Journal of Food Protection, Vol. 81, No. 6, 2018, Pages 1001–1014
doi:10.4315/0362-028X.JFP-17-379
Published 2018 by the International Association for Food Protection

Research Paper

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

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MS 17-379: Received 6 September 2017/Accepted 21 February 2018/Published Online 14 May 2018

ABSTRACT

We developed a quantitative risk assessment model to assess the risk of human nontyphoidal salmonellosis from consumption of pistachios in the United States and to evaluate the impact of *Salmonella* treatments (1- to 5-log reductions). The exposure model estimating prevalence and contamination levels of *Salmonella* at consumption included steps in pistachio processing such as transport from grower to huller, removal of the hull through wet abrasion, separation of pistachio floaters (immature, smaller nuts) and sinkers (mature, larger nuts) in a flotation tank, drying, storage, and partitioning. The risks of illness per serving and per year were evaluated by including a *Salmonella* dose-response model and U.S. consumption data. The spread of *Salmonella* through float tank water, delay in drying resulting in growth, increased *Salmonella* levels through pest infestation during storage (pre- and posttreatment), and a simulation of the 2016 U.S. salmonellosis outbreak linked to consumption of pistachios were the modeled atypical situations. The baseline model predicted one case of salmonellosis per 2 million servings (95% CI: one case per 5 million to 800,000 servings) for sinker pistachios and one case per 200,000 servings (95% CI: one case per 400,000 to 40,000 servings) for floater pistachios when no *Salmonella* treatment was applied and pistachios were consumed as a core product (>80% pistachio) uncooked at home. Assuming 90% of the pistachio supply is sinkers and 10% is floaters, the model estimated 419 salmonellosis cases per year (95% CI: 200 to 1,083 cases) when no *Salmonella* treatment was applied. A mean risk of illness of less than one case per year was estimated when a minimum 4-log reduction treatment was applied to the U.S. pistachio supply, similar to the results of the *Salmonella* risk assessment for almonds. This analysis revealed that the predicted risk of illness per serving is higher for all atypical situations modeled compared with the baseline, and delay in drying had the greatest impact on consumer risk.

Key words: Cross-contamination; Dry food; Pests; Pistachios; *Salmonella*; Tree nuts

The United States is the second-largest producer of pistachios in the world after Iran *(10),* with commercial production in California, Arizona, and New Mexico. The vast majority of the U.S. pistachio volume (99%) is grown in California's Central Valley *(1)*; 233,000 metric tons were produced in 2014 *(18)*. Major process steps include harvesting, transport from the grower to the huller, the removal of the hull with wet abrasion, separation of floaters (smaller, less mature nuts, which may have insect damage or adhering hull) and sinkers (mature nuts with large, fully developed nutmeat) in a flotation tank, drying, storage, and partitioning into units of smaller size (Fig. 1). Starting at the flotation tank and continuing throughout the production chain, the pistachio production process includes the two streams of product: floaters, which make up approximately 15% of the weight, and sinkers, which make up approximately 85% of the weight of a total annual production *(18)*.

Harris et al. *(18)* evaluated prevalence and levels of *Salmonella* on in-shell pistachios from samples collected from the storage silos up to 4 months after each of the 2010, 2011, and 2012 California harvests. Thirty-two of the 3,966 samples were positive for *Salmonella,* and a higher *Salmonella* prevalence (3-year average) was observed among floaters (2.0%; 95% confidence interval [CI], 1.3 to 3.1%) than among sinkers (0.37%; 95% CI, 0.21 to 0.67%). In the United States, pistachios or products containing pistachios were recalled because of the potential for *Salmonella* contamination in 2009, 2010, 2012, 2013, and 2016 *(24),* and foodborne outbreaks epidemiologically linked to consumption of pistachios or foods containing pistachios were reported in 2013 and 2015 to 2016 *(19)*.

We developed a quantitative risk assessment for *Salmonella* on pistachios. The first objective of the risk assessment was to estimate the potential impact on the risk of human salmonellosis from a *Salmonella* reduction treatment during processing of all U.S. pistachios. We considered treatments that resulted in a 1- to 5-log reduction in *Salmonella*. The second objective was to estimate the potential impact on the risk of human salmonellosis when an atypical situation occurs in the pistachio production system. Atypical situations that may occur both before and after a

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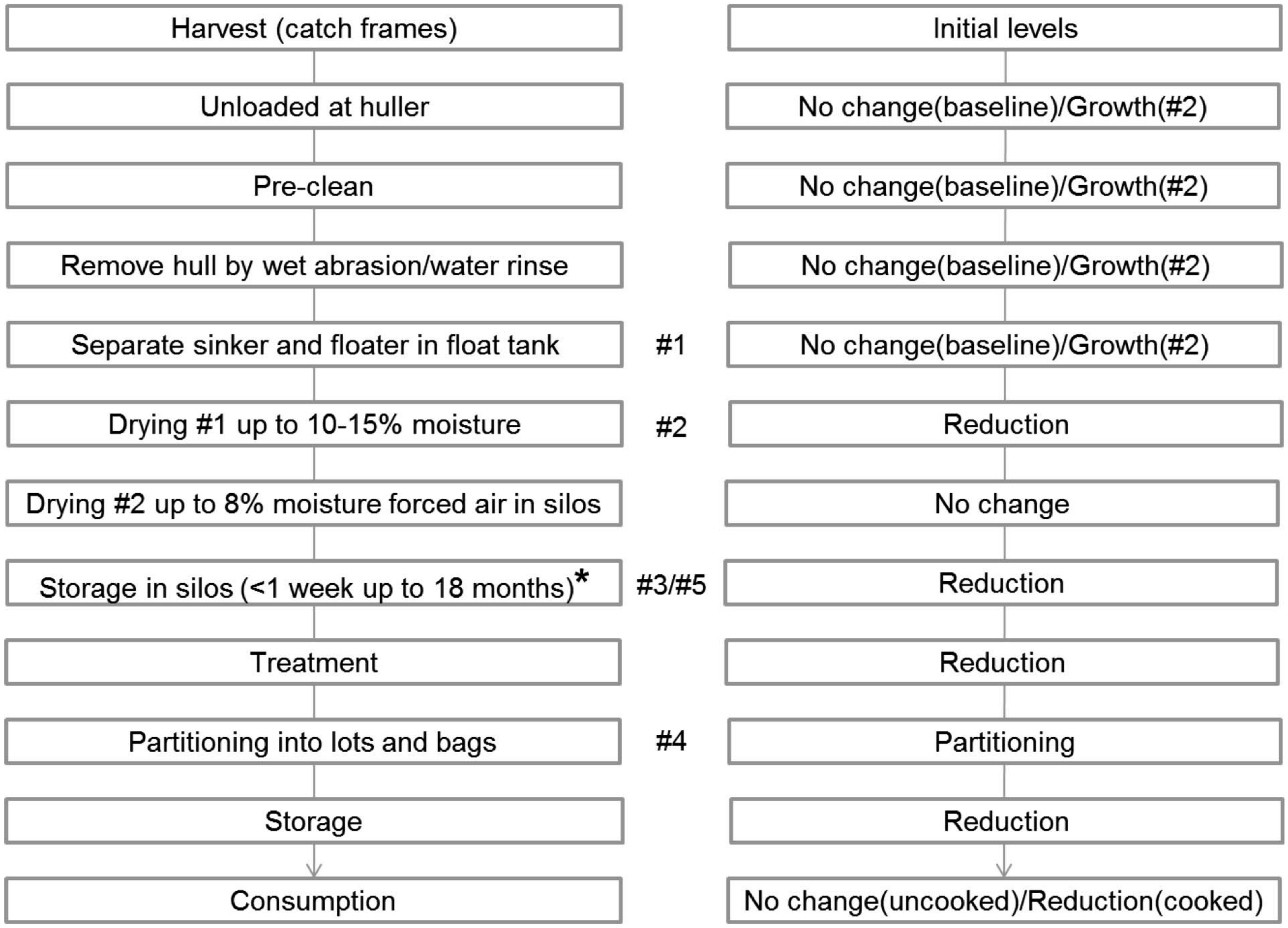

FIGURE 1. *Pistachio production steps (left) and expected changes in* Salmonella *levels (right) as a result of the corresponding production step. The step in the process in which an atypical situation was modelled is marked with a number: #1, cross-contamination in a float tank; #2, delay in drying; #3, contamination during pretreatment storage; #4, contamination during posttreatment storage; #5, 2016 salmonellosis outbreak. Modified from Frelka and Harris (13) according to Harris (16).*

Salmonella reduction treatment were examined, including one modeling the 2016 salmonellosis outbreak linked to pistachios in the United States. This risk assessment model improves upon previously published tree nut risk assessments *(20)*. The model includes a novel mathematical analysis for *Salmonella* probability of contamination (prevalence), *Salmonella* levels, and U.S. pistachio consumption patterns. As in previous U.S. Food and Drug Administration (FDA) tree nut risk assessment models *(30, 31)*, this model considers variability and uncertainty of parameters separately to accurately estimate risk and to provide a measure of the uncertainty of the estimated number of salmonellosis cases per year *(11, 14, 23)*. Probability of contamination and *Salmonella* levels for each step throughout the production process were also evaluated separately for better accuracy *(9, 27)*. The model includes the parameters of the survival model for *Salmonella* on tree nuts developed by our research group *(29)* quantifying variability and uncertainty.

