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Monte Carlo Simulations Assessing the Risk of Salmonellosis from Consumption of Almonds

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ABSTRACT

Recent outbreaks of salmonellosis associated with raw almonds have raised awareness of this food as a vector for foodborne illness. We performed a quantitative assessment of the risk of contracting salmonellosis from consumption of raw almonds, accounting for factors that become important after almonds reach the processor. We estimated the risk associated with the consumption of raw almonds and the risk reduction associated with almonds treated with a theoretical 5-log reduction process or treated with propylene oxide using a standard commercial process. Probability distributions were chosen to describe the chance of almond contamination and the effects of storage time, storage temperature, and processing from currently available data. A β-Poisson model for the dose-response relationship for *Salmonella* was obtained from published literature. The simulation estimated a 78% chance of one or more cases of salmonellosis per year from consumption of raw almonds. The application of a commercial propylene oxide treatment reduced this risk to 0.01%. Hypothetical 5-log reduction treatments with different standard deviations (±1, ±0.5, ±0.1, and ±0) reduced the predicted yearly risk of salmonellosis to 0.69, 0.35, 0.30, and 0.21%, respectively. These results suggest that the risk of one or more U.S. cases of salmonellosis per year from consumption of raw almonds can be reduced from 78% to less than 1% by using a process achieving a 5-log reduction in *Salmonella* with a process standard deviation as large as 1 log unit or by using a commercial propylene oxide treatment.

Salmonella is the third leading known cause of foodborne illness in the United States, with estimates of more than 1.3 million salmonellosis cases per year *(9)*. Recent increases in the global number of cases of salmonellosis are believed to be due to improved surveillance and resourcefulness of public health officials, the widespread occurrence of *Salmonella* in the natural environment, and inadequate sanitary practices during harvest, processing, and distribution of raw food and food ingredients *(5)*.

Outbreaks of salmonellosis associated with the consumption of raw whole almonds were reported in 2001 and in 2004 and have raised awareness of this tree nut as a vector for foodborne illness *(3, 11)*. Prior to this time, tree nuts other than shredded coconut *(15)* had not been associated with outbreaks of bacterial foodborne illness and had generally been considered low risk for disease transmission because they are consumed in a dried state, in which the water activity is below 0.7. *Escherichia coli* has been isolated from almonds, walnuts, and pecans *(10, 12–14, 16)*, but this contamination has not been investigated for possible correlations with the presence of foodborne pathogens.

There is interest in the effects of various interventions on the prevalence and concentration of *Salmonella* in almonds and on the risk associated with the consumption of raw almonds. A quantitative microbial risk assessment of salmonellosis from raw almonds using the currently available data would contribute to this understanding. The objective of this work was to develop a model using Monte Carlo simulation to describe the risk associated with consumption of raw almonds based on current distribution and consumption patterns in the United States. The model accounts for the factors that are important after almonds reach the processor, including consumer handling. By including bacterial reductions achieved by application of propylene oxide (PPO) using standard commercial processes or by application of a theoretical 5-log bacterial reduction process with different levels of process control, the reductions in salmonellosis risk following processing could be predicted.

MATERIALS AND METHODS

Table 1 provides an overview of the simulation variables and distributions affecting the survival of *Salmonella* on raw almonds that were used in the risk assessment model. To estimate the prevalence and concentration of *Salmonella* in raw almonds, data from a double-blind survey (unpublished data) were used. Almond samples from the 2001 through 2004 harvests were collected from seven almond handlers (processors) representative of small, medium, and large facilities located throughout the almond-growing regions of California. The approximately 400-g samples taken as the raw almonds were received were stored at 4°C until testing. A 100-g subsample was enriched for *Salmonella* using standard methods. When a sample was positive for *Salmonella,* additional subsamples (three subsamples of 25 g each, three of 2.5 g each, and three of 0.25 g each) were enriched using standard methods to determine the most probable number (MPN) of *Salmonella.* All *Salmonella* isolates were confirmed by standard biochemical means and serotyping. The prevalence of *Salmonella* in almond samples ranged from 0.6 to 1.15% (Table 2). In most cases, *Sal-*

