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Risk of salmonellosis from consumption of almonds in the North American market

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ABSTRACT

Salmonellosis outbreaks from consumption of raw almonds in 2001 and 2004 led to regulations that require mandatory treatment of almonds sold in North America to give a minimum 4-log reduction of *Salmonella*. This study aims to: 1) assess the risk of salmonellosis associated with almond consumption in North America, with current treatments in effect; 2) determine the resilience of the current production system to increases in prevalence or concentration of *Salmonella* on almonds; 3) assess the impact of treating less than 100% of the crop; and 4) investigate conditions that could explain the number of cases associated with the 2001 outbreak. Risk was assessed using a Monte Carlo simulation, based on an established dose–response relationship. Data for almond amounts sold, *Salmonella* prevalence and concentration on almonds, storage time and temperature at different handling steps, population reductions during storage at various temperatures and with different treatments, and consumer handling were based on data from published sources and almond industry or academic expert opinion. What-if scenarios were evaluated for *Salmonella* prevalence varying from 1 to 65%, concentrations of *Salmonella* varying from 1 to 120 MPN/100 g, and portions of untreated crop varying from 0 to 10%. The estimated incidence of salmonellosis in North America from almonds as currently treated is on average 0.008 cases per billion servings (with an estimated 6.6 billion servings consumed annually). Increases in *Salmonella* prevalence to 25%, mean concentrations above 25 MPN/100 g, or leaving 0.05% of the crop untreated all resulted in an arithmetic mean risk greater than 1 case/year (with geometric means remaining below 1 case/year for all variables). Assuming 4000 kg at a prevalence of 65% (observed in recalled lots) and an average concentration of 120 MPN/100 g in raw almonds (back calculated from levels in recalled almonds) predicted over the 2800 cases estimated for the 2001 outbreak. Applying a 4-log reduction to these almonds reduced the average number of predicted cases to less than a single case. The current regulation is effective in maintaining the risk of salmonellosis from consumption of almonds below an arithmetic mean of 1 case/year, although significant increases in either prevalence or concentration, or small increases in proportion of untreated almonds would frequently lead to exceeding this threshold.

1. Introduction

Outbreaks of salmonellosis associated with consumption of raw almonds were documented in North America in 2001 and 2004 and in Sweden in 2006 (CDC, 2004; Isaacs et al., 2005; Ledet Müller et al., 2007). Illnesses from *Salmonella* associated with other tree nuts have been limited to desiccated coconut (Ward, Brusin, Duckworth, & O'Brien, 1999). However, consumption of peanuts and peanut products, edible seeds, and many other low moisture foods have been linked to salmonellosis (Podolak, Enache, Stone, Black, & Elliott, 2010).

Salmonella has been shown to sporadically but consistently occur on harvested almonds; when present, levels are low (Bansal, Jones, Abd, Danyluk, & Harris, 2010; Danyluk et al., 2007). Limited surveys and documented recalls indicate that *Salmonella* can also be found in other nuts (Little, Rawal, de Pinna, & McLauchlin, 2010; Palumbo, Beuchat, Danyluk, & Harris, 2011). The sources of such contamination in nuts and seeds are not known, but likely arise from the pre-harvest environment and are carried through during harvest and postharvest handling (Danyluk et al., 2008; Podolak et al., 2010; Uesugi, Danyluk, Mandrell, & Harris, 2007). The extent of sporadic illness due to consumption of *Salmonella*-contaminated raw nuts is not well understood.

California-grown almonds account for about 80% of the world's and all of the U.S. commercial almond production (ABC, 2010). Documented outbreaks led to U.S. regulations, implemented in 2007, that require all California-grown almonds sold in North America (U.S., Canada, and Mexico) to be processed with a treatment capable of achieving a minimum 4-log reduction in *Salmonella* (Federal Register, 2007). Several treatments are available and used by the U.S. almond industry to achieve the mandated reductions of *Salmonella* (ABC, 2007), including oil and dry roasting (Du, Abd, McCarthy, & Harris, 2010), blanching (Harris, Uesugi, Abd, & McCarthy et al., in press), steam (ABC, 2007) and propylene oxide

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(Danyluk, Uesugi, & Harris, 2005). These inactivation methods have been individually validated, but with the exception of propylene oxide (Danyluk, Harris, & Schaffner, 2006) the predicted effects of their application on the incidence of salmonellosis have not been assessed.

Salmonella survival or decline on almond kernels depends on several factors, including temperature and water activity. Higher storage temperatures result in a faster decline of *Salmonella* on almonds (Uesugi, Danyluk, & Harris, 2006). However, the role of moisture is more complex: low moisture prevents bacterial growth and results in cell desiccation, but there is also evidence that low water activity can have a protective effect against inactivation, and can increase heat resistance (Podolak et al., 2010).

Risk assessment is a well-established tool to model human health effects associated with exposure to a hazard (Rocourt, BenEmbarek, Toyofuku, & Schlundt, 2003; Schlundt, 2000; Skovgaard, 2007). A quantitative risk assessment was carried out in 2006 to determine the risk of contracting salmonellosis from consuming raw almonds produced in California (Danyluk et al., 2006); an estimated 5% of all almonds in North America were consumed raw at that time. Since then, total annual North American almond consumption has increased from 140 million kg to 210 million kg (Birmingham, 2010), additional survey data are available (Bansal et al., 2010), and data have been generated to validate various traditional inactivation treatments applied to almonds, such as roasting (Du et al., 2010) and blanching (Harris et al., in press). With limited exception, untreated raw almonds are no longer sold; "raw-like" almonds are treated with one or more validated processes. New information is also available to support a model that more closely represents contamination levels and processing and handling practices from harvest to consumer.

The objectives of this study were to: (1) assess the risk of salmonellosis associated with almond consumption in North America under current treatment conditions; (2) determine the resilience of the current production system to increases in *Salmonella* prevalence or concentration of *Salmonella* on almonds; (3) assess the impact of treating less than 100% of almonds sold in North America; and (4) evaluate conditions that can predict the estimated number of cases associated with the 2001 outbreak.

2. Materials and methods

2.1. Estimating prevalence and concentration of Salmonella on almond kernels

Prevalence (in 2001–2007 and 2010) and concentrations of *Salmonella* (in 2002–2006) on almond kernels harvested by California growers were measured as described elsewhere (Bansal et al., 2010; Danyluk et al., 2007). Briefly, 13,972 samples of approximately 400 g each were collected upon receipt at seven processing facilities (small, medium, and large handlers) located throughout the almond-growing regions of California. Each sample originated from a different lot. One hundred grams of each 400-g sample were tested for the presence of *Salmonella* through enrichment as described by Danyluk et al. (2007). A total of 137 out of 13,972 samples were positive (0.98%); samples negative by enrichment were considered free from *Salmonella* and were not tested further. A most probable number (MPN) test was performed for a subset of positive samples ($n = 99$) to enumerate *Salmonella*. MPN values were calculated according to the preferred U.S. Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM) method (Garthright & Blodgett, 2003), based on the following dilution series: one 100-g replicate (the positive original enrichment), three 25-g replicates, three 2.5-g replicates, and three 0.25-g replicates.