MATERIALS AND METHODS

Overview of the exposure assessment model for *Salmonella* on pistachios. At harvest, pistachios are shaken into catch frames, transferred to trailers, and transported to a huller *(17)*; thus, pistachios are not in contact with the ground. This harvest process is different from that for other tree nuts (e.g., almonds and pecans), which fall to the ground during harvest. Following arrival at the huller, pistachio nuts are mechanically precleaned to remove field debris, and hulls are removed by mechanical wet abrasion (Fig. 1) *(18)*. Hulled pistachios are separated in a flotation tank into sinker and floater streams based on density *(18)* and then undergo two drying steps. The first drying step dries the nuts to 10 to 15% moisture content using heated forced air with various temperature and time schedules depending on the huller *(16)*. The second drying step occurs in the storage silos where forced air at ambient temperature (20 to 25°C) is used to achieve moisture levels of approximately 8% in the nutmeat *(16)*. Pistachios are then stored in the silos at ambient temperatures (20 to 25°C) from less than 1 week up to 18 months (Fig. 1).

The baseline exposure assessment model for this risk assessment includes the steps in the production process from the arrival of pistachios at the silo through storage in the silo (where the prevalence and contamination data were collected), treatment, partitioning, and posttreatment storage to the point of consumption. At every step in the exposure assessment model, the probability and levels of contamination of pistachios with *Salmonella* were estimated independently and separately for sinker and floater pistachios.

Probability of *Salmonella* contamination of pistachios and levels of contamination at the silo. In response to an FDA *(34)* request for data, information, and comments, data on *Salmonella* levels of contamination of pistachios at the silo were submitted by Dr. Linda Harris (Cooperative Extension Specialist, University of California, Davis) and the Administrative Committee for Pistachios and included results from the 2010, 2011, and 2012 surveys conducted by Harris et al. *(18)*. A comprehensive description of the sampling design used in these surveys was previously published *(18)*. Pistachios were collected from storage silos located at seven pistachio handlers. Twelve samples were collected from each silo except in six situations: in 2012, 24 samples were taken from each of three silos, 20 were taken from one silo, and 11 were taken from another silo; and in 2010, 11 samples were taken from a single silo. Subsamples (100 g) were analyzed for the presence of *Salmonella* by DFA of California (Safe Food Alliance, https://dfaofcalifornia.com/) using the AOAC official method 2001.09 (mini VIDAS assay system) *(2)*. Samples were collected within 4 months of harvest and were stored at 4°C for up to 6 months before analysis, with no change in *Salmonella* levels expected as a result of storage. Positive results were confirmed using culture methods and *Salmonella,* when present, was enumerated using a most-probable-number (MPN) determination following the FDA *Bacteriological Analytical Manual* method *(35)*.

Multiple models of *Salmonella* contamination for pistachio sinkers and floaters (separately) assuming either a Poisson or a lognormal distribution were fitted to the MPN patterns using a maximum-likelihood method. Models were compared using a maximum-likelihood ratio test when nested (results not shown). When models were not nested, the Akaike information criterion was used to determine the best applicable model (results not shown). A lognormal model with a common mean for all years and a standard deviation that differed by year was the best-fit model for both sinker and floater pistachios (Table 1). These models were translated in a Bayesian framework using JAGS through the rJags R library *(25)*. Uninformative priors were used for the mean (normal distribution with mean = 0 and standard deviation = 10 log CFU/g) and the standard deviation (independent uniform distribution of 0 to 10 log CFU/g). Convergence was confirmed using Gelman and Rubin's *(15)* convergence diagnostic, and a value of <1.1 was used as a sign of convergence.

The risk assessment model was integrated using a second-order Monte Carlo simulation. A unit was defined as a mass of pistachios (in shell or kernels, measured in grams) and was considered variable. The unit size at the silo in 64% of the cases followed a triangular distribution, with a minimum of 1,000 kg, a mode and maximum of 11,350 kg, and in 36% of the cases a uniform distribution of 11,350 to 22,700 kg *(16)*. For each iteration of the simulation, in the uncertainty dimension, one mean and standard deviation of the *Salmonella* level per unit was sampled from a coupled mean and standard deviation (μ_u and σ_u; to keep the correlation structure) of the Monte Carlo Markov chain. Estimates for the probability of contamination (probability of at least one *Salmonella* cell in the given food unit) and the 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. It was assumed that the *Salmonella* cells were Poisson distributed in a given unit (homogeneous distribution). The probability of contamination of a unit was defined as

$$\Pr(\text{cont}) = 1 - \exp(-\lambda_u \times s)$$

where $\log(\lambda_u) \sim N(\mu_u, \sigma_u)$ is the *Salmonella* level per gram in the unit and s (grams) is the size of the unit.

***Salmonella* survival during storage.** Santillana Farakos et al. *(29)* developed a mathematical model to predict survival of *Salmonella* on almonds, pecans, pistachios, and walnuts at ambient storage temperature (21 to 24°C) that considers variability and uncertainty separately. The Weibull model survival parameters specific to pistachios were used in this risk assessment. 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 to reduce the population by 1 log unit, ρ is a parameter that defines the shape of the curve, and t_1 and t_2 are two times since the beginning of the survival step (t_0). In the present assessment, t_0 was defined as the start of the storage step for pistachios at the silo. A binomial process restricted to positive values was used to evaluate the number of *Salmonella* cells 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 number of *Salmonella* cells in the contaminated unit at the end of survival (t_2) and N_1 is the number of *Salmonella* cells 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 *(27)* to

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

accounting for units in which there are no *Salmonella* cells ($N_2 = 0$) 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).

Storage of floater and sinker pistachios at the silo occurs at 20 to 25°C *(16),* and storage times (in weeks) differ. Based on data gathered by industry experts *(16),* we assumed the following distribution of storage times as representative of industry practice: 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 = 78 weeks).

***Salmonella* reduction treatment.** Scenarios modeled included those with either no treatment to reduce *Salmonella* or one of five possible *Salmonella* treatments (to achieve 1- to 5-log reductions). The *Salmonella* reduction treatments were defined per unit (in grams) of product treated. For the *Salmonella* reduction treatment step, it was assumed that each *Salmonella* cell had an identical and independent probability of inactivation. A binomial process restricted to positive values was used to evaluate the number of *Salmonella* cells at the end of this stage in positive units:

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

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

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

where L is the log reduction (1, 2, 3, 4, or 5 log CFU), $P_{\text{surv}T}$ is the probability of survival after each *Salmonella* reduction treatment,

TABLE 1. *Lognormal model outcome distributions representing* Salmonella *contamination on sinker and floater pistachios at the silo*

			Salmonella level (log CFU/g)					
				At uncertainty distributions of:				
Pistachio type	Parameter[a]	Year	Value	2.5%	25%	50%	75%	97.5%
Sinker	μ		−4.9	−5.9	−5.1	−4.9	−4.8	−4.3
	σ	2010	0.84	0.48	0.75	0.83	0.92	1.37
		2011	0.42	0.16	0.30	0.38	0.50	1.09
		2012	0.34	0.15	0.27	0.32	0.39	0.92
Floater	μ		−4.6	−5.8	−4.8	−4.5	−4.4	−3.9
	σ	2010	1.09	0.65	0.96	1.07	1.21	1.78
		2011	0.75	0.33	0.64	0.73	0.85	1.48
		2012	1.14	0.67	1.02	1.12	1.24	1.95

[a] Mean (μ; common for all years) and standard deviation (σ; varying by year) of the lognormal model.

P_{2T} is the probability of contamination posttreatment, P_{1T} is the probability of contamination pretreatment, N_{2T} is the number of *Salmonella* cells in the contaminated unit posttreatment, and N_{1T} is the number of *Salmonella* cells in the contaminated unit pretreatment. The minimum contamination in *Salmonella*-positive units is 1 CFU, and all *Salmonella* levels are a whole number.