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TABLE 1. *Simulation variables and distributions affecting the survival of* Salmonella *serovars on raw almonds*[a]

Node	Description	Source
Level of contamination in positive samples (MPN/100 g)	Low: Normal distribution between 1.2 and 2.9 MPN/100 g, with a mean (standard deviation) of 1.07 (0.01) MPN/100 g based on 63 MPN samples.	Unpublished data
	High: Triangular distribution with a most likely concentration of 562 MPN/100 g and minimum and maximum confidence intervals of 126 and 2,398 MPN/100 g, respectively. A 1 in 64 chance of a *Salmonella*-positive sample with a high MPN concentration.	Calculated estimate,[b] unpublished data *(17)*; conservative assumption, unpublished data
Handler storage time	Total time in handler storage (pre- and postprocess). Uniform distribution of 1–12 mo.	ABC[c] board members
Preprocess storage time	Total time of storage prior to processing at 24°C. [0–100% of handler storage time]	ABC board members
Preprocess reduction	Reduction in *Salmonella* that occurs during preprocess storage. [**preprocess storage time** × reduction/mo at 24°C (0.25 log CFU/mo)]	*(17)*
Postprocess storage time	Total time of storage prior to processing at 4°C. [**handler storage time** − **preprocess storage time**]	ABC board members and staff
Postprocess reduction	Reduction in *Salmonella* that occurs during postprocess storage. [**postprocess storage time** × reduction/mo at 4°C (0 log CFU/mo)]	*(17)*
Retail storage time	Storage time at 24°C after arrival at the retailer before sale. Triangular distribution with a minimum of 1 day, a maximum of 6 wk, and a most likely time of 2 wk.	ABC staff
Retail reduction	Reduction in *Salmonella* that occurs during retail storage. [**retail storage time** × reduction/mo at 24°C (0.25 log CFU/mo)]	*(17)*
Consumer storage time	Storage time after purchase but before consumption. Uniform distribution of 0–12 mo.	ABC staff
Consumer storage temperature	Storage temperature in consumer home. Equal likelihood of −20, 4, or 24°C.	ABC staff
Consumer reduction	Reduction in *Salmonella* that occurs during consumer storage. [**consumer storage time** × reduction/mo at **consumer storage temperature** (0, 0, or 0.25 log CFU/mo)]	ABC staff, *(17)*
***Salmonella* contamination/serving** (CFU/28 g)	Concentration of *Salmonella* per 28-g serving. [(**level of contamination** − **preprocess reduction** − **postprocess reduction** − **retail reduction** − **consumer reduction**)/28 g]	Calculated
Probability of illness/serving	Dose-response model (β-Poisson distribution) for *Salmonella* was used to predict the probability of illness. [1 − (1 + ***Salmonella* contamination/serving**/55)$^{-0.13}$]	*(8)*
U.S. consumption of raw almonds	Number of 100-g samples consumed raw annually in the United States [(141,595,859,009 g × 0.05)/100]	ABC staff
***Salmonella* prevalence** (% positive 100-g samples)	Uniform distribution between 0.6 and 1.15% of 100-g samples are contaminated.	Unpublished data
***Salmonella*-positive 100-g samples consumed**	Number of 100-g samples that are consumed raw and contain *Salmonella*. [**U.S. consumption of raw almonds × *Salmonella* contamination**]	Calculated
Contaminated servings in positive 100-g sample	Equal likelihood of one, two, three, or four servings in 100-g *Salmonella*-positive sample.	Assumption
Servings consumed containing *Salmonella*	Servings consumed annually containing *Salmonella* [***Salmonella*-positive 100-g samples consumed × contaminated servings in positive 100-g sample**]	Calculated
Predicted illnesses/yr	Number of illnesses/yr due to consumption of contaminated almonds. [**probability of illness/serving × servings consumed containing *Salmonella***]	Calculated

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[a] Node names are given in bold. MPN, most probable number.

