Tools for Evaluating the Human Health Risks Associated with Microbial Contamination of Apples

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Abstract

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This thesis incorporates laboratory methods, risk assessment modeling, and policy analysis to address food safety issues related to microbial contamination of apple crops. Chapter 1 provides an introduction to microbial food safety issues and apple production in the United States. It also broadly discusses the conceptual underpinnings for the three subsequent chapters and specific aims of the thesis. More robust background information is provided within the respective chapters. Chapter 2 provides an analysis of a novel method for the quantification of *E. coli* on apple surfaces. Chapter 3 aims to evaluate the health risks of apple contamination under varying harvest and cleaning conditions, utilizing three quantitative microbial risk assessment models. The policy relevance of the study is discussed in Chapter 4, incorporating history of food safety policy, broad policy process frameworks, and a discussion of the relevant policy development stages in which the study findings may apply. As a whole, this thesis is intended to provide an interdisciplinary approach to issues surrounding apple production and consumption.

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Acknowledgments

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1. Introduction

Foodborne illnesses are responsible for a significant amount of morbidity and mortality in the United States; 47.8 million cases are estimated to occur annually (Scallan et al. 2011a, 2011b). This burden of foodborne illness has significant economic impacts; the aggregated costs associated with foodborne illnesses are estimated at between $51.0 billion and $77.7 billion annually, due to medical expenses, productivity losses, and illness-related mortality (Scharff 2012). Over the past decades, fresh produce has been increasingly recognized as a major source of foodborne illness. Outbreaks linked to produce accounted for an increasing proportion of reported foodborne outbreaks associated with a known food item, rising from 0.7% in the 1970s to 6% in the 1990s (Sivapalasingam et al. 2004). From 1998-2008, 46% of foodborne illnesses in the United States were attributed to consumption of produce (Painter et al. 2013).

Several factors have be identified to explain the increase in produce-associated foodborne illnesses. Per capita consumption of fresh produce has increased substantially in the U.S. since the 1970s (Pollack 2001). The increasing number of produce-related outbreaks can also be attributed to larger scale production and more efficient distribution of fresh produce (Olaimat and Holley 2012). Additionally, as agriculture becomes more intensive, produce fields may be situated in closer proximity to animal production areas, and there may be increasing connectivity between wild animals, farm animals, and produce farms (Lynch et al. 2009). Yet, no single risk factor can explain the array of outbreaks associated with varying food items, production environments, processes, and pathogens; rather, foodborne illness outbreaks are likely due to a convergence of dynamic factors (Mandrell 2009).

The increasing number of foodborne illnesses associated with fresh produce has resulted in a growing concern about the production and distribution of fruits and vegetables. Diverse

stakeholders, including farmers, trading companies, consumer organizations, government authorities, and universities have prioritized the issue of bacterial pathogens on fresh produce (Van Boxstael et al. 2013). In response to growing concern over the safety of fresh produce, food safety policies have been increasingly focused on interventions that prevent human pathogens from contaminating fruits and vegetables. The passage of the Food Safety Modernization Act (FSMA) in 2011 represents an effort by the U.S. Government to address the safety of the nation's food systems. Under FSMA, the U.S. Food and Drug Administration (FDA) is charged with establishing science-based standards for the safe growing, harvesting, packing, and holding of produce on farms to reduce the incidence of foodborne illness cases associated with fruits and vegetables. At the time this thesis was written, the FDA was in the process of revising the proposed produce safety rule, titled "Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption" (FDA 2014). Among other mandates, the proposed produce rule requires the inspection, maintenance, monitoring, and follow-up actions related to agricultural water sources and water distribution systems. Furthermore, it requires control measures for water used to grow, harvest, and pack covered produce. While the proposed produce safety rule has the potential to reduce the incidence of produce-related foodborne illness, farmers and industry associations have voiced concerns that proposed standards are too stringent. In particular, apple growers have expressed concern over various provisions of the produce rule – such as proposed irrigation water quality standards – with the rationale that relatively few incidences of foodborne illness outbreaks have been attributed to apple consumption.

