pretreatment.

Partitioning. After the *Salmonella* reduction treatment and shelling, the units are redistributed into units of sizes from 45 to 45,000 kg *(20)*. The units are then further partitioned into consumer packages (shelled walnuts) from an 18-g snack pack to a 224-g (~0.5 lb) or 454-g (~1 lb) bag. To evaluate the change in *Salmonella* levels per subunit as a result of partitioning, one subunit (at random) is followed per iteration, and the probability of contamination and level of *Salmonella* for each step is estimated as follows:

$$N_2 \sim \mathrm{Binomial}\left(N_1, \frac{S_2}{S_1}\right), \text{ with } N_2 > 0$$

$$P_2 = P_1\left[1 - \left(1 - \frac{S_2}{S_1}\right)^{N_1}\right]$$

where N_1, N_2, P_1, and P_2 are defined as above and S_1 and S_2 refer to the subunit sizes before and after partitioning, respectively.

Further partitioning at the consumer level is the ingested dose, and that is a partition process from the size of the pack to the serving size (see "Consumption").

Cooking. Consumers can use walnuts as an ingredient in cooked products (e.g., when baking cakes or cookies). These walnuts are purchased as an uncooked ingredient (inshell or shelled) and are later cooked at home. No references were found with data specifically concerning *Salmonella* survival on walnuts during baking. Lathrop et al. *(26)* collected survival data for *Salmonella* in peanut butter during the baking of cookies. In that study, commercial peanut butter was artificially inoculated with a five-serovar cocktail of *Salmonella* (serovars Tennessee, Tornow, Hartford, Agona, and Typhimurium). The inoculated peanut butter was used to prepare peanut butter cookies using a standard recipe, and cookies were baked at 177°C for various times (10 to 15 min). A minimum of a 4.8-log decrease in *Salmonella* levels per cookie (25 g) were found after 10 min at 177°C (detection limit of 0.04 CFU/g). Cookies baked for 15 min had no detectable *Salmonella*. Peanut butter, similar to walnuts, is a low-water-activity product. Although the composition of peanuts and peanut-related products is different from that of walnuts, the main parameters influencing survival of *Salmonella* during heating of foods are temperature and water activity, which are assumed to be similar for peanuts and walnuts. In the absence of available data and based on the similarity in product type and water activity, we assumed that the expected log decrease in *Salmonella* levels during baking of walnuts approximates the minimum decrease that occurs during baking of peanut butter cookies. We used a fixed value of 5 log CFU for the reduction achieved during cooking for walnuts included as an ingredient in food products that undergo a cooking step in the home.

Consumption. Consumption of walnut kernels in the U.S. population was estimated using data originating from What We Eat in America (WWEIA), the dietary survey portion of the National Health and Nutrition Examination Survey (NHANES), for the 2003 to 2004, 2005 to 2006, 2007 to 2008, and 2009 to 2010 cycles *(9)*. Proportions of walnut ingredients in NHANES-WWEIA foods used in these analyses were based on "recipes" developed for the U.S. Environmental Protection Agency's food commodity intake database *(41)*. Empirical distributions representing serving sizes among consumers (eaters) and weighted by the NHANES-WWEIA dietary statistical sampling weights were used for walnuts consumed as a core product uncooked, as an ingredient uncooked, and as an ingredient cooked. We distinguished between three independent types of walnut products consumed (where the cooking step, when present, is assumed to happen in the home): (i) core walnut product (≥80% of the product ingredients are walnuts) consumed uncooked, (ii) walnut as an ingredient (<80% of the product ingredients are walnuts) consumed uncooked, and (iii) walnut as an ingredient (<80% of the product ingredients are walnuts) consumed cooked (e.g., in baked, fried, or boiled products). The cooking step in cooked walnuts is cooking by the consumer and would not include, for instance, walnuts sold as roasted. We estimated the number of servings per year, assuming that data reported in the NHANES-WWEIA 24-h dietary recalls (two per survey respondent, conducted 3 to 10 days apart) are representative of consumption over the whole year and estimating approximately 320 million individuals in the United States *(40)*. The number of walnut servings per year in the United States was estimated given NHANES-WWEIA data indicating that 1.51% of the population reported consumption of uncooked walnuts as a core product, 1.62% reported consuming uncooked walnuts as an ingredient, and 11.46% reported consuming cooked walnuts as an ingredient.

Modeling atypical situations in walnut handling. Atypical situations in the supply chain may change the risk of salmonellosis stemming from consumption of walnuts. Cross-contamination has been identified as a mechanism for pathogenic bacterial contamination of low-water-activity foods *(8, 29)*.

In this risk assessment, three atypical situations that could lead to increases in risk per serving were evaluated. These atypical situations included both pre- and posttreatment recontamination events and were modeled for walnuts consumed as a core product (i.e., consumed as bought or as an ingredient in a food that is at least 80% walnuts) not cooked at home. These atypical situations were not modeled for the entire U.S. walnut supply but as individual events impacting a portion of the supply. The number of salmonellosis cases per year linked to each atypical situation would be equal to the number of cases linked to one atypical situation multiplied by the number of such atypical situations in that year. Although it is not possible to predict the number of cases per year for each atypical situation because it is not known how many such events occur in a year, the risk estimates obtained for those situations we modeled provide an estimate of their significance compared with the baseline model scenario and of the impact the atypical situation could have on risk (changes in order of magnitude). In a fourth scenario, we evaluated risk estimates using the *Salmonella* contamination levels found in the 2014 to 2015 retail samples from U.S. retail markets *(43)*. The step in the process where the atypical situations (1, 2, and 3) are modeled to occur and where the retail model starts (situation 4) are marked with asterisks (*1, *2, *3, and *4) in Figure 1.