[b] Calculated MPN at time of shipment (February 2001) using 2000 to 2001 outbreak MPN data (9 MPN/100 g) from recalled almonds tested in July 2001.

[c] ABC, Almond Board of California.

TABLE 2. *Prevalence and concentration of* Salmonella *in raw almonds*

Harvest year	No. (%) of 100-g samples positive for *Salmonella*	*Salmonella* concn (MPN/100 g)
2000[a]	42/50 (84)	6.1–9.1
2001[b]	12/2,012 (0.60)	ND[c]
2002	23/2,000 (1.15)	<1.2–2.9
2003	15/1,764 (0.85)	<1.2–1.4
2004	13/1,644 (0.79)	<1.2–2.4

[a] Almonds were recalled from the 2000 to 2001 outbreak; % positive and MPN determinations were made July 2001.

[b] Almond Board of California survey data from random samples of almonds arriving at processing facility.

[c] ND, not determined.

monella was not detected upon retesting of positive samples with the MPN analysis. Therefore, these samples were estimated to have 1 MPN/100 g. When *Salmonella*-positive samples were found upon retesting, a single 25-g sample was typically positive, for an estimated population of 1 to 3 MPN/100 g. A normal distribution with a mean (standard deviation) of 1.07 (0.01) MPN/100 g (based on data for all 63 positive samples) was used to predict the concentration of *Salmonella* in contaminated samples.

In July 2001, raw almonds recalled from retail stores involved in the 2000 to 2001 outbreak *(11)* were tested to determine MPN values (unpublished data). The almonds had been sold in bulk, and 50 unopened 22.7-kg boxes were available for testing. A *Salmonella* concentration of 9 MPN/100 g was determined for these recalled raw almonds (Table 2). These almonds had been stored at ambient temperature from the time they were shipped from the handler in February 2001. Data describing the survival of *Salmonella* Enteritidis PT 30 under ambient conditions (23 ± 2°C) *(17)* were used to backcalculate the estimated population of *Salmonella* present on the almonds at the time these almonds would have been in retail stores in February 2001. Uesugi et al. *(17)* found that at ambient temperature *Salmonella* concentrations decline at a rate of 0.25 ± 0.04 log CFU/month. The estimated concentration in February 2001 was 562 MPN/100 g, with lower and upper confidence intervals of 126 and 2,398 MPN/100 g. A triangular distribution with a minimum of 126, a most likely value of 562, and maximum value of 2,398 MPN/100 g was used to estimate a high level of contamination per 28-g serving of almonds. The survey did not yield any almond samples with *Salmonella* concentrations greater than 3 MPN/100 g. Therefore, a very conservative approach was used to estimate the frequency with which almonds would have a high level of contamination. Because only 63 positive samples were detected in the 4-year survey, it was assumed that the 64th positive sample in the survey would have been contaminated at a high level equivalent to that calculated for the 2000 to 2001 outbreak (562 MPN/100 g), thereby giving a frequency of high-level contamination of 1 in 64.

Discussions with almond industry members indicated that almonds are not typically stored by the handler for longer than 12 months. A uniform distribution of 0 to 12 months was chosen to represent the total time almonds are stored by the handler. Almonds usually are stored at ambient temperatures before processing by the handler and sometimes under refrigerated conditions after processing by the handler. It was assumed that a uniform distribution described division of time between pre- and posthandling storage. To determine the reduction in *Salmonella* that occurred during preprocess storage, we multiplied the time of storage (uniform between 0 and 12 months) by the fraction of time spent in prehandling storage (uniform between 0 and 100%) and by the reduction that occurs at 24°C (0.25 log CFU/month) *(17)*. No reduction was predicted during postprocessing storage at 4°C *(17)*.