2.2. Risk assessment model

2.2.1. Framework

A Monte Carlo risk assessment framework was used to estimate the risk of salmonellosis associated with the consumption of almonds. Postharvest almond processing was modeled from receipt at the almond handlers through storage and consumption by consumers. The model assumes that contamination can occur only before almonds reach the processing facility, whereas subsequent treatments and storage can only lower *Salmonella* numbers as almonds are not generally subjected to conditions that could support *Salmonella* growth (Uesugi et al., 2006).

Microbial loads on almond kernels were simulated from handler to consumer ingestion to estimate the health risk associated with almond consumption. At each iteration of the Monte Carlo simulation, a *Salmonella* concentration (MPN/g) was drawn from the prevalence and concentration distributions. The concentration was multiplied by an amount consumed (in grams), drawn randomly from the statistical distribution, to obtain a dose of *Salmonella* (MPN). That dose was then followed from handler to consumer. Model variables are described in the following sections and summarized in Table 1. The number of simulation iterations was set at 100,000 to yield sufficiently stable results (across runs, the arithmetic mean number of cases per year varied up to approximately 24%, the median varied up to approximately 2%, and the geometric mean up to 1.5%). The simulation was implemented using a Latin Hypercube sampling scheme with @Risk software version 5.5.1 (Palisade, Inc., Ithaca, NY, USA).

2.2.2. Prevalence and concentration

Prevalence and concentration data were determined from almonds collected at handler receipt, the simulation starting point. Prevalence data from all survey years (Fig. 1) were pooled together and fit with a beta distribution. Concentration data from MPN tests (Fig. 2A) were pooled and fit with a lognormal distribution (Fig. 2B).

2.2.3. Storage at handler

The estimated total storage time at the handlers was based on information from staff at the Almond Board of California (ABC) and was distributed as follows: (1) the initial 5% of the crop received by the handler is shipped to the market within 5 weeks; (2) 90% of the crop is stored at the handler's facility from 5 to 52 weeks before processing, and almonds are processed at a relatively uniform rate over the year; and (3) the last 5% of the crop is shipped to the market between weeks 52 and 76, partially overlapping with the new crop. Accordingly, the pre-processing storage time was modeled with two triangular distributions (the first between 0 and 2 weeks, the second between 49 and 73 weeks) bracketing a uniform distribution between 2 and 49 weeks. The simulated storage time has 5% probability to be drawn from the first triangular distribution, 90% probability of being drawn from the uniform distribution, and 5% probability of being drawn from the second triangular distribution. After processing, treated almonds are assumed to be stored for a fixed time of 3 weeks before being shipped to retailers.

2.2.4. Salmonella decline during storage

Reduction of *Salmonella* counts on almond kernels was based on published results for *S.* Enteritidis PT 30 (Uesugi et al., 2006), and unpublished data for a six-strain cocktail of *Salmonella* (Enteritidis PT 30, Enteritidis PT 9c, Tennessee, Oranienburg, Anatum, and Montevideo). Reduction trends over time at room temperature were fit with a linear function, with the decimal logarithm of the *Salmonella* concentration as a dependent variable (Table 2). The distribution of rates across seven trials were fit with a normal distribution (Table 1). Similar reduction rates were observed across initial levels of *Salmonella* spanning several orders of magnitude (Table 2); therefore, decline at room temperature was modeled by pooling the results from seven trials into a single distribution.

2.2.5. Treatments to reduce Salmonella concentrations

California-grown almonds sold in North America are required to undergo treatments that guarantee a minimum 4-log reduction in *Salmonella* populations (Federal Register, 2007). The most common treatments are oil roasting, dry roasting, blanching, steam, and propylene

Table 1
Description and statistical distribution of the processes affecting the survival of *Salmonella* on almonds, considered in the risk simulation.

Variable	Description	Distribution	Source
Prevalence of positive samples	Proportion of samples positive after enrichment.	Beta distribution (s + 1, n − s + 1), where s: number of positive samples and n: total number of samples.	Danyluk et al., 2006; Bansal et al., 2010
Concentration in positive samples (MPN/100 g)	Concentrations calculated with the FDA-BAM method.	Lognormal distribution, μ: 1.114, σ: 0.6799.	Danyluk et al., 2006; Bansal et al., 2010
Handler storage time	Total time in handler storage.	Sum of pre-processing and post-processing storage times.	ABC[a] staff
Pre-process storage time	Total time in storage at 24 °C prior to processing.	Triangular (0, 2, 2) weeks with 5% probability, uniform (2, 49) weeks with 90% probability, triangular (49, 49, 73) weeks with 5% probability.	ABC staff
Pre-process reduction	Reduction in *Salmonella* during pre-process storage.	Linear log reduction per day at 23 °C: rate ~ Normal, μ: −0.0078388, σ: 0.00178.	Uesugi et al., 2006; unpublished data.
Treatment	Pasteurization treatments applied to reduce *Salmonella* concentrations.	Oil roasting (21.3% of crop): 127 °C for a time ~ Uniform (3, 15) min. log reduction: $-4.832 \cdot time^{0.494}$. Dry roasting (2.5% of crop): Assume 4-log reduction. Blanching (7.6% of crop): temperature ~ Uniform [88, 99] °C. Time linear from 5 min at 88 °C to 1 min at 99 °C, as a function of temperature. log reduction calculated: $D_{88°C} = 0.39$ min, z value = 35 Cº. Steaming (15.2% of crop): same as blanching. Propylene oxide (53.3% of crop): log reduction: triangular (4.8, 7, 7).	ABC staff; Du et al., 2010; Danyluk et al., 2005; Harris et al., in press, assumptions
Post-process storage time	Duration of storage after processing.	3 weeks fixed length.	ABC staff
Post-process reduction	Reduction in *Salmonella* during post-process storage.	80% of product stored at 23 °C, 20% at 4 °C. Linear reduction at 23 °C: rate ~ Normal, μ: −0.0078388, σ: 0.00178. No reduction at 4 °C.	ABC staff, Uesugi et al., 2006; unpublished data
Retail storage time	Duration of storage at 24 °C after arrival at the retailer and before sale.	Triangular (1 day, 2 weeks, 6 weeks).	ABC staff
Retail reduction	Reduction in *Salmonella* during retail storage.	Linear reduction at 23 °C: rate ~ Normal, μ: −0.0078388, σ: 0.00178.	Uesugi et al., 2006; unpublished data
Consumer storage temperature and storage time	Storage time between purchase and consumption.	43.6% stored at room temperature (23 °C), 24% in the refrigerator (4 °C), 32.4% in the freezer (−20 °C). Distributions of storage time (weeks): Room temp: inverse Gauss (μ: 7.77, λ: 1.67). Refrigerator: Gamma (k: 0.70, σ: 18.6). Freezer: uniform (0, 67.2).	Lee et al., 2011
Consumer reduction	Reduction in *Salmonella* during consumer storage.	Linear reduction at 23 °C: rate ~ Normal, μ: −0.0078388, σ: 0.00178. No reduction at 4 °C and −20 °C.	Uesugi et al., 2006; unpublished data
Serving size	Amount ingested in one eating session.	Pert: min 1 g, max 100 g, mode 25 g.	USDA, 2010; ABC staff
Probability of illness/exposure event	Dose response model linking dose ingested to probability of illness	β-Poisson model, α: 0.1324, β: 51.45.	FAO, 2002
Total almond consumption	Amount delivered to North American markets (2009 data).	458 million lbs = 208 million kg.	ABC staff
Number of exposure events per year	Number of individual almond servings consumed in a year.	Calculated: Total almond consumption divided by serving size.	
Illness cases per year	Number of illnesses/year due to consumption of contaminated almonds.	Calculated with the normal approximation to the binomial distribution ~ N(μ: n·p, σ: n·p·(1 − p)), where n is number of positive servings per year, and p is probability of illness per positive serving.	