Partitioning. Pistachio units typically are partitioned into smaller subunits twice. Following a *Salmonella* reduction treatment, the units are typically partitioned into smaller subunits of 45 to 45,000 kg *(16)*. The maximum size of the subunits after partitioning was set equivalent to the size of the unit before partitioning. These subunits are then further partitioned into consumer packages of 18 g (snack pack) to 454 g (bag) *(7)*. 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 number of *Salmonella* cells for each step is estimated as follows *(27)*:

$$N_3 \sim \text{Binomial}\left(N_2, \frac{S_2}{S_1}\right), \text{with } N_3 > 0$$

$$P_3 = P_2\left[1 - \left(1 - \frac{S_2}{S_1}\right)^{N_2}\right]$$

where N_3 is the number of *Salmonella* cells in the considered subunit of size S_2 (after partitioning), N_2 is the number of *Salmonella* cells in the considered unit of size S_1 (before partitioning), P_3 is the probability of contamination in the considered subunit of size S_2 (after partitioning), and P_2 is the probability of contamination in the considered subunit of size S_1 (before partitioning).

Cooking. Consumers often use pistachios as an ingredient in cooked products (e.g., cakes and cookies). These pistachios are purchased as an uncooked ingredient and are further cooked at home. No references were found for data on *Salmonella* survival specifically on pistachios during baking; however, Lathrop et al. *(21)* collected survival data for *Salmonella* in peanut butter during baking of cookies. In that study, commercial peanut butter was artificially inoculated with a five-serovar *Salmonella* cocktail (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). *Salmonella* populations were decreased by a minimum 4.8 log CFU per cookie (25 g) after 10 min at 177°C (detection limit of 0.04 CFU/g). Cookies baked for 15 min had no detectable levels of *Salmonella*. Similar to pistachios, peanut butter has low water activity (a_w). Although the composition of peanuts and peanut-related products is different from that of pistachios, the main parameters influencing survival of *Salmonella* during heating of foods are temperature and a_w, which were assumed to be similar (within 0.1 standard deviation). Although Lathrop et al. found a 4.8-log reduction in *Salmonella* per peanut butter cookie (25 g) after baking, those cells were not subjected to a microbial reduction treatment step before baking. In the absence of available data, for the purpose of this risk assessment it was assumed that the expected log decrease in *Salmonella* levels during baking of pistachios approximates that of the minimum level found in the baking of peanut butter cookies. A fixed value of 5 log CFU per unit (consumer packages after postprocessing and retail storage) was used for pistachios included as an ingredient in food products that undergo a cooking step in the home.

Modeling atypical situations in pistachio processing. Atypical situations in the food production system can potentially change the risk of salmonellosis from the consumption of pistachios. In many instances, contamination of low-a_w foods with pathogenic bacteria can be the result of cross-contamination *(8, 26)*. The main sources for cross-contamination in the processing facility are raw materials and the environment (including personnel, equipment, pests, dust, water, and air) *(4)*. The following types of root causes have been previously identified *(26)*: poor sanitation practices, poor facility and equipment design, lack of good manufacturing practices, and poor ingredient control and handling. In this risk assessment, five atypical situations that could lead to increases in risk per serving were evaluated. The atypical situations examined included both pretreatment and posttreatment events and were modeled for pistachios consumed as a core product not cooked in the home. These atypical situations are not modeled for the entire U.S. pistachio supply but rather as individual events. The number of salmonellosis cases per year linked to each atypical situation is 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 from the modeled atypical situations provide an estimate of the significance of such situations compared with the baseline model scenario and the impact it could have on risk (changes in the order of magnitude). The step in the process where these atypical situations are simulated to occur during production of pistachios are shown in Figure 1 (atypical situations #1, #2, #3, #4, and #5).

The first atypical situation, "cross-contamination in a float tank" (#1), assumes that a cluster of *Salmonella* cells (0.5 to 1.5 log CFU) contaminates a small number of pistachios before the flotation tank step (e.g., as a result of isolated contamination in the orchard environment). The aim of modeling this atypical situation was to evaluate the effect of spreading *Salmonella* throughout a unit of contaminated pistachios through float tank water without an effective sanitizing agent. For this atypical situation, it was assumed that the increased *Salmonella* levels do not follow a Poisson distribution (like the baseline model), and the flotation tank spreads this contamination throughout the unit of pistachios. To determine the prevalence and levels of *Salmonella* on pistachios at the flotation tank, back calculation from the values determined for the silo storage (baseline model) was done, taking into account the impact of drying (Fig. 1) based on information on heated forced air times and temperatures, which differ between operations. The following two variations were modeled: (i) ~6 h of drying in two stages (at ~82°C and then at ~77°C) and (ii) ~8 h of drying in four stages (~2 h each stage at 82, 71, 60, and 49°C) *(16)*. In the absence of data on *Salmonella* survival during drying of wet pistachios, log-linear declines of *Salmonella* during drying of wet pecan nutmeats at 80°C collected by Beuchat and Mann *(5)* were used to account for the effect of drying on *Salmonella* survival on sinker and floater pistachios. It was assumed that *Salmonella* would decrease during the first 2 h of drying (in both cases at temperatures close to 80°C). After these first 2 h, the nutmeat is sufficiently dry and no further moisture reduction occurs *(16)*. A bootstrap procedure was used to estimate the uncertainty around the log-linear estimate for *Salmonella* inactivation during drying. In the model, the float tank water served as a vehicle for the homogeneous distribution of *Salmonella* through a unit of pistachios. A drying step (using the same log-linear decline used for the back calculation) and subsequent steps described in the baseline exposure assessment model were modeled, including pretreatment storage (at the silos, applying the survival model), treatment (reductions of up to 5 log CFU), partitioning, posttreatment and retail storage (survival model), and consumption.

The second modeled atypical situation, "delay in drying" (#2), assumes a situation in which the drying step is delayed for a minimum of 6 h and a maximum of 48 h to simulate possible industry practices at the beginning or end of harvest when the flow of pistachios into the dryers would be slower as a result of fewer pistachios being harvested. It was assumed that both floater and sinker streams of pistachios were held under the higher moisture conditions ($a_w \geq 0.90$) during this period of time. Under these conditions, *Salmonella* growth is possible; the growth rate was estimated based on growth data published by Moussavi et al. *(22)* for *Salmonella* serovars Montevideo, Enteritidis, Senftenberg, Worthington, and Liverpool on hulled floater and sinker pistachios at 37°C and 90% relative humidity. Growth data were fitted to the Baranyi and Roberts growth model *(3)* using DMFit 2.0. R^2 values were used to evaluate the fit of the model to the data, and maximum specific growth rates were estimated. Estimated growth rates for hulled floater and sinker pistachios were 6.6 and 5.8 log CFU per day, respectively, and the maximum population density was 7 log CFU/g. A stochastic growth modeled as a Yule process was used *(36)*. To determine the prevalence and levels of *Salmonella* on pistachios at the flotation tank, following the steps of atypical situation #1, we used the values determined in the silo storage (baseline model) and back calculated, taking into account the impact of drying in the absence of a delay (Fig. 1). We back calculated *Salmonella* levels to that in the flotation tank, applied the growth model, and reapplied the drying step (using the same log-linear decline as that used for the back calculation). After the drying step, the model included the subsequent steps described in the baseline exposure assessment model, including pretreatment storage (at the silos, applying the survival model), treatment (reductions of up to 5 log CFU), partitioning, posttreatment and retail storage (survival model), and consumption.

In the third atypical situation, "contamination during pretreatment storage" (#3), increased levels of *Salmonella* in pistachios were modeled during pretreatment storage (0.5 to 1.5 log CFU) assuming a pest infestation in the storage silos. The rest of the process follows with the same steps as the baseline exposure assessment model: pretreatment storage, treatment, partitioning, and posttreatment and retail storage.

In the fourth atypical situation, "posttreatment contamination" (#4), increased levels of *Salmonella* in the contaminated units posttreatment (0.5 to 1.5 log CFU) were modeled assuming the same initial prevalence as the baseline exposure assessment model. The rest of the process follows with the same steps as the baseline exposure assessment model, including pretreatment storage, treatment, partitioning, and posttreatment and retail storage.

In the fifth atypical situation, "2016 outbreak" (#5), the model estimates risk using the prevalence and contamination levels (<0.36, <0.36, 0.92, 2.3, and 2.3 MPN/g for the five positive subsamples) in pistachios in preprocessing storage at the firm that was epidemiologically linked to the 2016 salmonellosis outbreak in the United States *(6)*. The five contamination levels included in the model were selected based on results of 5 of 30 subsamples from one of the three *Salmonella*-positive samples collected during the outbreak investigation *(6)*. A 2.3 MPN/g contamination level on pistachios in silo storage, as the highest level found in one of the subsamples (assuming ~10 million g per silo), is approximately 1 million times higher and 100,000 times higher than the levels in the baseline model (assuming no *Salmonella* reduction treatment has taken place) for sinker and floater pistachios, respectively. The rest of the process follows with the same steps as the baseline exposure assessment model, including pretreatment storage, treatment, partitioning, and posttreatment and retail storage.