Apples are the second most consumed fruit in the United States after bananas. In 2014, 11.2 billion pounds of apples were harvested in the U.S. The 2014 apple crop was valued at nearly $3 billion (NASS 2015). While unpasteurized apple juice and cider have been linked to

foodborne illness outbreaks, whole apples are regarded as a relatively safe crop. Only one major outbreak has been linked to whole, fresh apples; a multistate outbreak of listeriosis in the winter of 2014 prompted the recall of prepackaged caramel apples and specific varieties of packed, whole fresh apples, produced in California (CDC 2015).

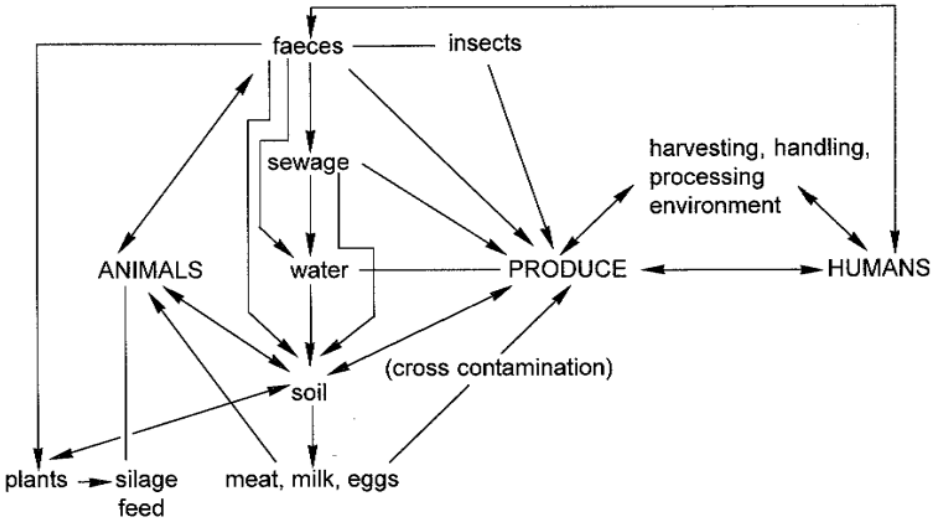
1.1. Sources and Control of Microbial Contamination

Several pathways for the contamination of produce prior to harvest have been proposed, including irrigation water, sewage, feces, soil, inadequately composted manure, insect vectors, wild and domestic animals, and air (Beuchat, 1996; Harris et al., 2003). Additionally, sources of postharvest contamination may include human handling, harvesting equipment, transport containers, wash and rinse water, and sorting and packing equipment (Harris et al., 2003). Figure 1.1 depicts some of the pathways by which fresh produce may become contaminated with microorganisms. Of the aforementioned pathways, plausible sources of bacterial contamination on apples include irrigation water used for spray irrigation or evaporative cooling, direct contact with feces or manure when apples are dropped, hand contact during harvest and contact with contaminated surfaces or washes during harvest and cleaning processes. Sources of contamination are reviewed in greater detail in Chapter 3.

To address potential contamination in the orchard, and to improve aesthetic and shelf-life, apples typically undergo several cleaning processes after harvest. These cleaning steps may include a dump tank with chlorine, chlorine or peracetic acid spray, flume systems with similar antimicrobials, spray bar systems with commercial cleaning agents or antimicrobials, and water rinses. After cleaning, apples are dried and waxed. All of these processes typically result in reduction of microbiological contaminants on the apple surface (Beuchat 1998; Herdt and Feng 2009; Kenney and Beuchat 2002). Pre-cleaning and post-cleaning storage also provides an

opportunity for microbial die-off (Janes et al. 2002). The lower risk of field contamination – given that tree fruit are less likely to contact the soil – as well as post-harvest cleaning procedures contribute to the observation that whole, fresh apples are rarely implicated in foodborne illness outbreaks. Cleaning processes are described in greater detail in Chapter 3.