In the atypical situation 1, cross-contamination at the float tank, water at the float tank becomes contaminated from contaminated walnuts and cross-contaminates other walnuts that pass through the float tank. We assumed that the prevalence of *Salmonella* was the same as in the baseline exposure assessment model but that levels were increased by a fixed amount of 0.5 to 1.5 log CFU per contaminated lot. The float tank step occurs before drying, whereas the contamination data we used in the baseline model were based on the levels after drying. To estimate prevalence and levels of contamination of *Salmonella* on walnuts at the float tank, a back-calculation from the values determined in the silo storage (baseline model) was done, taking into account the impact of drying. Drying was modeled based on data published by Frelka et al. *(16),* who found that *Salmonella* populations decreased by 2.57 log CFU (deterministic value) after drying under typical commercial conditions (warm air of $<43°C$ for ~12 h). The extra contamination with *Salmonella* of 0.5 to 1.5 log CFU (uniform distribution) was added to the back-calculated levels at the flotation tank. The drying step (using the same decline as that used for the back-calculation) was modeled followed by the subsequent steps in the baseline model: preshelling storage, shelling, *Salmonella* reduction treatment, partitioning, posttreatment storage, and consumption.

In atypical situation 2, additional contamination during preshelling storage, increased levels of *Salmonella* on walnuts during preshelling storage (0.5 to 1.5 log CFU per contaminated lot) were modeled to represent a pest infestation in storage silos. The same prevalence as the baseline exposure assessment model was assumed, but levels of contamination were increased. The rest of the process follows with the same steps as the baseline exposure assessment model: shelling, *Salmonella* reduction treatment, partitioning, postprocess storage, and consumption.

In atypical situation 3, posttreatment contamination, increased levels of *Salmonella* in contaminated units posttreatment but before partitioning into lots and bags (0.5 to of 1.5 log CFU per contaminated lot) were modeled assuming the same initial prevalence as the baseline exposure assessment model. The rest of the process follows with the same steps as those of the baseline exposure assessment model: partitioning, postprocess storage, and consumption.

In the retail risk assessment model, scenario 4, prevalence and levels found in the 2014 to 2015 FDA survey published by Zhang et al. *(43)* were assumed to be those at retail and thus at the point of consumption (1.22% prevalence; 375-g samples; 95% CI, 0.53 to 2.40%), and levels of contamination were <0.30 to 0.36 MPN/100 g. The assay used to analyze the 2014 to 2015 FDA survey samples is the same as that used to estimate initial levels of contamination in the baseline exposure assessment model *(42)*.

Hazard characterization. The dose-response model used in this risk assessment is equivalent to the β-Poisson dose-response model with parameters $\alpha = 0.1324$ (95% CI, 0.094 to 0.1817) and $\beta = 51.45$ (95% CI, 43.75 to 56.39) derived by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO-WHO) *(17)* adapted to the number of *Salmonella* cells, which in our model is an exact value (β-binomial dose-response model *(18)*). The risk estimates obtained when using the 2.5th and 97.5th percentile of the FAO-WHO *Salmonella* dose-response curve *(15)* resulted in mean estimated risks that were in the same order of magnitude as that when using the FAO-WHO expected values *(15)*. As such, no uncertainty in the dose-response was considered.

Risk characterization. Risk estimates per serving result from combining the FAO-WHO dose-response function *(15)* with the results of the exposure assessment module (levels of *Salmonella* per contaminated serving and prevalence of contaminated servings). Risk per year was then calculated by multiplying the number of servings by the risk per serving. The risk was assessed using a second-order Monte Carlo simulation *(17)*. Monte Carlo simulations were developed in R using the *mc2d* package *(31)*. The variability dimension was set to 10,001 replicates, and the uncertainty was set to 501 replicates (i.e., 501 replicates to evaluate uncertainty, and within each uncertainty loop 10,001 replicates to characterize variability in model parameters). The factors for which variability and uncertainty was considered were the probability of contamination, the *Salmonella* contamination levels, the survival model parameters, pretreatment and posttreatment storage times, and consumption patterns.

Sensitivity analysis. Spearman's rho statistic was determined, with risk per serving as the outcome variable and looking at risk estimates arising from consumption of walnuts as a core product uncooked at home for no treatment and a 4-log *Salmonella* reduction treatment level. Factors considered were those for which variability and uncertainty were estimated and included initial contamination levels, the time it takes to reduce the *Salmonella* population by 1 log CFU (δ), pretreatment and posttreatment storage times, and consumption patterns.

All statistical analyses were carried out using R 3.4.2 (R Core Team, Vienna, Austria). The R code is available on request by e-mail to FDAFoodSafetyRiskModel@fda.hhs.gov.