[a] ABC, Almond Board of California.

oxide. Based on information from ABC (Birmingham, 2010), the 2009 almond crop delivered to North American markets totals 208 million kg. Approximately 149 million kg of that crop will reach consumers directly, and the crop percentages estimated to undergo different treatments are provided in Table 1. Another 59 million kg of almonds are shipped untreated to other processors who are responsible to likewise provide a minimum 4-log reduction of *Salmonella* before or while incorporating the nuts into other products, such as granola bars, cereals, and candy. For the purposes of this assessment it was assumed that this portion of the crop is partitioned among different treatments in the same proportions as almonds reaching consumers directly (percentages in Table 1 refer to the portion of the crop that reaches consumers directly).

2.2.6. Storage by retailers

Almonds are generally stored by retailers for less than 6 weeks. Based on ABC information, retailer storage time was modeled using a triangular distribution (Table 1).

2.2.7. Storage by consumers

The probability of consumers storing almonds at different temperatures and the distribution of storage times were derived from survey results by Lee et al. (2011) (Fig. 3). Steamed, blanched, or "raw-like" almonds treated with propylene oxide were assumed to be stored at either freezer (−20 °C), refrigerator (4 °C) or room (23 °C) temperatures. Roasted almonds were assumed to be stored at room temperature.

2.2.8. Consumption patterns

The amount of almonds consumed per exposure event (serving amount) was assumed to range from a minimum of 1 g (e.g., in a granola or candy bar) to a maximum of 100 g (large out-of-hand serving), with a mode value of 25 g that approximates the USDA-recommended serving size of 28 g (1 oz) (USDA, 2010), and was modeled with a pert distribution. It was also assumed that consumers eat almonds as purchased, without further processing.

2.2.9. Dose–response relationship

The dose–response relationship linking the amount of *Salmonella* ingested to the probability of illness, which was adopted in this study, was derived by FAO/WHO using outbreak data (FAO, 2002). Percentiles of the curve parameters (2.5% and 98%) were used to estimate "low" and "high" confidence bands for the relationship, respectively.

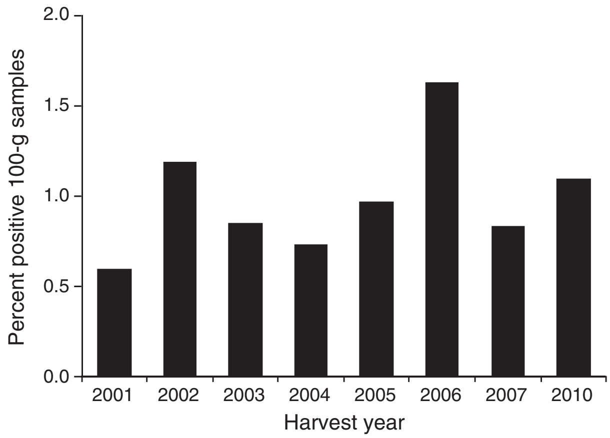

Fig. 1. Prevalence of positive 100-g samples, by harvest year. Modified from Danyluk et al. (2007).

2.2.10. Risk outcomes

The primary outcome of the risk assessment was the probability of illness, expressed as number of cases per billion exposure events (servings). We estimated the number of cases per year due to almond consumption in the North American market using the normal approximation to the binomial distribution as follows: at each iteration we calculated p = probability of illness per serving, estimated for positive servings only, and n = number of positive servings per year in North America; the number of cases per year was then drawn from a normal distribution of $\mu = np$ and $\sigma = np(1-p)$. The number of servings per year was calculated by dividing the total North American consumption (208 million kg) by the amount consumed per serving (pert distribution, see Table 1). The geometric mean of the incidence distribution was calculated by estimating n = mean of the distribution of log(cases per year), and computing 10^n.

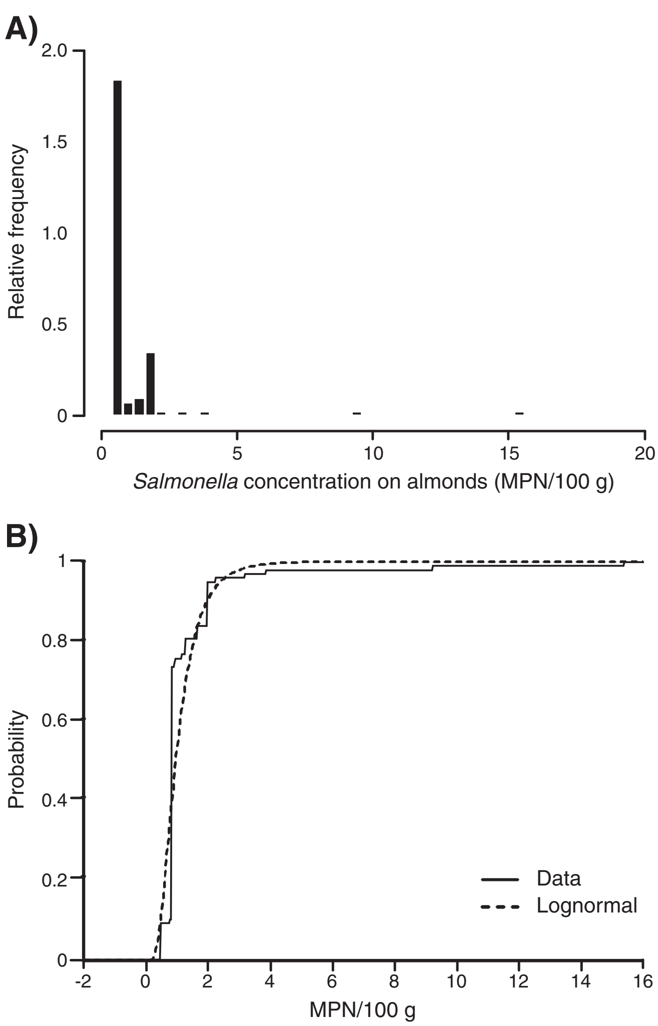

Fig. 2. *Salmonella* concentrations on almond kernels, assessed by MPN method in samples positive to enrichment. (A) Relative frequency histogram. (B) Cumulative frequency, fit with a Lognormal distribution.