Consumption. Consumption of pistachios by 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, for the 2003 to 2004, 2005 to 2006, 2007 to 2008, and 2009 to 2010 cycles *(10)*. Proportions of pistachio ingredients in WWEIA foods used in these analyses were based on "recipes" developed for U.S. Environmental Protection Agency Food Commodity Intake Database *(33)*. Empirical distributions representing serving sizes among consumers (eaters) and weighted by the WWEIA dietary statistical sampling weights were used for pistachios consumed as a core product (≥80% pistachio) uncooked in the home and as an ingredient cooked in the home. Assuming that data reported in the WWEIA 24-h dietary recalls (two per survey respondent, conducted 3 to 10 days apart) are representative of consumption over the whole year and considering that there are approximately 320 million individuals in the United States *(32)*, the number of servings per year was estimated. The estimated number of salmonellosis cases per serving and per year corresponds to an "average year" because the variability introduced in the study probability of contamination is integrated in the procedure. We distinguished between two types of pistachio-consumed products (where the "cooking" step, if present, is assumed to happen in the home): (i) core pistachio product (≥80% of the product ingredients are pistachios) consumed uncooked in the home (e.g., pistachios consumed as a snack) and (ii) pistachio as an ingredient (<80% of the product ingredients are pistachios) consumed cooked in the home (e.g., in baked, fried, or boiled products). The cooking step for cooked pistachios is done by the consumer in the home. The number of pistachio servings per year in the United States was estimated given data reported in the WWEIA over a 48-h period, which revealed that 0.63% of the population (~2 million individuals) reported consumption of pistachios as a core product uncooked in the home, and 0.11% (~350,000 individuals) reported consuming pistachios as an ingredient cooked in the home. Pistachio kernels are the only edible component of pistachios and account for up to 50% of the total weight *(17)*. However, most pistachios are sold as an in-shell product *(18)*. This percentage of edible portion was used as a multiplier when setting the unit and package sizes to accurately estimate the final weight of product consumed. The mean (±standard deviation) intake per serving (based on WWEIA data) is 35.6 (±30.4) g for pistachios consumed as a core product not cooked in the home and 3.1 (±3.2) g for pistachios consumed as an ingredient cooked in the home.

Hazard characterization. The dose-response model used in this risk assessment is equivalent to the beta-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) determined by the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO-WHO) *(12)* and adapted to the number of *Salmonella* cells, which in our model is an exact value (beta-binomial dose-response model). We assume all individuals are equally susceptible *to Salmonella* infection. The risk estimates obtained when using the 2.5th and 97.5th percentile of the FAO-WHO *Salmonella* dose-response curve resulted in mean estimated risks that were in the same order of magnitude as that obtained when using the FAO-WHO expected values. As such, uncertainty was not considered in the dose-response.

Risk characterization. The risk of salmonellosis per serving and per year was assessed using a second-order Monte Carlo simulation *(14)*. 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). Separate risks per serving estimate were calculated for sinker and floater pistachios assuming that all of the pistachios consumed were core product, either uncooked or cooked in the home, and were either all sinkers or all floaters. An estimate of the risk per year for pistachios consumed as a core product uncooked and cooked in the home also was calculated for sinker and floater pistachios combined (with 0.9 as a multiplier for 85% of the weight being sinkers and 0.1 as a multiplier for 15% of the weight being floaters) *(18)*. When reporting the risk estimates per year, the 95% CI reflects uncertainty in the number of salmonellosis cases. Variability in risk per year estimates is integrated in the procedure.

The risk assessment model was developed in R (3.3.1), and Monte Carlo simulations were run using the mc2d package *(28)*. The R code is available upon request (FDAFoodSafetyRiskModel@fda.hhs.gov).

RESULTS AND DISCUSSION

Baseline probabilities of *Salmonella* contamination and levels. The probability of *Salmonella* contamination and the levels of contamination on pistachios (sinkers and floaters) was estimated separately at each step of the pistachio production process from the silo to the point of consumption (Table 2; for 0-, 4-, and 5-log reduction treatments). The mean probability of *Salmonella* contamination and the levels in positive units at the end of each stage of the exposure assessment model for no treatment and for 1- to 5-log reduction treatments decrease throughout exposure for both sinker and floater pistachios. Sinker and floater pistachios have a very similar probability of contamination at silo storage, but the mean levels of *Salmonella* per contaminated unit are ~10-fold higher for floaters than for sinkers throughout the steps in the exposure assessment model. The levels of *Salmonella* per contaminated unit (Table 2) and per total units (contaminated plus noncontaminated) (Fig. 2; for 0-, 4-, and 5-log reduction treatments) decrease 10-fold for every additional log reduction treatment applied for both sinkers and floaters. Treatment rather than storage time has the greatest impact on the decrease in the probability of contamination and in the *Salmonella* levels in contaminated units (Fig. 2). The lower probability of contamination after partitioning results from the distribution of low *Salmonella* levels into a larger number of units of smaller unit size (i.e., an increase in the number of units that contain zero *Salmonella* cells). Partitioning results in an apparent decrease in the probability of contamination and in *Salmonella* levels. However, this apparent lower probability is a result of the redistribution of low *Salmonella* levels into a high number of units of smaller size. For example, if a 10,000-kg unit contains 100 CFU, the prevalence is 100% and the *Salmonella* level is 100 CFU per contaminated unit. After partitioning into 454-g bags, the per bag mean prevalence and level of *Salmonella* will be lower because some bags will contain no *Salmonella* and the 100 CFU will be distributed among multiple bags.

Risk estimates per serving. The distribution of the estimated salmonellosis risk per serving of sinker and floater pistachios represents the probability of acquiring human salmonellosis due to consumption of sinker or floater servings in the U.S. population (Table 3). The predicted risk is calculated by integrating the FAO-WHO *(12)* *Salmonella* dose-response function with the results of the exposure assessment module (levels of *Salmonella* per contaminated serving and the prevalence of contaminated servings in Table 2). We calculated the risk per serving for pistachio sinkers and floaters separately, and these values are calculated assuming the total pistachio mass in a serving is 100% sinkers or 100% floaters. To estimate the risk per serving for pistachios including the production weight percentages of floaters and sinkers, the production percentages of each pistachio stream can be included as a multiplier, and both risk estimates are summed to obtain a total.

The risk characterization results contain six sets of statistics, one for each treatment level (no treatment and 1-,

TABLE 2. *Mean probability of* Salmonella *contamination and mean* Salmonella *levels for sinker and floater pistachios at each stage of the exposure assessment model for 0-, 4-, and 5-log reduction treatments*

			Salmonella assessments (mean ± SD)				
Parameter	Pistachio type	*Salmonella* reduction treatment (log CFU)	Initial silo[a]	After storage[b]	After reduction treatment	After partitioning to bags[c]	Posttreatment storage[d]
Mean wt/unit (g)			10,161,226	10,161,226	10,161,226	224	224
Mean probability of *Salmonella* contamination	Sinkers	0	0.99 ± 0.05	0.83 ± 0.26	0.83 ± 0.26	6.6E−04 ±4.1E−04	4.1E−04 ±3.2E−03
		4			0.0030 ± 0.02	7.0E−08 ±4.3E−08	4.3E−08 ±3.5E−07
		5			0.00032 ± 0.002	6.9E−09 ±4.2E−09	4.2E−09 ±3.4E−08
	Floaters	0	0.98 ± 0.09	0.86 ± 0.28	0.86 ± 0.28	5.6E−03 ±3.8E−03	3.8E−03 ±3.0E−02
		4			0.02 ± 0.08	8.8E−07 ±5.3E−07	5.3E−07 ±9.2E−06
		5			0.003 ± 0.03	8.9E−08 ±5.4E−08	5.4E−08 ±9.0E−07
Mean *Salmonella* level (CFU in positive units[e])	Sinkers	0	219 ± 1,242	33 ± 251	33 ± 251	1 ± 0.02	1 ± 0.01
		4			1 ± 0.04	1	1
		5			1 ± 0.01	1	1
	Floaters	0	2,980 ± 37,798	438 ± 6,459	438 ± 6,459	1 ± 0.1	1 ± 0.08
		4			1 ± 0.64	1	1
		5			1 ± 0.07	1	1

[a] Starting *Salmonella* levels at the silo.