RESULTS AND DISCUSSION

Baseline probabilities of *Salmonella* contamination and levels of contamination throughout steps in the exposure assessment stage. Examination of the estimated mean probability of *Salmonella* contamination and *Salmonella* levels (for contaminated units) at the end of each stage of the exposure assessment model for no treatment and for 1-, 2-, 3-, 4-, and 5-log *Salmonella* reduction treatments revealed a decrease in both of these quantities throughout the exposure model (Table 1). Gradually increasing reduction treatments (from 1 to 5 log CFU) result in gradually decreasing levels of *Salmonella* per unit, with an

TABLE 1. *Probability of* Salmonella *contamination and contamination levels for each stage of the exposure assessment model for walnuts treated with a simulated 0-, 1-, 2-, 3-, 4-, and 5-log* Salmonella *reduction treatment*

Exposure assessment stage	Unit size (g)	Mean probability of contamination after reduction treatment of[a]: 0 log	1 log	2 log	3 log	4 log	5 log	Mean contamination (CFU/unit) after reduction treatment of[b]: 0 log	1 log	2 log	3 log	4 log	5 log
Preshelling storage	10,161,226	0.32						20.71					
Posttreatment	10,161,226	0.068	0.019	0.0042	0.00072	0.000095	0.0000097	1.81	1.06	1	1	1	1
Partition into units	4,715,836	0.045	0.012	0.0024	0.00039	0.000048	0.0000049	1.37	1	1	1	1	1
Partition into packages	224	3.75E−05	3.81E−06	3.78E−07	3.74E−08	3.76E−09	3.82E−10	1	1	1	1	1	1
Postprocess and retail storage	224	1.84E−05	1.83E−06	1.81E−07	1.79E−08	1.85E−09	1.86E−10	1	1	1	1	1	1

[a] Probability that a unit is contaminated with *Salmonella*; a contaminated unit is defined as having at least 1 CFU.
[b] *Salmonella* level in each contaminated unit at the end of each exposure assessment stage (the minimum value is 1 CFU).

approximately 10-fold decrease for every additional log reduction (Fig. 2). Among the factors considered in the baseline model, these results indicate that treatment has the largest impact on the probability of *Salmonella* contamination and on levels in contaminated units (Table 1 and Fig. 2). Although the mean level is 1 CFU per contaminated unit for all treatments at the partitioning-into-packages stage, the levels are not necessarily independent of treatment and partitioning does not necessarily result in a decrease in *Salmonella* levels. Rather, contamination levels are expressed per contaminated unit, and the units are partitioned to such a degree that they contain the minimum *Salmonella* level to be considered positive, which is 1 CFU. The impact of the treatment is thus mostly reflected in the probability of contamination for a given unit (Table 1). The lower probability of contamination per unit after partitioning (Table 1) is a result of the increase in the number of units that contain zero *Salmonella* cells (which results from the redistribution of *Salmonella* cells into a higher number of units of smaller unit size).

Consumption. The mean (±SD) intakes per serving (based on NHANES-WWEIA 2003 to 2010 data) are 20.6 (±16.6) g for walnuts consumed as a core product uncooked at home, 4.77 (±5) g for walnuts consumed as an ingredient uncooked at home, and 1.52 (±2.34) g for walnuts consumed as an ingredient cooked at home.

Risk estimates per serving. The distribution of the estimated risk per serving of walnuts represents the probability of acquiring human salmonellosis in the U.S. population due to the consumption of a walnut serving (Table 2 and Fig. 3). Table 2 contains six sets of statistics (one for each *Salmonella* treatment level: no treatment and 1-, 2-, 3-, 4-, and 5-log reduction) on risk from consuming three types of walnut products: walnuts consumed as a core product uncooked at home, walnuts consumed as an ingredient uncooked at home, and walnuts consumed as an ingredient cooked at home. In Figure 3, risk of human salmonellosis per serving for consumption of walnuts given a 1-, 2-, 3-, 4-, and 5-log *Salmonella* reduction treatment relative to the risk per serving from consumption of walnuts as an ingredient cooked at home (i.e., having received an additional 5-log *Salmonella* reduction treatment through cooking) is shown. The highest risk is associated with walnuts consumed as a core product uncooked at home (walnuts that receive no cooking step at home), followed by walnuts consumed as an ingredient uncooked at home, and to a lesser extent walnuts cooked at home before consumption (Table 2 and Fig. 3). As the treatment efficiency increases from a 1- to 5-log reduction, the mean risk of salmonellosis per serving in the U.S. population decreases for all three types of walnut products consumed (Table 2 and Fig. 3). Variability (columns in Table 2) represents heterogeneity in the risk per serving (not reducible by data collection), and uncertainty (rows in Table 2) represents lack of knowledge (which can be reduced by additional data collection). The considered variability and uncertainty is included in the probability of

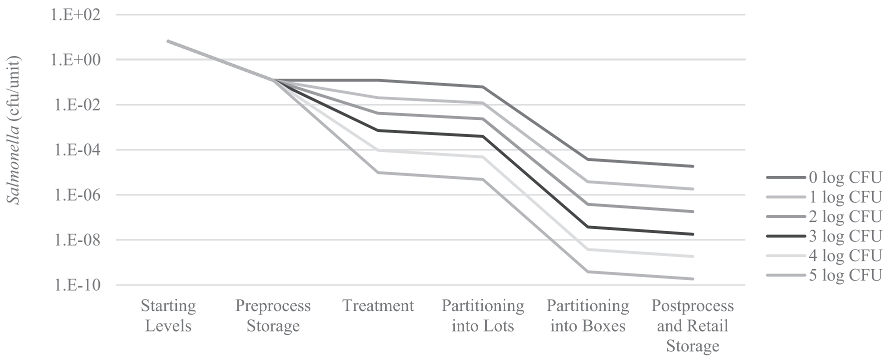

FIGURE 2. Salmonella *levels (CFU per unit) in each exposure assessment stage for the 0-, 1-, 2-, 3-, 4-, and 5-log* Salmonella *reduction treatments.*