Discussions with the Almond Board of California staff indicated that although exact storage times in the retail environment are unknown, they appear to be short. Storage times were estimated to be a minimum of 1 day, a maximum of 6 weeks, and a most likely time of 2 weeks, and a triangular distribution was used to describe this pattern. Reduction in *Salmonella* concentration during retail storage was predicted by multiplying the time of storage by the reduction at ambient temperature (0.25 log CFU/month) *(17)*. Discussions with Almond Board of California staff revealed that consumer storage could be up to 1 year before consumption, and this storage might occur under ambient, refrigerated, or frozen conditions. Reduction in *Salmonella* concentration during consumer storage was predicted by multiplying the time of storage by the reduction during storage at −20, 4, or 24°C (0, 0, or 0.25 log CFU/month, respectively). It was assumed that each of the three storage conditions was equally likely.

The average concentration of *Salmonella* in each 28-g (1-oz) serving (size recommended by the U.S. Department of Agriculture) was determined by subtracting the reductions during preprocessing, postprocessing, retail, and consumer storage from the initial concentration and dividing this number by 28. A β-Poisson model for the dose-response relationship that was obtained from the published literature *(8)* was used to estimate the probability of illness based on the ingested dose. The Food and Agricultural Organization dose-response model *(8)* was developed for a risk assessment of *Salmonella* in eggs and broiler chickens based on information from 20 documented outbreaks involving seven serovars of *Salmonella.*

The total weight of raw almonds consumed in the United States in 2003 was calculated by multiplying the 2003 total U.S. consumption of almonds (312,160,183 lb [14,172,072 kg] of kernels) *(2)* by the fraction (0.05) of that amount that the Almond Board of California staff estimated was consumed raw *(1)*. This value was converted into grams and divided by 100 to yield the number of 100-g samples of raw almonds consumed per year.

Almond Board of California survey data from 2001 to 2004 (unpublished data and Table 2) were used to estimate the percentage of *Salmonella*-positive 100-g samples of raw almonds; the range was 0.6 to 1.15%, with a mean of 0.85%. A uniform risk distribution between 0.6 and 1.15% was used to predict the percentage of contaminated 100-g samples. The predicted number of 100-g samples consumed per year was multiplied by the predicted percentage of contaminated 100-g samples to provide the number of *Salmonella*-positive 100-g samples consumed.

Survey data and data generated during the testing of recalled almond samples from the 2000 to 2001 outbreak suggest that *Salmonella* cells are unevenly distributed throughout the product. Therefore, within each *Salmonella*-positive 100-g sample consumed, it was assumed that there was an equal likelihood of one, two, three, or four positive subsamples (25 g each). One 25-g subsample was assumed to be one serving. The number of servings containing *Salmonella* was predicted by multiplying the number of 100-g *Salmonella*-positive samples by the number of contaminated subsamples within that sample (discrete uniform distribution: one, two, three, or four). To predict the number of expected illnesses per year, the probability of illness per serving was then multiplied by the number of contaminated servings consumed per year.

Figure 1 provides the data from our laboratory on the distri-

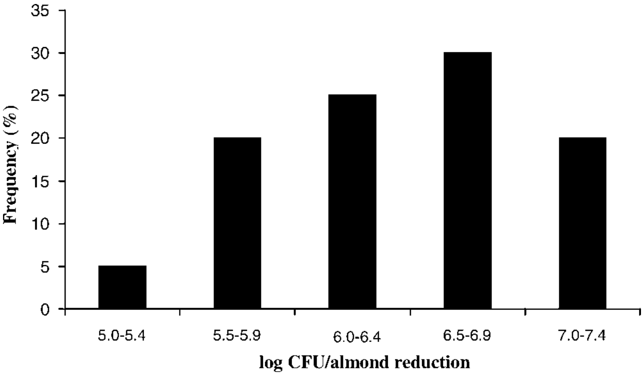

FIGURE 1. *Distribution of mean log reductions in* Salmonella *obtained with PPO treatment. The histogram is shown (5, 7: {1, 4, 5, 6, 4}).*

bution of reductions of *Salmonella* Enteritidis PT 30 on inoculated almonds after treatment with PPO *(4).* To evaluate process variability, 5-log reductions with normal distributions and standard deviations of 0, 0.1, 0.5, and 1 also were used (Table 3).