2.3. Comparison with previous almond risk assessment

The current model differs from the previously published almond risk assessment (Danyluk et al., 2006) in both structure and key variables. To assess the impact of model variables on risk outcome, the previously-published model was run with original and with updated variables based on new information used for the current model (Table 1). The variables updated were total amounts consumed, prevalence, concentration, assumed storage times and temperature distributions at all steps, and decline rates for *Salmonella*. The distinction between high and low concentrations in the 2006 model was maintained, with a probability of 1 in 64 of high concentration, and high concentrations following a triangular distribution with a mode value of 562 MPN/100 g, minimum of 126 MPN/100 g, and maximum of 2398 MPN/100 g.

Furthermore, to assess the impact of model structure on risk outcome, the new model was run with Danyluk et al. (2006) variables as applicable. In this case, only the “low” concentration levels observed during the almond survey were considered (Danyluk et al., 2006).

2.4. Scenario exploration

2.4.1. Increased prevalence

To test the sensitivity of the risk model to shifts in *Salmonella* prevalence, risk was estimated while shifting the percent of positive samples to fixed values between 1% and 100%, while all other variables were kept as in the baseline scenario (Table 1).

2.4.2. Increased concentration

To test the sensitivity of the risk model to shifts in *Salmonella* concentration, risk was estimated while setting the mean of *Salmonella* concentrations to between 5 and 120 MPN/100 g, while the variance of the concentration distribution and all other variables were kept as in the baseline scenario (Table 1).

2.4.3. Proportion of crop pasteurized

Assuming that the sale of raw untreated almonds was still legal, the risk associated with consuming up to 10% of the domestic crop untreated was estimated. The remaining portion of the crop was assumed to be treated as described in Section 2.2.5.

2.4.4. Outbreak scenario

Salmonella counts on almond kernels recalled during the 2001 outbreak were derived from Danyluk et al. (2006, 2007) to compare risk during outbreak conditions to baseline risk. A prevalence of 65% (using the FDA-BAM enrichment method) was determined from enrichments of 100-g samples taken from 50 recalled boxes of almonds representing four different lots (Table 1 in Danyluk et al., 2007). In July 2001, MPN were determined for the 26 positive boxes and five of these boxes were retested in August 2001. These data were used in combination to calculate levels of *Salmonella* of 3.4 MPN/100 g (21 samples), 5.6 MPN/100 g (1 sample), and 7.9 MPN/100 g (4 samples). As tests were conducted several months after the almonds were shipped, the original concentration for shipments received at the retailer was back-calculated from early August 2001 (test date) to mid February 2001 (initial shipment dates) assuming that samples had

Table 2
Decline of *Salmonella* on almond kernels at different temperatures, modeled with linear and exponential functions.

Temperature (°C)	Serotype	Trial duration (days)	Inoculum (log CFU/g)	Linear model[a]		Exponential model[b]			Reference
				Slope (log CFU/month)	p-value[c]	Span (log CFU)	k (1/days)	p-value (k)	
23	PT 30	171	7.1	−0.32	<0.001	2.3	0.0084	0.020	Uesugi et al., 2006
	PT 30	336	8.5	−0.21	<0.001	2.4	0.0078	<0.001	Unpublished
	PT 30	559	7.9	−0.24	<0.001	7.6	0.0015	<0.001	Uesugi et al., 2006
	PT 30	161	7.3	−0.16	<0.001	0.84	0.029	0.060	Uesugi et al., 2006
	PT 30	161	4.8	−0.21	<0.001	1.1	0.040	0.010	Uesugi et al., 2006
	PT 30	161	3.1	−0.25	<0.001	1.3	0.021	0.080	Uesugi et al., 2006
	PT 30	161	1.2[d]	−0.20	<0.001	1.0	0.028	0.65	Uesugi et al., 2006
	Cocktail[e]	172	5.8	−0.29	<0.001	1.6	0.017	0.0080	Unpublished
4	PT 30	171	7.2	−0.052	0.0030	0.40	0.13	0.10	Uesugi et al., 2006
	PT 30	336	8.5	−0.039	<0.001	0.50	0.0080	0.0020	Unpublished
	PT 30	559	7.9	−0.018	<0.001	0.44	0.021	0.0030	Uesugi et al., 2006
	Cocktail[e]	172	5.8	−0.019	0.67	NA	NA	NA	Unpublished
−20	PT 30	559	7.9	0.0027	0.45	0.48	0.18	0.048	Uesugi et al., 2006
	Cocktail[e]	172	5.8	−0.043	<0.001	NA	NA	NA	Unpublished

[a] The linear model was expressed as: log(concentration) = log (initial concentration) + rate·days.
[b] The fitted exponential curve was expressed as: log(concentration) = plateau + span·exp(−k·days).
[c] The 95% confidence intervals never included zero, except for the last trial at 4 °C, and the first trial at −20 °C.
[d] The results of this trial were excluded from the calculation of the average reduction rate at 23 °C for risk assessment purposes, due to several non-detects.
[e] The *Salmonella* cocktail included the following serotypes: Enteritidis PT 30, Enteritidis PT 9c, Tennessee, Oranienburg, Anatum, and Montevideo (unpublished).