contamination, the *Salmonella* contamination levels, the survival model parameters, and all process conditions that are part of the exposure assessment model (e.g., times and temperatures during storage). The impact of variability is much larger than the impact of the considered uncertainty. Variability in estimated risk (from the 2.5th to the 97.5th quantile of variability) spans over 5 log, whereas the uncertainty (from the 2.5th to the 97.5th quantile of uncertainty) for a given statistic spans 2 to 3 log (Table 2). The distributions, notably in the variability dimension, are skewed as can be inferred by the position of the mean, which is much closer to the 97.5% quantile of variability than to the 2.5% quantile.

Mean risk estimates per contaminated serving among the contaminated servings eaten by individuals in the U.S. population correspond to one case of salmonellosis per 100 million servings (95% CI, one case per 3 million to one case per 2 billion servings) of walnuts consumed as a core product uncooked at home with no *Salmonella* reduction treatment applied. However, the 4- and 5-log *Salmonella* reduction treatments reduce the risk per serving for these uncooked walnuts to one case of salmonellosis per 1 trillion servings (95% CI, one case per 50 billion to one case per 10 trillion servings) and one case per 10 trillion servings (95% CI, one case per 100 billion to one case per 100 trillion servings), respectively. Walnuts consumed as an ingredient cooked at home have an average of ∼2,000,000-fold lower risk per serving compared with walnuts consumed as a core product uncooked at home. Differences in estimated risk for walnuts consumed as a core product uncooked at home versus as an ingredient cooked at home can be mainly attributed to the additional *Salmonella* reduction step (cooking) when consuming cooked walnuts and, to a lesser degree, to differences in serving size when walnuts are consumed as an ingredient. Minimal differences in estimated risk for walnuts consumed as a core product uncooked at home versus as an ingredient uncooked at home (an average of fivefold lower when consumed as an ingredient) can be attributed to differences in the walnut serving size when walnuts are consumed as an ingredient.

Risk estimates per year. As estimated from the NHANES-WWEIA data, 1.51% of the U.S. population consumed walnuts as a core product uncooked at home (7.9×10^8 servings per year), 1.62% consumed walnuts as an ingredient uncooked at home (9.5×10^8 servings per year), and 11.46% consumed walnuts as an ingredient cooked at home (6.7×10^9 servings per year). The estimated salmonellosis occurrence in the United States (Table 3) for walnuts consumed as a core product uncooked at home without a *Salmonella* reduction treatment was 6 cases per year (95% CI, <1 to 278 cases). A 1- and 2-log *Salmonella* reduction treatment level reduces the mean risk for these walnuts to less than 1 case per year, with 95% CIs of <1 to 20 and <1 to 2 cases per year, respectively. A minimum 3-log *Salmonella* reduction treatment for these walnuts results

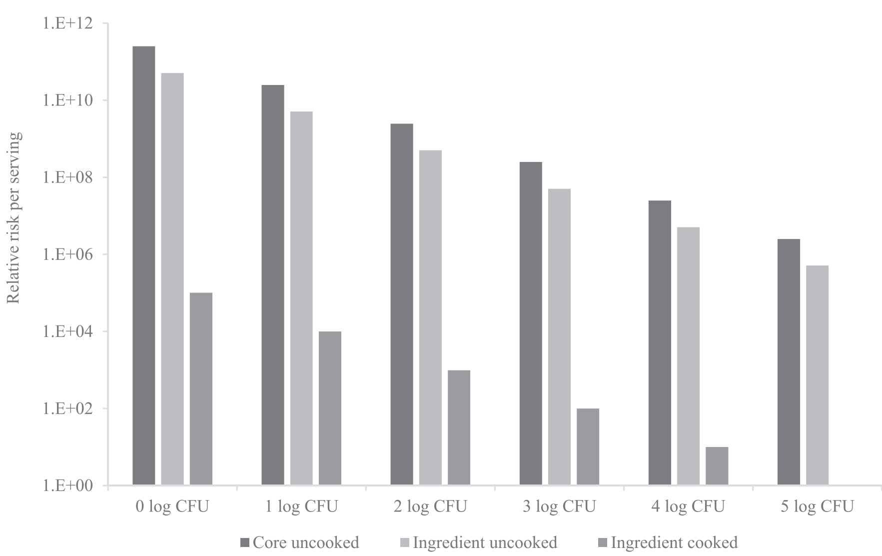

FIGURE 3. *Risk of human salmonellosis per serving for consumption of walnuts given a 1-, 2-, 3-, 4-, and 5-log* Salmonella *reduction treatment relative to the risk per serving from consumption of walnuts as an ingredient cooked at home (i.e., having received an additional 5-log reduction treatment through cooking). In a core walnut product, ≥80% of the ingredients are walnuts; when walnuts are an ingredient, <80% of the product ingredients are walnuts. Uncooked walnuts are not further cooked at home; cooked walnuts receive a cooking step at home (e.g., baking).*

TABLE 2. *Salmonellosis risk per serving for consumption of walnuts in the U.S. population*[a]