[b] *Salmonella* levels after storage in the silo at 20 to 25°C for various times, where 90% of crop storage follows a uniform distribution (minimum = 2, maximum = 49 weeks), 5% follows a triangular distribution (minimum = 49, mode = 49, maximum = 78 weeks), and 5% follows a triangular distribution (minimum = 0, mode = 2, maximum = 2 weeks).

[c] Individual package sizes of 18, 224, and 454 g.

[d] Storage for 3 weeks plus retail period follows a triangular distribution (minimum = 1 day, mode = 2 weeks, maximum = 6 weeks) where 80% of storage occurs at 23°C and 20% occurs at 4°C.

[e] Minimum *Salmonella* level for the unit to be considered positive was 1 CFU.

2-, 3-, 4-, and 5-log reduction treatments) (Table 3). Variability (columns in Table 3) represents the heterogeneity of the data (not reducible by data collection), and uncertainty (rows in Table 3) represents lack of knowledge (which can be reduced by collection of additional data). The considered uncertainty includes uncertainty in the probability of contamination, the *Salmonella* contamination levels, and the survival model parameters. The impact of variability is much higher than the impact of the considered uncertainty. Variability in estimated risk (from the 2.5th to the 97.5th quantile of variability) spans over 4 to 6 log units, whereas the uncertainty (from the 2.5th to the 97.5th quantile of uncertainty) for a given statistic spans over roughly 1 log unit (Table 3).

Sinker pistachios are associated with a 10-fold lower risk of salmonellosis per serving compared with floater

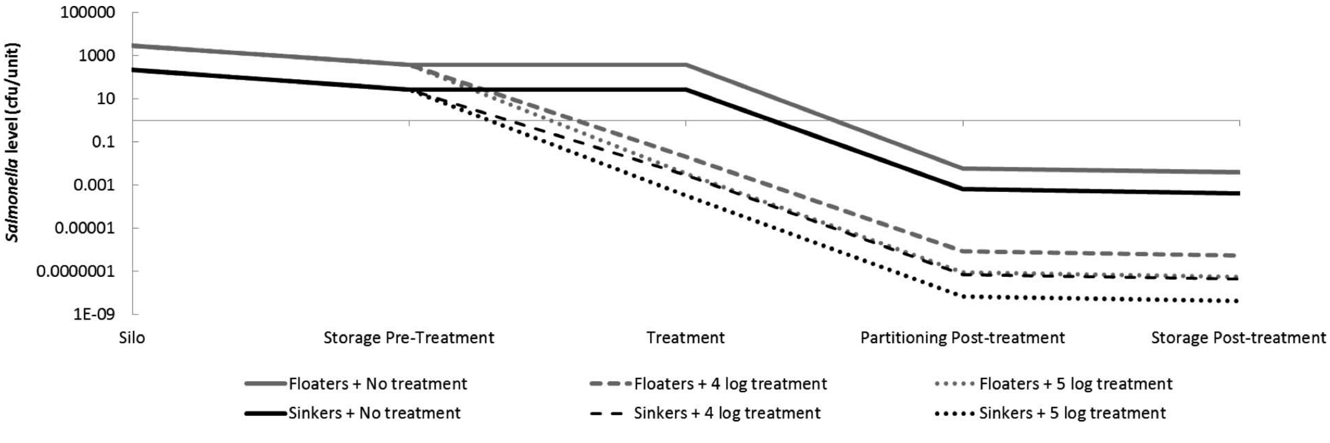

FIGURE 2. Salmonella *levels (CFU per unit) in each exposure assessment stage including a 0-, 4-, and 5-log* Salmonella *reduction treatment and two pistachio streams (sinkers and floaters).*

TABLE 3. *Salmonellosis risk per serving from consumption of pistachios in the United States*

	Pistachios, core uncooked[b]					Pistachios, ingredient cooked[c]				
			Quantile of variability of:					Quantile of variability of:		
Salmonella reduction treatment[a]	Mean	SD	2.5%	50%	97.5%	Mean	SD	2.5%	50%	97.5%
Pistachio sinkers										
0 log CFU										
Estimate	4.88E−07	4.58E−06	3.23E−10	3.43E−08	2.83E−06	3.05E−13	2.71E−12	1.89E−16	2.14E−14	1.75E−12
95% CI	2.21E−07	1.49E−06	5.71E−11	1.01E−08	1.26E−06	1.35E−13	8.94E−13	3.29E−17	6.37E−15	7.78E−13
	1.22E−06	4.11E−05	1.13E−09	1.08E−07	6.10E−06	7.25E−13	2.14E−11	6.78E−16	6.80E−14	3.73E−12
1 log CFU										
Estimate	4.93E−08	4.70E−07	3.26E−11	3.42E−09	2.90E−07	3.03E−14	2.79E−13	1.91E−17	2.13E−15	1.81E−13
95% CI	2.22E−08	1.52E−07	5.74E−12	9.89E−10	1.21E−07	1.35E−14	9.44E−14	3.30E−18	6.30E−16	7.59E−14
	1.15E−07	3.24E−06	1.19E−10	1.01E−08	6.31E−07	7.05E−14	1.76E−12	7.00E−17	6.36E−15	3.88E−13
2 log CFU										
Estimate	4.96E−09	4.92E−08	3.27E−12	3.56E−10	2.65E−08	3.05E−15	2.93E−14	1.92E−18	2.21E−16	1.64E−14
95% CI	2.23E−09	1.54E−08	5.74E−13	1.02E−10	1.17E−08	1.35E−15	9.19E−15	3.30E−19	6.46E−17	7.03E−15
	1.20E−08	3.70E−07	1.23E−11	1.10E−09	5.88E−08	7.08E−15	2.09E−13	7.19E−18	6.85E−16	3.63E−14
3 log CFU										
Estimate	4.88E−10	5.20E−09	3.27E−13	3.62E−11	2.73E−09	3.09E−16	2.90E−15	1.92E−19	2.26E−17	1.71E−15
95% CI	2.21E−10	1.60E−09	5.74E−14	1.03E−11	1.24E−09	1.31E−16	9.63E−16	3.30E−20	6.52E−18	7.37E−16
	1.17E−09	4.00E−08	1.23E−12	1.13E−10	5.87E−09	7.09E−16	2.39E−14	7.21E−19	7.08E−17	3.56E−15
4 log CFU										
Estimate	4.94E−11	4.62E−10	3.27E−14	3.63E−12	2.78E−10	3.07E−17	2.70E−16	1.92E−20	2.26E−18	1.73E−16
95% CI	2.25E−11	1.50E−10	5.74E−15	1.03E−12	1.26E−10	1.37E−17	9.04E−17	3.30E−21	6.53E−19	7.48E−17
	1.17E−10	3.67E−09	1.23E−13	1.13E−11	6.04E−10	7.58E−17	2.32E−15	7.21E−20	7.10E−18	3.68E−16
5 log CFU										
Estimate	4.86E−12	4.69E−11	3.27E−15	3.63E−13	2.79E−11	3.04E−18	2.69E−17	1.92E−21	2.26E−19	1.73E−17
95% CI	2.23E−12	1.52E−11	5.74E−16	1.03E−13	1.26E−11	1.36E−18	8.96E−18	3.30E−22	6.53E−20	7.50E−18
	1.15E−11	2.79E−10	1.23E−14	1.13E−12	6.05E−11	6.92E−18	1.97E−16	7.21E−21	7.11E−19	3.67E−17
Pistachio floaters										
0 log CFU										
Estimate	6.24E−06	1.18E−04	2.26E−10	9.07E−08	2.66E−05	4.03E−12	7.30E−11	1.34E−16	5.73E−14	1.69E−11
95% CI	2.60E−06	2.75E−05	9.22E−12	1.26E−08	1.17E−05	1.55E−12	1.57E−11	5.84E−18	7.75E−15	7.37E−12
	2.28E−05	1.07E−03	1.19E−09	2.90E−07	5.08E−05	2.43E−11	1.77E−09	7.36E−16	1.81E−13	3.20E−11
1 log CFU										
Estimate	6.29E−07	1.02E−05	2.31E−11	8.54E−09	2.71E−06	3.99E−13	6.49E−12	1.36E−17	5.30E−15	1.71E−12
95% CI	2.61E−07	2.71E−06	9.22E−13	1.23E−09	1.19E−06	1.60E−13	1.54E−12	5.84E−19	7.70E−16	7.52E−13
	3.09E−06	1.70E−04	1.23E−10	2.58E−08	5.25E−06	2.56E−12	2.04E−10	7.52E−17	1.62E−14	3.23E−12
2 log CFU										
Estimate	6.37E−08	1.00E−06	2.32E−12	9.01E−10	2.75E−07	4.05E−14	6.96E−13	1.36E−18	5.61E−16	1.71E−13
95% CI	2.63E−08	2.74E−07	9.22E−14	1.28E−10	1.19E−07	1.61E−14	1.54E−13	5.84E−20	8.01E−17	7.36E−14
	3.88E−07	2.83E−05	1.27E−11	2.77E−09	5.15E−07	2.35E−13	1.49E−11	7.80E−18	1.73E−15	3.24E−13
3 log CFU										
Estimate	6.35E−09	1.03E−07	2.32E−13	9.43E−11	2.48E−08	4.05E−15	6.74E−14	1.36E−19	5.90E−17	1.57E−14
95% CI	2.57E−09	2.85E−08	9.22E−15	1.30E−11	1.08E−08	1.62E−15	1.62E−14	5.84E−21	8.10E−18	6.82E−15
	3.00E−08	1.91E−06	1.27E−12	2.92E−10	4.67E−08	2.38E−14	1.75E−12	7.84E−19	1.83E−16	3.02E−14
4 log CFU										
Estimate	6.23E−10	1.10E−08	2.32E−14	9.49E−12	2.60E−09	3.96E−16	7.06E−15	1.36E−20	5.95E−18	1.64E−15
95% CI	2.60E−10	2.68E−09	9.22E−16	1.30E−12	1.15E−09	1.60E−16	1.63E−15	5.84E−22	8.10E−19	7.20E−16
	3.26E−09	2.26E−07	1.27E−13	2.98E−11	4.93E−09	2.47E−15	1.71E−13	7.85E−20	1.86E−17	3.07E−15