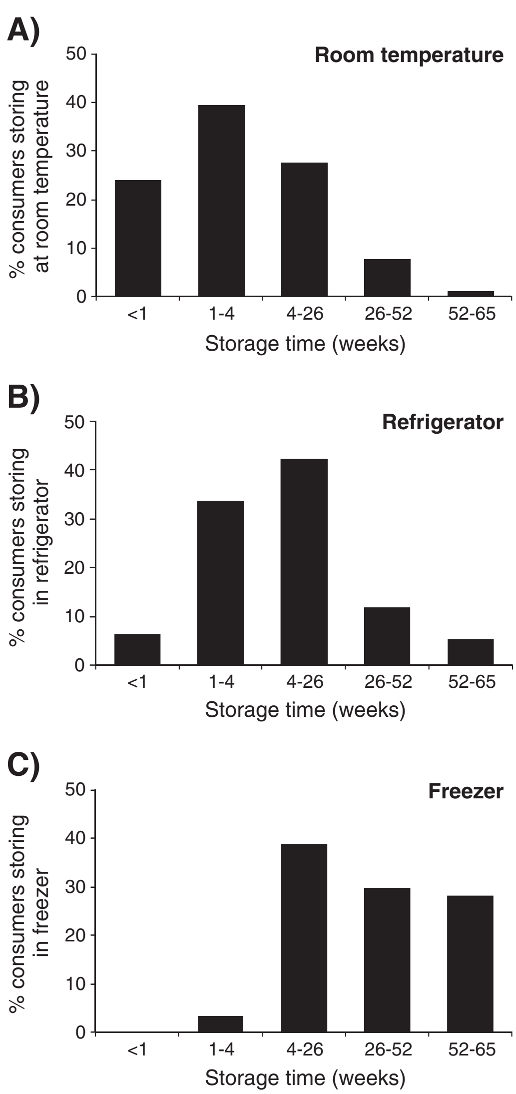

Fig. 3. Storage time at consumers' homes, by storage temperature: (A) room, (B) refrigerator, and (C) freezer temperatures. In the model, these temperatures were assumed to be 23 °C, 4 °C, and −20 °C, respectively. Note that the x-axis categories are not uniform time intervals. (adapted from Lee et al., 2011).

been stored at room temperature for 6 months. The cumulative frequency of the 26 back-calculated concentration data was fit with a lognormal distribution (μ = 117.6 MPN/100 g, σ = 37.0 MPN/100 g).

Several outbreak scenarios were simulated by considering different concentration distributions and raw almond amounts consumed. Prevalence was assumed to be 65%, as observed in the recalled samples tested. The total amount of almonds consumed was assumed to range from 2000 kg to 30,000 kg, which was the amount shipped from California to retail establishments associated with the 2001 outbreak between February 16 and April 4 minus the documented amount recovered (1000 22.7-kg boxes) during the recall that was initiated April 12 (Isaacs et al., 2005; Uesugi & Harris, 2006).

Only the second phase of the 2001 outbreak between approximately February 25 and the end of June was considered, as such cases were more likely associated with the almond lots that were recalled and tested. The number of cases of salmonellosis occurring between February 25 and the end of June 2001 was estimated as 2800, which corresponds to 29 times the 95 cases reported (Isaacs et al., 2005), accounting for the CDC under-reporting factor of 1 case reported every 29.3 (Scallan et al., 2011).

The simulation was initiated at the retail level, where almonds were assumed to be stored at room temperature for a uniform duration between 0 and 12 weeks (six weekly shipments were made from February 16 to April 4). Consumers were assumed to store raw almonds as described in Section 2.2.7, but such that the cumulative storage time by retailer and consumer would be no more than 4 months (roughly the maximum time that almonds could have been stored by retailer or consumer, from mid February until mid June). Simulated storage times beyond 4 months were ignored. Additional simulations were conducted to assess if treatments achieving up to 4-log reduction in *Salmonella* concentrations could have prevented the outbreak. The model was run three times at 10,000 simulation iterations (each model run used a different seed). The intermediate result of these three runs was reported.

3. Results

3.1. Baseline risk under current treatment practices

The estimated mean risk of salmonellosis due to consumption of almonds, under the current mandatory requirement to treat almonds to achieve a 4-log reduction of *Salmonella*, is well below a mean of one case per year (Table 3). The number of servings consumed per year in

Table 3
Scenario exploration. Effect of change in prevalence, concentration, or portion of untreated crop consumed on the estimated risk of illness.

Prevalence	Mean number of cases per billion servings[a]	Arithmetic mean	Geometric mean
		Number of cases per year	Number of cases per year
Baseline[b]	0.0084	0.060	0.00016
Prevalence (%)			
3	1.9	0.14	0.00049
10	5.1	0.46	0.0016
25	13	1.6	0.0041
65	33	3.5	0.011
Concentration (MPN/100 g)			
5	0.018	0.24	0.00052
25	0.14	1.1	0.0019
80	0.32	3.8	0.0051
120	0.72	5.1	0.0074
Percent untreated			
0.02	0.0026	0.90	0.00016
0.05	0.0073	2.8	0.00017
5	38	340	0.00038
10	96	680	0.00090

[a] The number of servings consumed per year in North America was estimated as 6.6 billion.
[b] The baseline scenario is based on Table 1.

the North American market was estimated as 6.6 billion. The mean probability of illness per serving, based on all samples analyzed, was estimated as approximately 0.0084 cases per billion servings (standard deviation: 0.35 cases per billion servings). The estimated incidence of salmonellosis presented a highly skewed distribution (Fig. 4), with an arithmetic mean of 6.0 cases per 100 years (one case every 17 years on average, standard deviation: 180 cases per 100 years, skew: 11,300 cases per 100 years), and a geometric mean of 1.6 cases per 10,000 years (Table 3). The sensitivity analysis conducted on the baseline scenario indicated that the main variables affecting the risk outcome were treatment method and storage time at handler (Fig. 5).

3.2. Comparison with the risk assessment by Danyluk et al. (2006)

The current model is different from Danyluk et al. (2006) in both structure and key assumptions. The 2006 model with updated (2010) variables predicted risks that were 2.6-fold higher compared to the 2006 model run with the 2006 variables (Table 4). A 5-fold increase

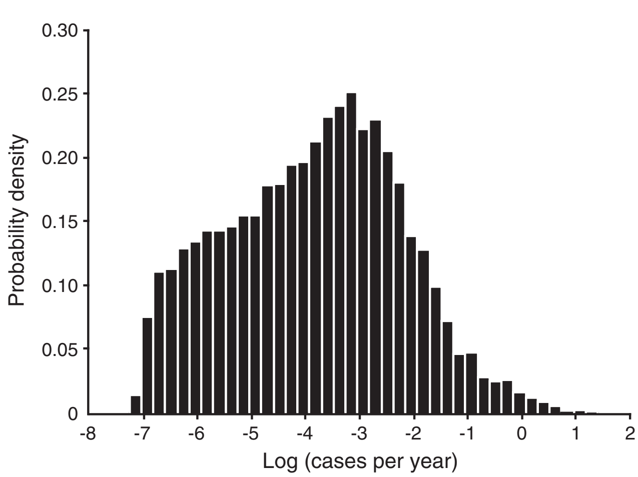

Fig. 4. Number of salmonellosis episodes per year, as decimal logarithm, under the current production and treatment scenario.

was observed when comparing the new model, run with 2010 variables to that run with 2006 variables. Changes in model structure from Danyluk et al. (2006) to the new model resulted in a lower arithmetic mean number of cases per year of about an order of magnitude, although they did not significantly affect the geometric mean.

3.3. Scenario exploration: shift in prevalence

An increase in *Salmonella* prevalence from the observed level of approximately 1% up to 10% led to an increase in the arithmetic mean number of cases of about an order of magnitude, while remaining below one case per year (Table 3). The arithmetic mean number of cases reached levels of approximately one case per year (standard deviation: 47 cases per year) at a prevalance of 25%. The predicted geometric mean number of cases per year remained low: four cases per 1000 years at a prevalence of 25%. The sensitivity analysis indicated that the observed prevalence distribution was not significantly correlated with the risk outcome.