Treatment (log reduction)	Statistic	Walnut core uncooked[b]				Walnut ingredient uncooked[c]				Walnut ingredient cooked[d]			
				Quantiles of variability				Quantiles of variability				Quantiles of variability	
		Mean	SD	2.5%	97.5%	Mean	SD	2.5%	97.5%	Mean	SD	2.5%	97.5%
0	Estimate	7.15E−09	1.11E−07	1.62E−13	1.26E−08	1.44E−09	2.32E−08	3.13E−14	2.60E−09	2.86E−15	4.31E−14	2.39E−20	4.86E−15
	95% CI	5.50E−10	4.69E−09	8.38E−25	6.70E−17	1.35E−10	1.05E−09	1.66E−25	1.41E−17	1.08E−16	2.66E−15	1.12E−31	1.85E−23
		3.15E−07	2.86E−05	1.65E−10	8.58E−08	5.54E−08	4.72E−06	3.29E−11	1.73E−08	7.79E−14	7.33E−12	2.07E−17	3.67E−14
1	Estimate	7.08E−10	1.24E−08	1.62E−14	1.27E−09	1.44E−10	2.40E−09	3.13E−15	2.62E−10	2.84E−16	4.38E−15	2.39E−21	4.82E−16
	95% CI	5.02E−11	4.66E−10	8.38E−26	6.70E−18	1.49E−11	1.09E−10	1.66E−26	1.41E−18	1.10E−17	2.39E−16	1.12E−32	1.85E−24
		2.29E−08	2.05E−06	1.66E−11	8.42E−09	5.52E−09	4.65E−07	3.31E−12	1.73E−09	7.07E−15	6.80E−13	2.08E−18	3.61E−15
2	Estimate	6.98E−11	1.10E−09	1.62E−15	1.31E−10	1.43E−11	2.29E−10	3.13E−16	2.64E−11	2.79E−17	3.76E−16	2.39E−22	4.95E−17
	95% CI	5.44E−12	4.61E−11	8.38E−27	6.70E−19	1.12E−12	1.08E−11	1.66E−27	1.41E−19	1.07E−18	2.04E−17	1.12E−33	1.85E−25
		2.04E−09	1.85E−07	1.67E−12	8.64E−10	4.87E−10	4.16E−08	3.31E−13	1.76E−10	6.67E−16	6.30E−14	2.10E−19	3.69E−16
3	Estimate	7.09E−12	1.08E−10	1.62E−16	1.31E−11	1.42E−12	2.14E−11	3.13E−17	2.65E−12	2.84E−18	4.38E−17	2.39E−23	4.95E−18
	95% CI	5.52E−13	4.61E−12	8.38E−28	6.70E−20	1.25E−13	1.06E−12	1.66E−28	1.41E−20	1.14E−19	2.60E−18	1.12E−34	1.85E−26
		2.45E−10	2.22E−08	1.67E−13	8.75E−11	4.80E−11	4.01E−09	3.31E−14	1.77E−11	6.78E−17	6.50E−15	2.10E−20	3.68E−17
4	Estimate	7.05E−13	1.07E−11	1.62E−17	1.31E−12	1.44E−13	2.40E−12	3.13E−18	2.65E−13	2.84E−19	4.34E−18	2.39E−24	4.95E−19
	95% CI	7.66E−14	4.61E−13	8.38E−29	6.70E−21	1.34E−14	1.06E−13	1.66E−29	1.41E−21	1.13E−20	2.61E−19	1.12E−35	1.85E−27
		2.21E−11	1.99E−09	1.67E−14	8.76E−12	5.20E−12	4.68E−10	3.31E−15	1.77E−12	6.84E−18	6.38E−16	2.10E−21	3.69E−18
5	Estimate	7.09E−14	1.08E−12	1.62E−18	1.31E−13	1.46E−14	2.40E−13	3.13E−19	2.65E−14	2.84E−20	4.29E−19	2.39E−25	4.95E−20
	95% CI	5.39E−15	4.61E−14	8.38E−30	6.70E−22	1.35E−15	1.06E−14	1.66E−30	1.41E−22	1.16E−21	2.61E−20	1.12E−36	1.85E−28
		2.20E−12	1.94E−10	1.67E−15	8.76E−13	4.97E−13	4.43E−11	3.31E−16	1.77E−13	5.63E−19	5.25E−17	2.10E−22	3.70E−19

[a] Columns (e.g., mean, SD, 2.5%, and 97.5%) characterize variability, and rows (estimate, 95% CI) characterize uncertainty in the estimates. 95% CI represents the range of values in which there is a 95% probability of finding the true value.
[b] Walnuts consumed as a core product uncooked at home.
[c] Walnuts consumed as an ingredient in a product uncooked at home.
[d] Walnuts consumed as an ingredient in a product cooked at home.