Data were entered into Excel (Microsoft, Redmond, Wash.) spreadsheets. When appropriate, data were binned into histograms with Excel and analyzed with Best Fit (Palisade Corporation, Newfield, N.Y.) to create probability distribution functions. These functions were combined using the assumptions detailed above, and @RISK software (Palisade) was used to perform Monte Carlo simulations of 10,000 iterations.

RESULTS AND DISCUSSION

The risk assessment model predicted a 78% chance of more than 1 case of salmonellosis per year associated with consumption of raw almonds in the United States, with a mean of 8 cases per year and a maximum of 4.4 × 10^5 cases per year (Fig. 2a and Table 4). Two documented outbreaks of salmonellosis have been associated with consumption of raw almonds, involving 168 (2000 and 2001) and 29 (2003 and 2004) cases *(3, 11).* However, given the low concentration of *Salmonella* typically found in almonds and the apparent uneven distribution, it is likely that most cases of salmonellosis from consumption of raw almonds are sporadic rather than linked to outbreaks.

In September 2004, the U.S. Food and Drug Administration (FDA) reviewed validation data for a standard PPO process *(4).* Bulk almond kernels treated in this manner may be labeled "pasteurized" without objection from the FDA. When the PPO distribution data (Fig. 1) were used in the simulation to provide a kill step, the probability of salmonellosis from consumption of almonds was greatly reduced; the chance of more than one illness per year was predicted to 0.01% (Fig. 2b).

TABLE 3. *Variability of processing treatments used to reduce the presence of* Salmonella *on raw almonds*

Node	Description
PPO treatment	Histogram (5, 7, {1, 4, 5, 6, 4})
5 ± 1.0-log reduction	Normal (5, 1)
5 ± 0.5-log reduction	Normal (5, 0.5)
5 ± 0.1-log reduction	Normal (5, 0.1)
5 ± 0-log reduction	Normal (5, 0)

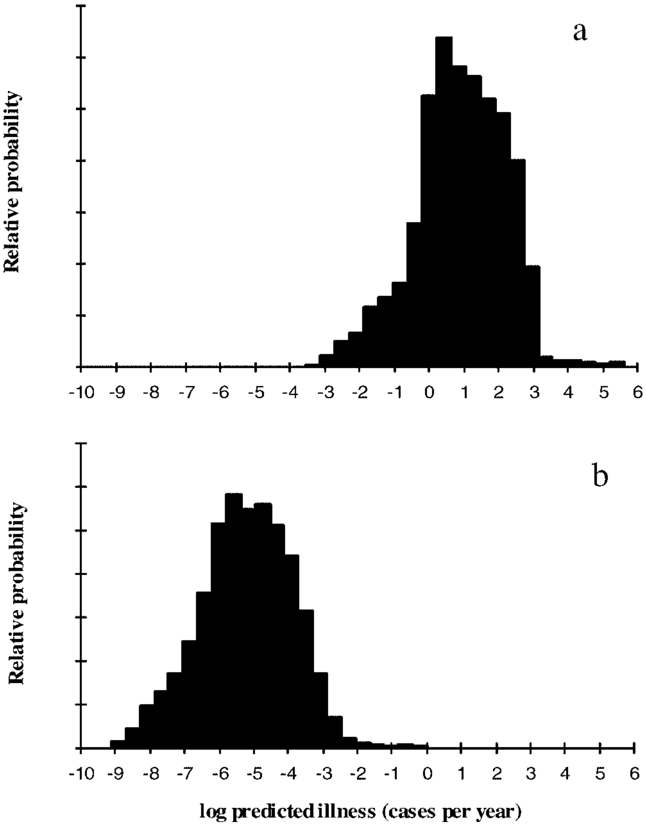

FIGURE 2. *Probability of salmonellosis from (a) consumption of raw almonds and (b) consumption of almonds treated with PPO. Results of @Risk simulations (10,000 iterations).*