3.4. Scenario exploration: shift in average concentration

A shift in average concentrations between the observed levels (mean 1.1 MPN/100 g) and 120 MPN/100 g led to an increase in the mean number of cases per year of approximately two orders of magnitude, from 0.060 to 5.1 cases per year (standard deviation: 100 cases per year) (Table 3). The sensitivity analysis indicated that the observed concentration distribution was only slightly correlated (Spearman Rank correlation coefficient = 0.14) with the risk outcome, expressed as probability of illness (Fig. 5).

3.5. Scenario exploration: percentage of the crop not treated

The simulated consumption of 0.05% of raw untreated almonds led to an increase in the mean number of cases per year by almost two orders of magnitude compared to the baseline (where the entire crop was treated), from 0.060 cases per year to 2.8 cases per year (standard deviation: 570 cases per year) (Table 3). Increasing the percentage of untreated almonds to 5% led to a further increase of approximately two orders of magnitude to an average of 340 cases per year. The risk distribution was distinctly bimodal; the geometric mean of the number of cases per year remained below one case per year even when 10% of the crop was left untreated.

3.6. Simulated 2001 outbreak

Several combinations of *Salmonella* concentrations and amounts consumed predicted a total number of cases close to 2800, corresponding to the number reported during the outbreak corrected for reporting bias (Table 5). With a prevalence of 65% and within the range of concentrations of *Salmonella* compatible with available information about the 2001 outbreak, between 2000 and 4000 kg of consumed raw almonds could have caused the outbreak (Table 5). Several other scenarios, either smaller amounts at higher concentration levels, or larger amounts at lower concentrations, could predict an outbreak of the magnitude observed. Among such scenarios, both amounts as small as 1000 kg contaminated at an average level of 500 MPN/100 g and 30,000 kg contaminated at mean concentrations as low as 10 MPN/100 g predicted approximately 3000 cases (data not shown).

If the current regulations had been in place at the time of the outbreak and a 4-log treatment had been applied, the number of predicted cases would have dropped to below one in most of the scenarios we evaluated (Table 5). For example, at the concentration levels and distribution observed in the recalled samples, and with a consumed amount of 4000 kg, the estimated number of cases would have decreased from approximately 3400 without any treatment to

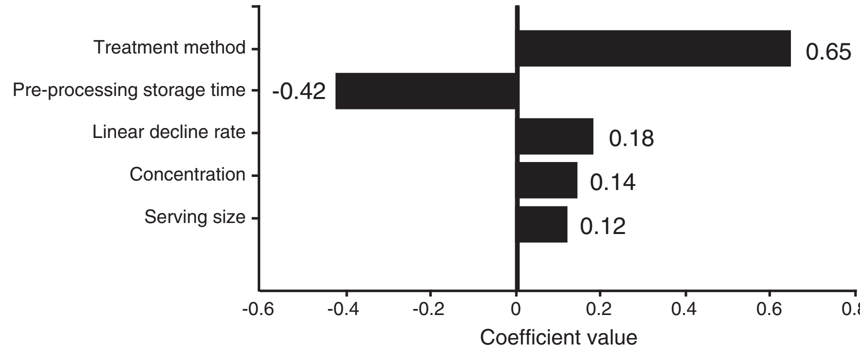

Fig. 5. Sensitivity analysis for the baseline risk assessment model. Values in the plot refer to the Spearman correlation coefficient, with probability of illness per positive serving as the outcome variable.

<1 case. At a uniform concentration of 500 MPN/100 g and a consumed amount of 2000 kg, the number of cases estimated would have dropped from 3900 to <1.

4. Discussion

The current risk of salmonellosis from consumption of U.S. almonds in North America is low, based on estimates presented in this study. The model is likely conservative, as additional *Salmonella* reductions that would be realized at the consumer level, such as roasting, cooking, or baking, were not taken into consideration (Lee et al., 2011). Our simulations predict that the probability of becoming ill from a single serving of almonds is about eight in one trillion. The current required treatments applied to reduce *Salmonella* levels are the primary reason for this low predicted risk. The simulations support the idea that current treatments are effective in preventing almond-related salmonellosis, either epidemic or sporadic, compared to the situation before the introduction of mandatory treatment in the U.S. (Federal Register, 2007). While no outbreaks have been reported in North America since the introduction of the regulation, a direct comparison of sporadic incidence before and after the regulation is not possible, since sporadic cases of salmonellosis are not usually reported nor are the associated foods identified. Many treatments, such as oil roasting and blanching, achieve significantly more than the 4-log minimum reduction of *Salmonella* (Table 1). Even when a fixed 4-log reduction was substituted for the current treatments in the model (results not shown) the predicted arithmetic mean number of cases was reduced to 0.71 per year; a fixed 3-log reduction predicted a mean of 7.2 cases per year.

Almonds are the only nut or seed with a mandatory *Salmonella* reduction program. However, in the aftermath of a large peanut butter salmonellosis outbreak (CDC, 2009) and a separate recall of pistachios for the presence of *Salmonella* in 2009 (FDA, 2009c), the U.S. Food and Drug Administration issued guidance documents for peanuts and pistachios that recommend applying a kill step capable of reducing *Salmonella* levels by a minimum of 5 log (FDA, 2009a, 2009b). Little is known about natural contamination rates in other nuts. If prevalence, levels, and survival of *Salmonella* are determined to be similar to or less than those found in almonds, performance standards of less than 5 log may provide adequate reduction of risk.

Risk outcomes, expressed both as the probability of illness per serving or the mean number of cases per year, present a high variance and are significantly right-skewed, highlighting the fact that while the vast majority of servings are *Salmonella*-negative and carry no risk, and the majority of *Salmonella*-positive almond servings carry very low risk, a small proportion of servings carry a high likelihood of causing illness. In a right-skewed distribution, the arithmetic mean is strongly affected by the higher right-tail values. Therefore, conclusions should not be based solely on average risk metrics, but consider the entire distribution.

Risk outcomes for the baseline scenario were most affected by the treatment method, followed by storage time at handler before processing, and were marginally affected by the distribution of the linear decline rate, the serving size, and concentration levels (Fig. 5). The importance of treatment to predicted model outcomes is explained by the fact that different pasteurization methods achieve very different reduction levels, from the minimum of 4 logs prescribed by the regulations up to the greater than 10-log reductions potentially achieved by standard commercial oil roasting (Du et al., 2010). Predicted outcomes were also correlated to the duration of storage at room temperature at handlers before processing because, in the model, it is only at room temperature (about 23 °C) that *Salmonella* is observed to decline on almonds (Table 2), and storage of almonds at handlers is most often the longest time that almonds are kept at room temperature. Conversely, almonds are usually stored at retail for only a few weeks, and consumers will refrigerate or freeze almonds that they want to store for more than a short time (Lee et al., 2011). It is also likely that some almonds are stored at temperatures above 23 °C or between 4 and 23 °C for various lengths of time, depending on location and time of the year. Sufficient information on the distribution of almonds and the reduction of *Salmonella* at other storage temperatures is not available at this time to assess the impact on risk.