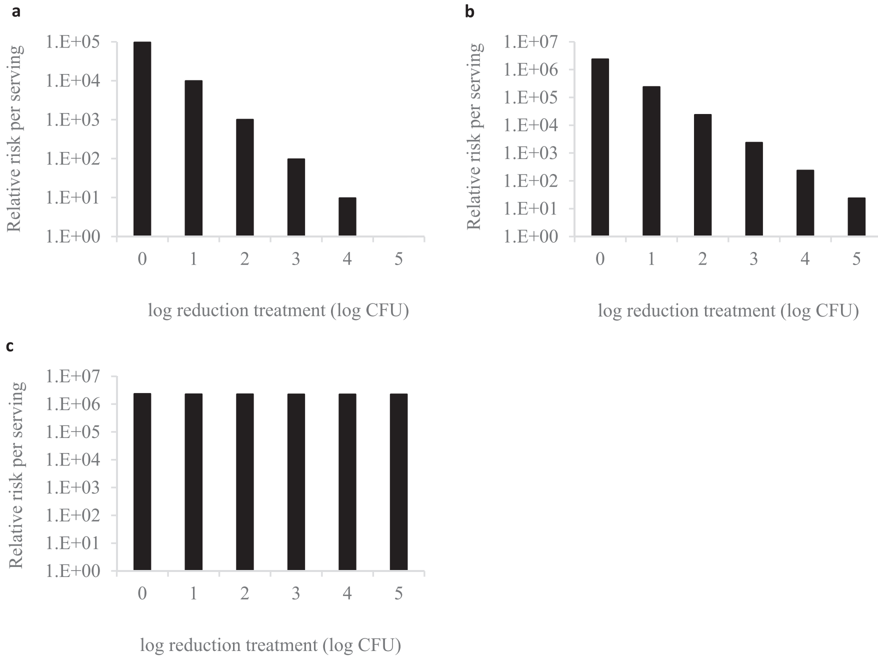

FIGURE 4. *Risk per serving of walnuts consumed as a core product uncooked at home assuming various* Salmonella *reduction treatments (0- to 5-log reductions) relative to the risk per serving for the baseline model given a 5-log* Salmonella *reduction treatment (7.1×10^{-14}). (a) Atypical situation 1: cross-contamination with* Salmonella *at the float tank; (b) atypical situation 2: contamination with* Salmonella *during preshelling storage; (c) atypical situation 3: posttreatment recontamination with* Salmonella *before partitioning into lots and bags.*

in a mean risk of <1 case per year, including an upper uncertainty bound of the mean estimated cases of <1. For walnuts consumed as an ingredient uncooked at home, a minimum 2-log *Salmonella* reduction treatment results in a mean risk of <1 case per year, with an upper uncertainty bound of the mean estimated cases of <1. Cooking walnuts significantly decreases the risk estimate, with the number of cases per year estimated to be <1 for all treatments when walnuts are cooked in the home.

Estimated risk from the modeled pre- and posttreatment atypical situations. For each of the three atypical situations considered, the estimated risk of salmonellosis arising from consumption of walnuts as a core product uncooked at home is compared with the risk for the baseline model (with no atypical situation) in Figure 4. The number of cases per year linked to each kind of atypical situation would be equal to the number of cases linked to the atypical situation multiplied by the number of such atypical situations in that year (which is currently unknown).

For a pretreatment recontamination event at the float tank (with an increase in *Salmonella* by 0.5 to 1.5 log CFU in contaminated units predrying), no difference in risk was found compared with the baseline model (Fig. 4a). This is because drying is estimated to mitigate the risk. A ~3-log recontamination would have to occur in this type of atypical situation to see a significant increase in risk estimates compared with the baseline model. Because the atypical situation occurs prior to treatment, there is a significant difference in risk estimate among reduction treatments, with the risk decreasing as the reduction treatment level increases from 1 to 5 log CFU.

When a pretreatment recontamination event occurs during preshelling storage (with an increase in levels of *Salmonella* of 0.5 to 1.5 log CFU), a significant difference in risk estimates for the different reduction treatments is predicted (Fig. 4b). The risk estimate for this atypical event decreases as the reduction treatment increases from 1 to 5 log CFU. The risk estimates are an average of ~24 times higher mean risk per serving when compared with the baseline for each treatment level (Fig. 4b). This increased risk is due to contamination that occurs after drying but before a *Salmonella* reduction treatment.

When contamination with *Salmonella* occurs posttreatment before partitioning into lots and packages with the same increase in levels of *Salmonella* of 0.5 to 1.5 log CFU as assumed in the other two atypical situations, a significant increase in risk is predicted. When no treatment is applied, the increase in mean risk per serving is predicted to be ~20 times higher than the risk estimated for the baseline model

TABLE 3. *Estimated number of salmonellosis cases per year from consumption of walnuts in the United States*

	No. of salmonellosis cases					
	Walnut core uncooked[a]		Walnut ingredient uncooked[b]		Walnut ingredient cooked[c]	
Treatment (log reduction)	Mean	95% CI[d]	Mean	95% CI	Mean	95% CI
0	6	<1–278	1	<1–52	<1	<1
1	<1	<1–20	<1	<1–5	<1	<1
2	<1	<1–2	<1	<1	<1	<1
3	<1	<1	<1	<1	<1	<1
4	<1	<1	<1	<1	<1	<1
5	<1	<1	<1	<1	<1	<1

[a] Walnuts consumed as a core product uncooked at home (8×10^8 servings per year).
[b] Walnuts consumed as an ingredient in a product uncooked at home (9×10^8 servings per year).
[c] Walnuts consumed as an ingredient in a product cooked at home (7×10^9 servings per year).
[d] 95% CI represents the range of values in which there is a 95% probability of finding the true value.