Because no step in food processing is ever 100% effective, it is important to acknowledge the variability associated with each process. A highly variable process with a high mean reduction in bacteria may be less useful than a process with a slightly lower mean kill but a more narrow range of variability, which is less likely to fail *(7).* Almonds are a particulate food for which a high chance of process variability is anticipated and has been observed. To evaluate the impact of variability in a process on the overall risk of salmonellosis from almonds, treatments producing 5-log *Salmonella* reductions with decreasing levels of variability (Table 3) were included in the risk assessment model to provide a kill step. The shape of the predicted probability

TABLE 4. *Summary of results from @RISK simulations predicting the risk of salmonellosis from consumption of raw almonds*

		Probability of (%):		
Treatment	Mean no. of cases/yr	>1 case/yr	>10 cases/yr	>100 cases/yr
Raw	8	78	48	21
PPO	6 × 10^{-6}	0.01	<0.01	<0.01
5 ± 1.0-log reduction	8 × 10^{-5}	0.69	0.18	0.01
5 ± 0.5-log reduction	8 × 10^{-5}	0.35	0.07	<0.01
5 ± 0.1-log reduction	7 × 10^{-5}	0.30	0.03	<0.01
5 ± 0-log reduction	7 × 10^{-5}	0.21	0.02	<0.01

TABLE 5. *Summary of results from @RISK simulations with lower frequencies of high levels of* Salmonella *predicted*

Treatment	Frequency of high contamination	Probability of (%):		
		>1 case/yr	>10 cases/yr	>100 cases/yr
Raw	1/64	78	48	21
	1/200	78	48	20
	1/500	77	48	20
	1/1,000	77	46	20
5 ± 1.0-log reduction	1/64	0.69	0.18	0.01
	1/200	0.31	0.05	<0.01
	1/500	0.31	0.05	<0.01
	1/1,000	0.16	<0.01	<0.01

distributions obtained from the simulations varies slightly with the different standard deviations tested, especially in the length of the right tails (data not shown). However, the probability of more than one case of salmonellosis per year for 5 ± 1-, 5 ± 0.5-, 5 ± 0.1-, and 5 ± 0-log reductions are remarkably similar: 0.69, 0.35, 0.30, and 0.21%, respectively (Table 4). In each scenario, the mean number of salmonellosis cases was 7×10^{-5} to 8×10^{-5} per year. These data indicate that the variability of a 5-log reduction process for *Salmonella* in almonds, based on our estimates of the existing contamination levels, may not be of critical importance.

Although the 1 in 64 chance of high levels of *Salmonella* contamination represents the data currently available, discussions with members of the almond industry indicated that this frequency may grossly overestimate the frequency of high levels of *Salmonella* contamination. To evaluate the importance of the frequency assumption of *Salmonella* contamination in raw almonds, the model was modified to look at a 1 in 200, 1 in 500, and 1 in 1,000 chance of high levels of contamination. A 78% chance of more than one case of salmonellosis per year from consumption of raw almonds was estimated at both 1 in 64 and 1 in 200 chances of high levels of *Salmonella* (Table 5). The probability of more than one case per year dropped slightly to 77% when the chance of high levels of *Salmonella* decreased to 1 in 500 and 1 in 1,000. Very slight differences also were seen when estimating the probability of more than 10 cases per year due to consumption of raw almonds. In these simulations, the probability of more than 10 cases per year decreased from 48 to 46% as the chance of high concentrations of *Salmonella* decreased. Similarly, the probability of more than 100 cases per year was estimated to be 21% when the chance of high contamination was 1 in 64, whereas this estimate fell to 20% for all the other chances of high contamination.

Evaluating the effect of changing the frequency of high levels of contamination on almonds undergoing a 5 ± 1-log kill step revealed small differences between 1 in 64, 1 in 200, 1 in 500, and 1 in 1,000 in the probability of greater than one case per year. Results for 5 ± 0.5-, 5 ± 0.1-, and 5 ± 0-log kill steps and PPO were similar to those of 5 ± 1-log reduction (data not shown). Although the 1 in 64 frequency of high levels of contamination may overestimate the actual level of contamination, it did not significantly influence the output of the risk model.