TABLE 3. *Continued*

	Pistachios, core uncooked[b]					Pistachios, ingredient cooked[c]				
			Quantile of variability of:					Quantile of variability of:		
Salmonella reduction treatment[a]	Mean	SD	2.5%	50%	97.5%	Mean	SD	2.5%	50%	97.5%
5 log CFU										
Estimate	6.23E−11	1.03E−09	2.32E−15	9.49E−13	2.67E−10	3.94E−17	6.89E−16	1.36E−21	5.95E−19	1.69E−16
95% CI	2.58E−11	2.63E−10	9.22E−17	1.30E−13	1.16E−10	1.55E−17	1.53E−16	5.84E−23	8.10E−20	7.35E−17
	2.94E−10	1.76E−08	1.27E−14	2.98E−12	5.10E−10	2.23E−16	1.65E−14	7.85E−21	1.86E−18	3.21E−16

[a] Pistachios from the sinker stream (larger, fully developed nuts that make up approximately 85% of the weight of a total annual production) and the floater stream (smaller and less mature nuts that make up approximately 15% of the weight). Estimate represents the median value; 95% CI (confidence interval) represents the range (top = lower value; bottom = upper value) in which the true value can be found with a 95% probability.

[b] Pistachios consumed as a core product, uncooked in the home.

[c] Pistachios consumed as an ingredient in a product cooked in the home.

pistachios for equal *Salmonella* reduction treatments applied. Pistachios consumed as an ingredient cooked in the home are associated with a mean ~2,000,000-fold lower level of risk per serving compared with pistachios consumed as a core product uncooked in the home (Table 3 and Fig. 3). Differences in estimated risk for the different types of pistachio products consumed (as a core product uncooked in the home versus as an ingredient cooked in the home) can be mainly attributed to the additional *Salmonella* reduction step (cooking) when consuming cooked pistachios and, to a lesser degree, to differences in the pistachio serving size when consuming pistachios as an ingredient. These mean risk estimates per contaminated serving among the contaminated servings eaten by individuals in the U.S. population corresponds to one case of salmonellosis per 2 million servings (95% CI, one case per 5 million to 800,000 servings) of sinker pistachios consumed as a core product uncooked in the home with no *Salmonella* reduction treatment applied. The 4- and 5-log reduction treatments reduce the risk per serving to one case of salmonellosis per 20,000 million servings (95% CI, one case per 44,000 million to 9,000 million servings) and one case per 200,000 million servings (95% CI, one case per 400,000 million to 90,000 million servings), respectively, for uncooked sinker pistachios. Floater pistachios had higher mean estimates of risk, with an estimated one case of salmonellosis per 200,000 servings with no *Salmonella* reduction treatment and consumed as a core product uncooked in the home (95% CI, one case per 400,000 to 40,000 servings). The 4- and 5-log reduction treatments for floaters reduced the risk per serving to one case of salmonellosis per 2,000 million servings (95% CI, one case per 4,000 million to 300 million servings) and one case per 20,000 million servings (95% CI, one case per 40,000 million to 3,000 million servings), respectively. Consumption of cooked pistachios as an ingredient reduces the salmonellosis risk per serving by ~1,000,000-fold given that the rest of the process remains the same.

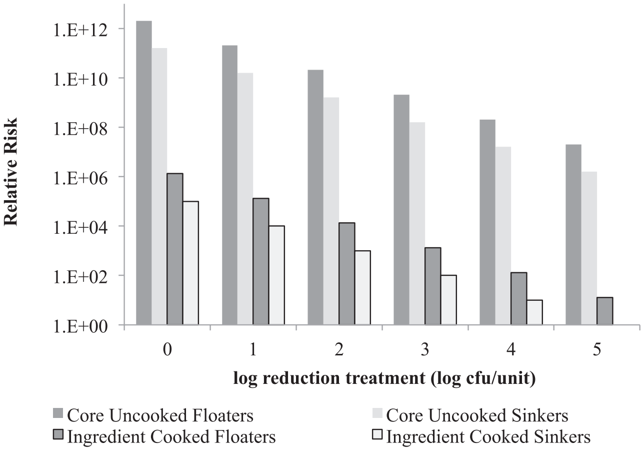

FIGURE 3. *Relative risk of human salmonellosis cases per serving due to consumption of pistachio sinkers and floaters given a 1-, 2-, 3-, 4-, and 5-log* Salmonella *reduction treatment and relative to the risk per serving from consumption of pistachios as an ingredient cooked in the home (i.e., having received an additional 5-log* Salmonella *reduction treatment). Core, pistachio product in which ≥80% is pistachios (including whole pistachios); ingredient, product in which <80% is pistachios; uncooked, pistachios not further cooked in the home; cooked, pistachios receive a cooking step in the home (e.g., baking in a cookie).*

Risk estimates per year. The estimated numbers of servings of pistachios consumed as a core product uncooked in the home and as an ingredient cooked in the home were 3.7×10^8 and 6.4×10^7, respectively. These serving numbers combined with the risk per serving considering that pistachios at retail are made up of approximately 85% sinkers (0.9 multiplier) and 15% floaters (0.1 multiplier) by volume provide the predicted number of U.S. salmonellosis cases per year (Table 4). If the pistachio supply does not receive a *Salmonella* reduction treatment, the model estimates a mean of 419 cases per year for pistachios consumed as a core product not cooked in the home (95% CI, 200 to 1,083 cases). The estimated mean number of cases decreases roughly 10-fold as the *Salmonella* reduction treatment is increased by 1 log unit for all pistachio products (Table 4). Cooking pistachios in the home significantly decreases the risk estimate, with the number of cases per year estimated as less than one for all treatments. For

TABLE 4. *Salmonellosis cases per year from consumption of pistachios in the United States*

Salmonella reduction treatment[a]	No. of salmonellosis cases/yr Pistachios, core uncooked[b]	 Pistachios, ingredient cooked[c]
0 log CFU		
Estimate	419	<1
2.50%	200	<1
97.50%	1,083	<1
1 log CFU		
Estimate	43	<1
2.50%	21	<1
97.50%	141	<1
2 log CFU		
Estimate	4	<1
2.50%	2	<1
97.50%	17	<1
3 log CFU		
Estimate	<1	<1
2.50%	<1	<1
97.50%	1	<1
4 log CFU		
Estimate	<1	<1
2.50%	<1	<1
97.50%	<1	<1
5 log CFU		
Estimate	<1	<1
2.50%	<1	<1
97.50%	<1	<1

[a] Estimate represents the median value; 2.50 and 97.50% represent the range (lower and upper values, respectively) in which the true value can be found with a 95% probability.

[b] Pistachios consumed as a core product, uncooked in the home (0.6% of individuals; 3.7×10^8 servings).

[c] Pistachios consumed as an ingredient in a product cooked in the home (0.1% of individuals; 6.4×10^7 servings).

pistachios consumed uncooked in the home but treated to produce a minimum 4-log reduction in *Salmonella,* the lower uncertainty bound of the mean estimated cases per year is less than one. A 3-log reduction treatment for pistachios and pistachio products uncooked in the home results in a mean risk per serving estimate of less than one case per year (95% CI, <1 to 1 case).