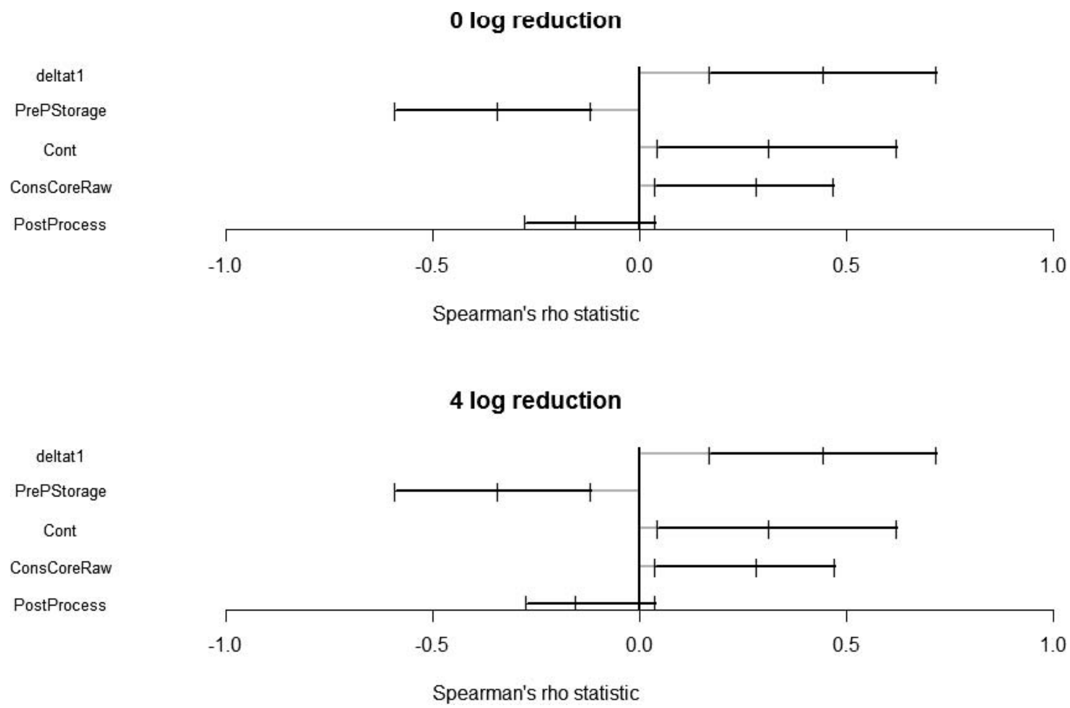

FIGURE 5. *Spearman rho statistic for the baseline risk assessment model considering no* Salmonella *reduction treatment and a 4-log reduction treatment, with risk per serving from consumption of walnuts as a core product uncooked at home as the outcome variable. Delta1 is the time (weeks) it takes to reduce the* Salmonella *population by 1 log CFU per contaminated unit at 23°C, PrePStorage is the pretreatment storage time (weeks), Cont is the initial* Salmonella *contamination, ConsCoreRaw is the serving size for walnuts consumed as a core product uncooked at home, and PostProcess is the posttreatment storage time (weeks).*

(without treatment). When a 5-log reduction treatment is applied, the increase in risk estimated for this atypical event is predicted to be $\sim 2 \times 10^6$ times higher than that for the baseline model. This difference is a result of the fact that estimated mean risk of illness per serving for this atypical event is nearly independent of the log reduction treatment level (Fig. 4c) because contamination takes place after the reduction treatment and is being compared with the baseline model assuming a 5-log reduction treatment.

These results indicate that generally, when a treatment is in place, the contribution to the overall salmonellosis risk of atypical events that lead to pretreatment contamination of walnuts will be small compared with the contribution of atypical events that lead to posttreatment contamination. When no *Salmonella* reduction treatment is applied, the relative risk is essentially the same whether recontamination occurs early or late in the process.

Sensitivity analysis results. The results of the sensitivity analysis indicate that the time it takes to reduce *Salmonella* by 1 log CFU has the greatest impact on mean risk per serving estimates followed by pretreatment storage time at any treatment level (Fig. 5). Longer storage times result in decreasing levels of mean estimated risk per serving (*Salmonella* tends to decrease at 20 to 25°C), which is the reason for the negative Spearman rho values found for these factors (i.e., pretreatment and posttreatment storage). Initial contamination levels and U.S. consumption patterns follow in decreasing order of impact. Postprocess storage (including retail storage time) has the lowest impact on risk estimates.

Estimated risk when using *Salmonella* contamination data from the 2014 to 2015 retail survey. Data on prevalence and levels of *Salmonella* on tree nuts (including walnuts) at retail in the United States were collected from 2014 to 2015 by a commercial testing laboratory under contract with the FDA *(43)*. Prepackaged shelled walnut samples (617 conventional and 41 organic) were collected from various types of retail markets (major chain, small chain, discount store, drug store, etc.) and from various regions throughout the continental United States. Roasted nuts, nut butters, nut mixes, or nuts coated with seasonings, chocolate, or candy were excluded. A mean *Salmonella* prevalence of 1.22% (95% CI, 0.53 to 2.40%) was found for shelled walnuts as a ready-to-eat product. When using the 2014 to 2015 retail survey data, the risk estimate per serving for walnuts consumed as a core product uncooked at home leads to a risk estimate of one salmonellosis case per 600,000 servings (95% CI, one case per 800,000 to one case per 400,000 servings). This risk per serving estimate using the retail contamination data is approximately two orders of magnitude higher than that found for the baseline walnut risk assessment model, which used contamination data at the handler and assumed no *Salmonella* reduction treatment. It is not known whether the walnuts sampled at retail had undergone a *Salmonella* reduction treatment because this information is not provided on the package and treatment currently is not required.