The model predicts that current almond treatments will prevent salmonellosis even with increases in *Salmonella* prevalence or concentration significantly over that observed in surveys. Increasing either the prevalence to approximataly 25% or average concentration to 25 MPN/100 g, was necessary to raise the predicted mean incidence above one case per year. Separately increasing the prevalence for the entire crop to 65% or concentration levels to 120 MPN/100 g (measured or predicted for almonds involved in the 2001 outbreak) predicted a mean of 3.5 and 5.1 cases per year, respectively.

Table 4
Comparison between previous almond risk assessment (Danyluk et al., 2006) and updated model.

Model[a]	Arithmetic Mean	Geometric mean
	Number of cases/year	Number of cases/year
Danyluk 2006	1000	6.1
Danyluk 2006 with updated variables[b]	2600	24
New model with 2006 variables[c]	72	8.0
New model with updated variables[d]	360	31

[a] For comparison purposes, all models in this table assume the source of exposure to be 5% of the crop, untreated, as in Danyluk et al. (2006).
[b] A model with structure as in Danyluk et al. (2006) was run with updated variables as of 2010.
[c] A model with the structure presented in this paper was run with variables as in Danyluk et al. (2006).
[d] A model with the structure presented in this paper was run with updated variables as of 2010 (baseline scenario), but considering only 5% of the crop, untreated, as the source of exposure.

Table 5
Estimated number of salmonellosis cases, based on *Salmonella* concentration scenarios in the range inferred from testing almonds recalled during the 2001 outbreak[a].

Salmonella mean concentration (MPN/100 g)	Amount of almonds consumed (kg)							
	30,000		10,000		4000		2000	
	Untreated	Treated[b]	Untreated	Treated	Untreated	Treated	Untreated	Treated
500[c]	62,000	12	23,000	4.8	8900	1.80	3900	0.82
200[c]	30,000	5.4	12,000	1.8	4600	0.75	2400	0.33
120[c], estimated distribution[d]	24,000	3.2	8200	1.1	3400	0.44	1500	0.21
50[c]	12,000	1.4	3800	0.43	1600	0.20	810	0.087
20[c]	5000	0.52	1700	0.16	690	0.072	330	0.034

[a] The number of cases reported between February 25 and the end of June (2001) was 95 (Isaacs et al., 2005). It is estimated that one case in 29.3 is reported (Scallan et al., 2011), for a total number of actual cases in the order of 2800.
[b] Treatment accomplishing a fixed 4-log reduction was assumed.
[c] Approximately 55,000 kg of almonds arrived at retailers between February 16 and April 4, 2001, when the second spike of the outbreak occurred and before almonds were recalled on April 12. Prevalence was assumed to be 65%, as in the samples tested (Danyluk et al., 2007); 30,000 kg correspond to the entire 55,000 kg shipped minus 1000 boxes of 22.7 kg recalled (minimum documented amount recalled) (Danyluk et al., 2007). Results in this table are based on 10,000 model iterations.
[d] Based on 26 positive samples from recalled almonds tested in 2001, the concentration of positive almonds was modeled with a lognormal distribution ($\mu = 117.6$ MPN/100 g, $\sigma = 37.0$ MPN/100 g).

The conditions that resulted in the 2001 outbreak are believed to be an unusual combination of both high prevalence and high concentration, at least in a portion of the shipments that reached the North American market between February and April 2001. It is unknown what led to such conditions (Isaacs et al., 2005), and as such it is unclear if the causes of the high level contamination have been addressed throughout the industry. In their model, Danyluk et al. (2006) assigned a high concentration (average 500 MPN/100 g) to 1.6% (1 in 64) of positive samples. However, neither high concentrations of *Salmonella* nor high prevalence were incorporated into the current baseline model as they were assumed to be rare events. At the 65% prevalence observed for almonds recalled in 2001, several combinations of concentration levels and amount consumed could predict the number of cases estimated during the second phase of the 2001 outbreak, although it is less likely that a small batch (e.g., 1000 kg) was responsible for the entire outbreak, since cases were observed over several months and in several geographical locations (Isaacs et al., 2005). If the almonds associated with the outbreak had been treated to a minimum 4-log reduction as currently required, the model predicted that the outbreak would have been significantly reduced or completely prevented. Since the conditions that led to the outbreak are currently unknown, and therefore impossible to control, applying a uniform treatment is the most protective solution at this time.

This risk simulation is based on the best current knowledge on almond postharvest processing, and both uncertainty in the input variables and intrinsic variability in the processing and handling conditions contribute to the high variance of the risk outcomes. In particular, since *Salmonella* reduction due to different treatment methods and the distribution of storage time at room temperature strongly affect risk outcomes, a more detailed characterization of these variables would increase the precision of the model results. Uncertainty in the dose–response function, not accounted for in this model, could also have major impacts on risk results. Results are also affected by model assumptions. In particular, the distribution of the probability of illness, here expressed as number of cases per billion servings, was estimated by simulating exposure to 100,000 individual servings. Incidence, however, was calculated in a simplified fashion by treating each simulated year as the outcome of a binomial distribution of parameters p (p: probability of illness per positive serving) and n (n: number of positive servings per year), thus assuming that the probability of illness was the same for each serving. This outcome is thus less precise than the probability of illness per serving, and should be considered with caution when deriving conclusions, especially at the right tail of the distribution. A major assumption of the model is that contamination can occur only before or during harvest. While *Salmonella* levels at the handler's receipt are well documented, it is recognized that under certain conditions the product might also become contaminated during handling or post processing. Assessing these contamination routes was outside the scope of this study.

In conclusion, this new model predicted that the risk of salmonellosis from consuming U.S. almonds sold in North America was at exceptionally low levels (eight cases per trillion servings on average). The current almond production system is predicted to prevent illness even with substantial increases in prevalence and concentration over that determined in surveys. However, failing to treat even a small portion of the crop raised the predicted risk significantly above the baseline. The conditions leading to the 2001 outbreak appear to be unusual but demonstrate that relatively small amounts of almonds contaminated at both high prevalence and concentration can lead to an outbreak. However, even under these circumstances, a treatment achieving a 4-log reduction could have dramatically reduced the magnitude of or prevented the outbreak.