A sensitivity analysis was performed to further evaluate which inputs and assumptions have the greatest effect on the predicted annual number of cases of salmonellosis from consumption of raw almonds (Table 6). Correlation coefficients (R^2) farther from zero indicate a greater influence of the parameter on the model output, with positive R^2 values representing variables that increase the risk with increasing value and negative R^2 values representing variables that decrease the risk with increasing value. The analysis revealed that the primary factors influencing uncertainty surrounding the estimate of number of salmonella cases per year from consumption of raw almonds were total handler storage time, reduction during consumer storage (i.e., consumer storage temperature), and consumer storage time. Factors such as retail storage time and initial concentration of *Salmonella* were less important in the estimate of the number of cases of salmonellosis per year. When a similar sensitivity analysis was run for the models involving a 5 ± 1-log reduction treatment, all factors appear to have a relatively small influence on the predicted cases of salmonellosis annually (small R^2 values). Tornado plots for models involving 5 (±0.5, ±0.1 and ±0)-log reductions and PPO treatment were virtually indistinguishable (plots not shown) from the results provided in Table 6. These data indicate that when a highly effective treatment is used, other factors become much less important in estimating the number of salmonellosis cases per year.

Although the simulation model proposed here provides a good framework for understanding and estimating the risks associated with almonds, many questions remain un-

TABLE 6. *Sensitivity analysis: relative significance of variables for models*

Rank	Raw almonds		Almonds with 5 ± 1-log reduction treatment	
	Variable	R^2	Variable	R^2
1	Handler storage time	−0.70	Handler storage time	−0.026
2	Reduction during consumer storage	−0.58	Reduction during consumer storage	0.023
3	Consumer storage time	−0.20	Percentage of *Salmonella*-positive 100-g samples	−0.020
4	Chance of low concentration of *Salmonella*	−0.19	Chance of high concentration of *Salmonella*	0.019
5	Number of contaminated servings	0.14	Chance of low concentration of *Salmonella*	0.019
6	Percentage of *Salmonella*-positive 100-g samples	0.074	Consumer storage time	−0.016
7	Retail storage time	−0.025	Preprocessing storage time	0.016
8	Chance of high concentration of *Salmonella*	0.023	Number of contaminated servings	0.015

answered. Our model assumes that only 5% of almonds (those currently consumed raw) are associated with illness and evaluates the effect that a 5-log reduction treatment or PPO treatment of raw almonds would have on reducing that risk. The majority of almonds (the remaining 95%) are neglected in this model because they are assumed to not contribute to illness. The validity of this assumption should be tested. Most of the remaining 95% of domestically consumed almonds are roasted (either in oil or a dry roaster) or blanched (to remove the skins). Both oil roasting and blanching treatments have been validated to achieve at least a 5-log reduction of *Salmonella* Enteritidis PT 30 *(6, 18)*. However, the reductions achieved during various dry roasting processes have not been published. More recent data exist for treatments other than PPO that reduce *Salmonella* while maintaining the sensory qualities of raw almonds; these data could be entered in the existing simulation model to evaluate the impact of these treatments on the predicted risk.

We have assumed a serving size of 28 g (1 oz), whereas in reality serving sizes for raw almonds range from two almonds (in some confections) to more than 28 g (for some individuals that eat large quantities of almonds as a snack).

The results of the Monte Carlo simulation model suggest that both PPO and other treatments are promising for controlling the disease risk associated with the consumption of raw almonds. Additional improvements in the simulation model are anticipated, including variation in the level of initial contamination and a more detailed analysis of handler storage practices. Sensitivity analysis revealed that some assumptions made for these calculations (i.e., consumer almond storage practices) should be more accurately defined. Additional research and modeling of the variability and effectiveness of less harsh processing treatments is warranted.

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