The difference in estimates can be traced to observed prevalence. The mean prevalence found for the 2014 to 2015 samples at retail (1.22%, 8 of 658 total samples were positive for *Salmonella*) was approximately 10 times higher than that found for the 2010 to 2013 inshell walnuts during storage at the handler (preshelling) (0.14%, 3 of 3,838 samples were positive) *(12)*. The observation of a higher prevalence at retail than at the handler is unexpected because all typical walnut processing stages are expected to either reduce the prevalence and levels of *Salmonella* or leave them unchanged (Fig. 1). We suggest that the higher prevalence observed in the 2014 to 2015 retail survey is either a reflection of variability in *Salmonella* contamination across the supply or an atypical event affecting a portion of the supply. For example, raw walnuts at harvest from 2014 to 2015 may have been significantly more contaminated than those sampled during the 2010 to 2013 survey. In research on other tree nuts, *Salmonella* prevalence and levels on these nuts (which are harvested once per year) can vary significantly among harvest years *(11, 21, 34, 36)*. However, for this scenario to explain the 2014 to 2015 prevalence estimate at retail, the prevalence and/or levels of contamination at harvest would have to have been orders of magnitude higher than the observations in the multiyear 2010 to 2013 survey, taking into account the subsequent decreases expected from drying (~2.6 log CFU) and from pre- and postprocess storage (~3 log CFU). A more likely scenario is that there was *Salmonella* contamination during processing in one or more facilities in 2014, most likely posttreatment when a *Salmonella* reduction treatment was applied. This hypothesis is also consistent with preliminary data from a follow-up survey conducted under contract with the FDA during 2015 to 2016, in which no *Salmonella*-positive samples were found among 498 retail samples examined *(19)*. Other scenarios or factors could explain the observed contamination levels, but a thorough analysis is beyond the scope of the present study.

Whether the relatively high prevalence of *Salmonella* on walnuts during the 2014 to 2015 retail survey reflects variability in contamination among raw walnuts or atypical situations leading to contamination during processing, these data indicate that contaminated walnuts that pose a public health risk to U.S. consumers can reach the retail market.

Comparison with previous tree nut risk assessment models. Previous risk assessments for *Salmonella* on tree nuts include those for almonds *(25, 36),* pecans *(35),* and pistachios *(24, 34)*. In these studies, the estimated mean risk of salmonellosis arising from U.S. consumption of almonds, pecans, or pistachios was less than one case per year when a minimum 4-log reduction treatment was applied to the entire U.S. supply. The current walnut risk assessment results predict that a minimum 3-log reduction treatment would result in a risk per year of <1 case of salmonellosis, when the initial contamination levels of inshell walnuts during storage at the handler are similar to those reported by Davidson et al. *(12)*. This 10-fold difference in risk between walnuts and the other tree nuts can mainly be attributed to the lower prevalence of *Salmonella* on walnuts found at the handler, which is on average 10 times lower than that found for almonds, pecans, and pistachios at the equivalent step in their corresponding production processes.

In the FDA's previously published risk assessments *(34–36),* we also modeled examples of atypical situations that have the potential to lead to increased risk of illness, including simulation of two U.S. outbreak events, one associated with almonds in 2001 and one with pistachios in 2016. Atypical events examined included higher initial contamination levels at the pecan handler *(35)*; growth of *Salmonella* due to a delay in drying, leading to higher *Salmonella* levels prior to the simulated *Salmonella* reduction treatment in almonds and pistachios *(34, 36)*; and *Salmonella* recontamination events pre- and postreduction treatment for almonds, pecans, and pistachios *(34–36)*. In all these simulated atypical events for almonds, pecans, and pistachios and in the current walnut risk assessment, risk per serving estimates increased as a result of the atypical situations. Although process control through *Salmonella* reduction treatments is predicted to significantly reduce the risk in the baseline models, our results indicate that potential atypical situations that occur post- and in some cases pretreatment could lead to increased risk; such situations could explain the outbreak events that occurred in the United States involving almonds and pistachios *(34, 36)*.

The model and results of this assessment are limited to *Salmonella,* walnuts, and the United States. Data on the probability of *Salmonella* contamination and on *Salmonella* levels at harvest would allow development of models of exposure from harvest to silo storage. Data on whether *Salmonella* transfers through the shell to the kernel and during shelling and the transfer rates associated with each would aid in estimating risk from consumption. Characterizing the time-temperature profiles for relevant cooking processes at the consumer level would provide a better means of estimating the risk of salmonellosis from consumption of walnuts as an ingredient in products cooked at home. As data become available on the distribution of log reductions achieved from a targeted treatment and during drying, the effect of the variability in both the treatment and the drying step could be quantified using the results of this risk assessment. If consumption of walnuts were to increase, a proportional increase in the number of salmonellosis cases would occur, assuming all other factors remain the same.

The current risk assessment predicts that a minimum 3-log *Salmonella* reduction treatment would result in less than one case of salmonellosis linked to the consumption of walnuts per year under typical conditions. However, the relatively high prevalence of *Salmonella* on walnuts observed during the 2014 to 2015 retail survey suggests that contaminated walnuts that pose a health risk to U.S. consumers can reach the retail market, probably as a result of one or more atypical events. Scenarios examining the impact of atypical events on the risk of illness indicate that process control through preventive treatments can be insufficient, particularly when contamination takes place posttreatment.

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