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References

(ABC) Almond Board of California (2007). Pasteurization treatments. Available from: http://www.almondboard.com/Handlers/Documents/Pasteurization-Treatments.pdf. Accessed February 2011.
(ABC) Almond Board of California (2010). The 2010 almond almanac. Available from: http://www.almondboard.com. Accessed Jan 2011.
Bansal, A., Jones, T. M., Abd, S. J., Danyluk, M. D., & Harris, L. J. (2010). Most-probable-number determination of *Salmonella* levels in naturally contaminated raw almonds using two sample preparation methods. *Journal of Food Protection*, *73*(11), 1986–1992.
Birmingham, T. (personal communication, 2010). Almond production and treatments. Available from: the author at ljharris@ucdavis.edu.
(CDC) Centers for Disease Control and Prevention (2004). Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds—United States and Canada, 2003–2004. *MMWR Weekly*, *53*(22), 484–487.
(CDC) Centers for Disease Control and Prevention (2009). Multistate outbreak of *Salmonella* infections associated with peanut butter and peanut butter-containing products—United States, 2008–2009. *MMWR Weekly*, *58*(4), 85–90.
Danyluk, M. D., Harris, L. J., & Schaffner, D. W. (2006). Monte Carlo simulations assessing the risk of salmonellosis from consumption of almonds. *Journal of Food Protection*, *69*(7), 1594–1599.
Danyluk, M. D., Jones, T. M., Abd, S. J., Schlitt-Dittrich, F., Jacobs, M., & Harris, L. J. (2007). Prevalence and amounts of *Salmonella* found on raw California almonds. *Journal of Food Protection*, *70*(4), 820–827.
Danyluk, M. D., Nozawa-Inoue, M., Hristova, K. R., Scow, K. M., Lampinen, B., & Harris, L. J. (2008). Survival and growth of *Salmonella* Enteritidis PT 30 in almond orchard soils. *Journal of Applied Microbiology*, *104*(5), 1391–1399.

Danyluk, M. D., Uesugi, A. R., & Harris, L. J. (2005). Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *Journal of Food Protection*, *68*, 1613–1622.

Du, W. -X., Abd, S. J., McCarthy, K. L., & Harris, L. J. (2010). Reduction of *Salmonella* on inoculated almonds exposed to hot oil. *Journal of Food Protection*, *73*, 1238–1246.

Federal Register (2007). Almonds grown in California; outgoing quality control requirements. *Federal Register*, *72*, 15021–15036 Available from:http://www.gpoaccess.gov/fr/retrieve.html. Accessed February 2011.

(FAO) Food and Agriculture Organization of the United Nations (2002). Risk assessments of *Salmonella* in eggs and broiler chickens. Available from:ftp://ftp.fao.org/docrep/fao/005/y4392e/y4392e00.pdf. Accessed February 2011.

Garthright, W. E., & Blodgett, R. J. (2003). FDA's preferred MPN methods for standard, large or unusual tests, with a spreadsheet. *Food Microbiology*, *20*(4), 439–445.

Harris, L. J., Uesugi, A. R., Abd, S. J., & McCarthy, K. L. (in press). Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds in hot water treatments. *Food Research International*.

Isaacs, S., Aramini, J., Ciebin, B., Farrar, J. A., Ahmed, R., Middleton, D., et al. (2005). An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis. *Journal of Food Protection*, *68*(1), 191–198.

Ledet Müller, L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Plym Forshell, L., et al. (2007). Cluster of *Salmonella* Enteritidis in Sweden 2005–2006—Suspected source: Almonds. *Eurosurveillance*, *12*(6), 153–155.

Lee, L. E., Metz, D., Giovanni, M., & Bruhn, C. M. (2011). Consumer knowledge and handling of tree nuts: Food safety implications. *Food Protection Trends*, *31*(1), 18–27.

Little, C. L., Rawal, N., de Pinna, E., & McLauchlin, J. (2010). Survey of *Salmonella* contamination of edible nut kernels on retail sale in the UK. *Food Microbiology*, *27*, 171–174.

Palumbo, M., Beuchat, L. R., Danyluk, M. D., & Harris, L. H. (2011). Recalls of tree nuts and peanuts in the US, 2001 to present. Available from:http://ucfoodsafety.ucdavis.edu/files/26473.pdf Accessed February 2011.

Podolak, R., Enache, E., Stone, W., Black, D. G., & Elliott, P. H. (2010). Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *Journal of Food Protection*, *73*, 1919–1936.

Rocourt, J., BenEmbarek, P., Toyofuku, H., & Schlundt, J. (2003). Quantitative risk assessment of *Listeria monocytogenes* in ready-to-eat foods: The FAO/WHO approach. *FEMS Immunology and Medical Microbiology*, *35*(3), 263–267.

Scallan, E., Hoekstra, R. M., Angulo, F. J., Tauxe, R. V., Widdowson, M. -A., Roy, S. L., et al. (2011). Foodborne illness acquired in the United States—Major pathogens. *Emerging Infectious Diseases*, *17*(1), 7–15.

Schlundt, J. (2000). Comparison of microbiological risk assessment studies published. *International Journal of Food Microbiology*, *58*(3), 197–202.

Skovgaard, N. (2007). Risk assessment of *Vibrio vulnificus* in raw oysters, Interpretative summary and technical report. *International Journal of Food Microbiology*, *119*(3), 358–359.

(USDA) U.S. Department of Agriculture (2010). Protein foods. Available from:http://www.mypyramid.gov/pyramid/meat.html. Accessed December 2010.

(FDA) U.S. Food and Drug Administration (2009a). Guidance for industry: Measures to address the risk for contamination by *Salmonella* species in food containing a pistachio-derived product as an ingredient; draft guidance. Available from:http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm169160.htm. Accessed February 2011.

(FDA) U.S. Food and Drug Administration (2009b). Guidance for industry: Measures to address the risk for contamination by *Salmonella* species in food containing a peanut-derived product as an ingredient. Available from:http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm115386.htm. Accessed February 2011.

(FDA) U.S. Food and Drug Administration (2009c). Pistachio product recalls. Available from:http://www.fda.gov/Safety/Recalls/MajorProductRecalls/Pistachio/default.htm. Accessed February 2011.

Uesugi, A. R., Danyluk, M. D., & Harris, L. J. (2006). Survival of *Salmonella* Enteritidis phage type 30 on inoculated almonds stored at −20, 4, 23, and 35 °C. *Journal of Food Protection*, *69*(8), 1851–1857.

Uesugi, A. R., Danyluk, M. D., Mandrell, R. E., & Harris, L. J. (2007). Isolation of *Salmonella* Enteritidis phage type 30 from a single almond orchard over a 5-year period. *Journal of Food Protection*, *70*(8), 1784–1789.

Uesugi, A. R., & Harris, L. J. (2006). Growth of *Salmonella* Enteritidis phage type 30 in almond hull and shell slurries and survival in drying almond hulls. *Journal of Food Protection*, *69*(4), 712–718.

Ward, L., Brusin, S., Duckworth, G., & O'Brien, S. (1999, 18 Marchh). *Salmonella* java phage type Dundee—Rise in cases in England: update. *Eurosurveillance Weekly*, *3*(12), 1435.