Estimated risk from the modeled atypical situations. The estimated salmonellosis risk per serving from the modelled atypical situations (#1 through #4 in Fig. 1) in pistachio production and the risk estimates when modeling the atypical *Salmonella* levels found in the silos from the recalled units of the 2016 U.S. salmonellosis outbreak (#5 in Fig. 1) linked to pistachios result in risk estimates per serving that are all higher than the risk per serving for pistachio sinkers as a core product uncooked in the home with no *Salmonella* reduction treatment. Figures 4 and 5 present the risk per serving of each atypical situation relative to the risk per serving for the sinker stream baseline model given a 5-log *Salmonella* reduction treatment.

The atypical situation that resulted in the highest risk is a delay in drying (#2) because *Salmonella* growth was modeled to occur (for a minimum of 6 h and a maximum of 48 h) prior to the simulated *Salmonella* reduction treatment. This growth step resulted in significantly higher levels of *Salmonella* per contaminated unit compared with the levels in the baseline model and resulted in risk estimates per serving that were a minimum of 2×10^4-fold to 2×10^5-fold (with no *Salmonella* reduction treatment) to a maximum of 2×10^5-fold to 5×10^8-fold (with a 4- to 5-log reduction treatment) higher than the risk estimates obtained when using the baseline model (Fig. 4).

The atypical situation of cross-contamination in a float tank (#1), in which the flotation tank spreads *Salmonella* of 0.5 to 1.5 log CFU homogeneously throughout the unit of pistachios from a cluster of pistachios containing *Salmonella* cells, resulted in risk estimates per serving that were similar (no difference for floaters and only 1.1-fold higher for sinkers) to those found when using the baseline process model. A mean of 10 to 15 *Salmonella* cells were added per contaminated unit as a result of this situation, which explains the slight increase observed in risk per serving. However, should this situation occur, this dispersal of *Salmonella* cells from a previously isolated to a cluster of pistachios to a larger volume is expected to result in a greater number of illnesses because more servings will become contaminated.

Similar to atypical situation #1, in atypical situation #3, contamination during pretreatment storage, the same increase of 0.5 to 1.5 log CFU resulted in risk estimates per serving that are the same (floaters) and ~1.5-fold higher (sinkers) than those with the baseline model. Similar to the baseline model, each 1-log increase in the reduction treatment reduced the risk by 10-fold. Thus, the risk estimate is affected by the *Salmonella* reduction treatment for all three atypical situations (#1, #2, and #3) that occur pretreatment.

In atypical situation #4, contamination during posttreatment storage, the same increase in levels of *Salmonella* (0.5 to 1.5 log CFU) as modeled for atypical situations #1 and #3 was applied but after a *Salmonella* reduction treatment. This additional contamination is thus not affected by the *Salmonella* reduction treatment, and risk estimates per serving are affected by treatment to the degree to which treatment affects the decrease in *Salmonella* levels that takes place before the contamination. This atypical situation resulted in risk estimates that were very dependent on the *Salmonella* reduction treatment modeled. Equal estimates of salmonellosis risk per serving (floaters with no *Salmonella* reduction treatment or a treatment of 1 log CFU) and up to ~60,000-fold higher estimates (sinkers with a 5-log *Salmonella* reduction treatment) were obtained compared with the baseline exposure model (Fig. 4).

In atypical situation #5, the same prevalence and levels of *Salmonella* contamination were simulated as those found on pistachios in the storage silos at the production facility epidemiologically linked to the 2016 U.S. salmonellosis outbreak. The most contaminated sample contained *Salmo-*

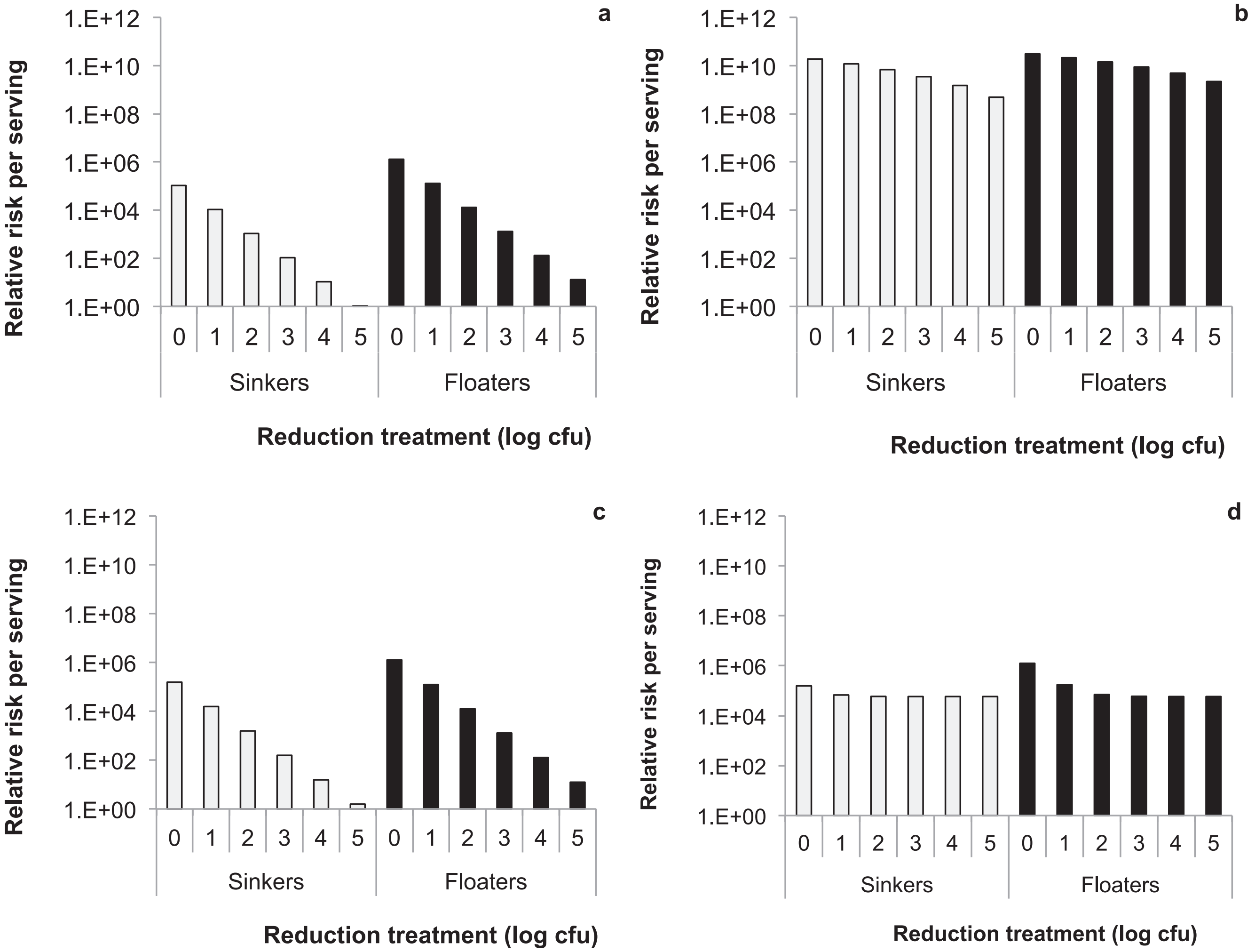

FIGURE 4. *Risk per serving of pistachios consumed as a core product uncooked in the home assuming various* Salmonella *reduction treatments (0- to 5-log reductions) relative to the risk per serving for the sinker stream baseline model given a 5-log reduction treatment. (a) Atypical situation assuming a cross-contamination event with* Salmonella *before the flotation tank step and spreading of the contamination throughout the product in the flotation tank; (b) atypical situation assuming a delay in drying after the flotation tank, leading to growth of* Salmonella*; (c) atypical situation assuming contamination during storage pretreatment; (d) atypical situation assuming contamination during storage posttreatment.*

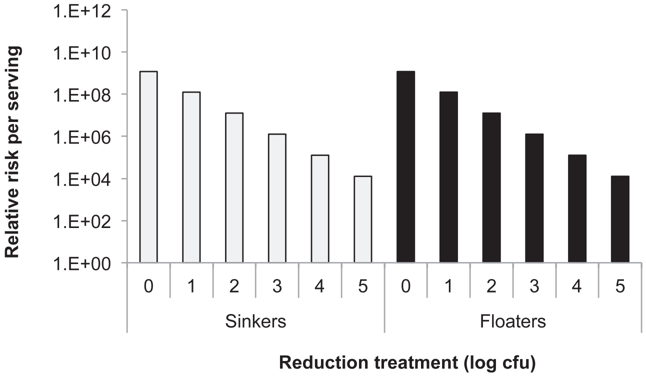

FIGURE 5. *Salmonellosis risk per serving of pistachios consumed as a core product uncooked in the home assuming the prevalence and levels of contamination found in the recalled units of the 2016 U.S. salmonellosis outbreak and given various* Salmonella *reduction treatments (0- to 5-log reductions) relative to the risk per serving for sinkers using the baseline model with a 5-log* Salmonella *reduction treatment.*

nella levels ~1 million- and ~100,000-fold higher than levels in the baseline model for sinkers and floaters, respectively. This atypical situation assumed a homogeneous distribution of *Salmonella* cells within the contaminated units and resulted in a ~9×10^2-fold and ~1×10^4-fold increase in risk estimates per serving for floaters and sinkers, respectively, when compared with the baseline process model (Fig. 5). The mean number of estimated salmonellosis cases per silo for sinkers was 25,824 (95% CI, 2,578 to 303,418) with no *Salmonella* reduction treatment, 2,715 (95% CI, 256 to 49,556) with a 1-log reduction treatment, and 275 (95% CI, 26 to 5,434) and 27 (95% CI, 3 to 561) for 2- and 3-log reduction treatments, respectively. Results in the same order of magnitude were observed for floaters. We estimated 3 (95% CI, 0 to 56) cases per silo for pistachios (sinkers or floaters) after a 4-log reduction treatment. A 5-log reduction treatment resulted in an equal estimated mean of zero cases (95% CI, 0 to 6) per silo for sinkers or floaters. Modeling this same atypical situation with the 2016 U.S. outbreak prevalence and levels of contamination and assuming the distribution of *Salmonella* cells was not homogeneous throughout the silo resulted in risk estimates per serving and number of cases per silo that are twofold lower when no microbial treatment is in place, and when assuming homogeneous distribution the estimates for all reduction treatments are of the same order of magnitude as those modeled.

Similar to the results seen in our group's previous tree nut risk assessments *(30, 31),* the results of modeling atypical situations including the prevalence and levels of contamination found in the 2016 outbreak recall units highlight that process control through *Salmonella* reduction treatment may not be sufficient to prevent illnesses or outbreaks, especially when the atypical situations occur after a *Salmonella* reduction treatment has been applied. The number of salmonellosis cases per year linked to atypical situations of these types is 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 from the modeled atypical situations provide an estimate of the significance of such a situation compared with the baseline model scenario and the impact these atypical situations may have on increasing the risk (changes in the order of magnitude). Modeling atypical situations such as these also sheds light on which steps of the process, when controlled, can prevent increase in the predicted risk. Similar to the results of the risk assessment of *Salmonella* on almonds *(31),* the estimated mean risk of salmonellosis arising from consumption of pistachios in the United States is less than one case per year when a minimum 4-log reduction treatment is applied to the entire U.S. pistachio supply.

Comparison with previous pistachio risk assessment models. Lambertini et al. *(20)* developed a model to calculate the risk of salmonellosis associated with the consumption of pistachios in the U.S. population using prevalence data from 2010, 2011, and 2012 *(18)*. The baseline risk assessment model includes steps from storage at the silo to the point of consumption, assuming no decline in *Salmonella* levels during storage and no *Salmonella* reduction treatment *(20)*. The estimated mean risk of illness from consumption of pistachios using the baseline model was approximately 14 cases per million servings, resulting in an mean of 2,900 cases per year. The authors reported a markedly left-skewed year-to-year variability in the expected number of cases, spanning over ~5 log units. By considering each of their Monte Carlo iterations as a 1-year realization, the authors made the explicit assumption that their parameters (e.g., serving size and *Salmonella* level in positive samples) were constant in a given year, which might be an unrealistic assumption. However, the arithmetic mean is preserved in this framework. A 4-log reduction treatment resulted in an average of 1.4 cases per billion servings and <1 case per year. Our current risk assessment model (for consumption of pistachio sinkers as a core product uncooked in the home) predicts mean risk estimates per serving that are 33-fold lower and mean risk estimates per year that are 10-fold lower than those obtained with the Lambertini et al. model. This unequal difference when comparing mean risk per serving and mean risk per year estimates of the current model and that of Lambertini et al. arises from the fact that in that model the number of pistachio servings not cooked at home were fixed at 0.79 (in-shell) and 0.29 (kernels) billion servings based on market data in the United States. The current model was based on a distribution of consumption data from the WWEIA and made a distinction for whether pistachios were consumed as a core product or as an ingredient (cooked in the home), accounted for the proportion of the pistachio that is kernel versus shell, and modeled only consumption of kernels (assuming all pistachios at retail are in-shell). Use of pistachio sales data to account for consumption would not change the risk estimates per serving but would overestimate the risk per year. Similar to the results obtained by Lambertini et al., a 10-fold decrease in risk is observed with our model for every 1-log increase in the reduction treatment applied, and a mean risk of illness of less than one case per year was estimated when a minimum 4-log reduction treatment was applied to the U.S. pistachio supply. A 3-log reduction treatment resulted in a mean risk of illness per year of less than one case (95% CI, <1 to 1). Lambertini et al. reported a 10-fold decrease in risk when including *Salmonella* declines during storage as a "what-if" scenario (including consumer home storage). In our model, decline during storage is considered part of the baseline risk assessment model, and we do not assume log-linear declines of *Salmonella*. Our model does not include consumer home storage because it cannot be counted on as a risk mitigation strategy (consumers may eat pistachios immediately after purchase). If the consumer stored pistachios at room temperature (20 to 25°C) or at refrigeration or freezing temperatures after purchase and before consumption, *Salmonella* levels on pistachios would be decreased or maintained, making the estimated risk of salmonellosis lower. The differences in risk estimates when comparing the Lambertini et al. model with our risk assessment model are mainly the result of the fact that our model includes *Salmonella* declines during pretreatment and posttreatment storage in the baseline exposure assessment, has a different model to estimate initial prevalence and contamination levels, uses WWEIA consumption data to account for the serving size and number of servings, and separates variability and uncertainty in risk estimates. This risk assessment is the first for *Salmonella* on pistachios that has separated variability and uncertainty in its risk estimates.

The model and results of this assessment are limited to *Salmonella,* pistachios, and the United States. Data on *Salmonella* probability of contamination and *Salmonella* levels at harvest would provide the means with which to model exposure from harvest to silo storage. Data on transfer rates of *Salmonella* across the pistachio shell to the kernel would aid in estimating risk from kernel consumption. With additional data on different types of exceptional situations and the frequency with which they occur, we could better estimate the impact on public health. Characterization of the time-temperature profiles for relevant cooking processes at the consumer level would provide a better means of estimating the risk of salmonellosis caused by consumption of pistachios as an ingredient in products cooked in the home. As data become available on the distribution of log-reduction levels achieved for a targeted treatment, the effect of the variability in treatment could be quantified using the results of this risk assessment.

The impact of 1- to 5-log *Salmonella* reduction treatments on the predicted risk of human salmonellosis arising from the consumption of pistachios in the United States was evaluated in this study. The 4- and 5-log reduction treatments resulted in an estimated mean risk of illness of less than one case (95% CI, less than one case) per year in the United States. The impact of variability is much higher than the impact of the considered uncertainty on risk estimates, indicating that additional data collection will not increase the certainty of these risk estimates. These results are similar to those obtained by the FDA risk assessment for *Salmonella* on almonds *(31),* in which an estimated mean risk of salmonellosis of less than one case per year was predicted for a minimum 4-log reduction treatment applied to the entire U.S. pistachio supply. The modeled atypical situations resulted in a higher predicted risk of illness per pistachio serving, and *Salmonella* growth due to a delay in drying had the greatest impact on risk estimates. The 2016 outbreak atypical situation model suggests that a minimum 5-log *Salmonella* reduction treatment would have been sufficient to prevent the outbreak. If the pistachios linked to the 2016 outbreak had undergone a *Salmonella* reduction treatment, the applied treatment may have been insufficient to adequately reduce *Salmonella* levels, the *Salmonella* levels on raw pistachios may have been higher than those recovered during the investigation and subsequently modeled, there could have been a *Salmonella* reduction treatment process failure, or postprocess contamination may have occurred. Although process control through *Salmonella* reduction treatment is predicted to significantly reduce the health risk associated with pistachio consumption, atypical situations that occur pre- and posttreatment may lead to increased risks.

ACKNOWLEDGMENTS

The authors thank Dr. Linda Harris for providing raw data and information and Jenny Scott for her careful review of the current and previous versions of the manuscript. This study used the computational resources of the High Performance Computing clusters at the FDA Center for Devices and Radiological Health.

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