Figure 1.1 – Pathways of Fresh Produce Contamination (Beuchat 1996)



This study will focus on *Escherichia coli O157:H7*, *Salmonella* spp., and *Listeria monocytogenes* as pathogens of concern in apple production. Strains of these bacteria have been implicated in foodborne illnesses associated with fresh produce (Harris et al., 2003; Lynch et al., 2009) and are recognized as pathogens of concern (Beuchat, 1996). Foodborne outbreaks have been linked to unpasteurized apple cider contaminated with *E. coli* O157:H7 (Hilborn et al. 2000; CDC 1996; CDC 1997; Besser et al. 1993) and *Salmonella typhimurium* (CDC 1975). The 2014 outbreak associated with caramel apples was caused by *L. monocytogenes* (CDC 2015). Additionally, numerous recalls of apple products have occurred due to potential *L. momocytogenes* contamination, although illnesses were not directly linked to these incidents (Stearns 2014).

1.2. Methods for the Detection of Microbial Contamination on Apples

Given the potential for microbial contamination of apples in the field, analytical methods are essential for determining levels of bacterial load on the fruits. Quantification methods serve two main purposes: (a) monitoring of apples during harvest and processing for regulatory or quality control purposes, or (b) evaluating the risks of microbial contamination under varying field conditions and cleaning processes. The methods discussed in this thesis relate to the latter purpose; to better understand the potential human health risks of apple contamination, reliable and efficient laboratory methods are needed to generate data on apple production, which in turn may inform best management practices for regulatory standards.

While several quantification methods have been developed, there is no standard method for the enumeration of microbiological agents on and in apples. The US Food and Drug Administration (FDA) provides the agency's preferred laboratory procedures for microbiological analyses of foods in the Bacteriological Analytical Manual (FDA BAM). However, the FDA BAM method – which utilizes multiple tube fermentation – is time-consuming and labor intensive. Furthermore, researchers who examine the microbiological aspects of apple growing and post-harvest processes tend to tailor their methodologies to address their specific research question. For example, Fatemi et al. (2006) developed a method of coring and sectioning apple samples to examine the penetration of *E. coli* into the fruit tissue, while Kreske et al. (2006) utilized a rinsing method to quantify *Bacillus* spores on the apple surface. A rapid, accurate, and easy-to-perform method for quantifying *E. coli* on apples may improve future food safety investigations that examine apples.

1.3. Estimating the Health Impacts of Apple Contamination

Because apples have not been implicated in many foodborne illness outbreaks – compared to crops more commonly associated with outbreaks such as lettuce, tomatoes, and sprouts – the food safety literature lacks risk assessments that quantitatively estimate the health outcomes associated with apple consumption. This study will utilize a quantitative microbial risk assessment (QMRA) framework to estimate the probability of illness associated with the consumption of contaminated apples. The purpose of this investigation is to estimate the foodborne illness risks associated with apple contamination and to characterize the relative contribution of contamination sources and cleaning steps to the risks of foodborne illness. Three QMRA models are provided, respectively examining three microorganisms of concern: *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*.

Risk assessment is the qualitative or quantitative characterization and estimation of potential adverse health effects associated with exposure to hazards (Haas et al. 1999). Risk assessment is a component of risk analysis, which incorporates risk assessment, risk management, and risk communication. Risk assessment has developed as a tool for characterizing human and ecological health risks associated with physical, chemical, and biological hazards in the environment. The origin of risk assessment is tied to congressional mandates of the 1970s, namely the Clean Air Act and the Safe Drinking Water Act Amendments. The National Academy of Sciences formally recognized the field of risk assessment in 1983 with the publication of the "red book" (NRC 1983). Although the field of risk assessment has been critiqued over the years (Graham 1995), it continues to evolve and serve as a tool for both academics and government agencies.

Charles Haas was the first to quantitatively examine microbial risks associated with drinking waters based on dose-response modeling (Haas 1983). QMRA has since emerged as a sub-field of risk assessment that specifically examines the health outcomes associated with exposure to microbial hazards. The QMRA framework is explained in greater detail in Chapter 3. While utilizing the same framework as chemical risk assessment, QMRAs require modifications to account for the differences between chemical and microbiological hazards. For example, QMRAs must address the growth and die-off pathogens and microbiologically-relevant dose-response models (Haas et al. 1999).

1.4. Policy Relevance

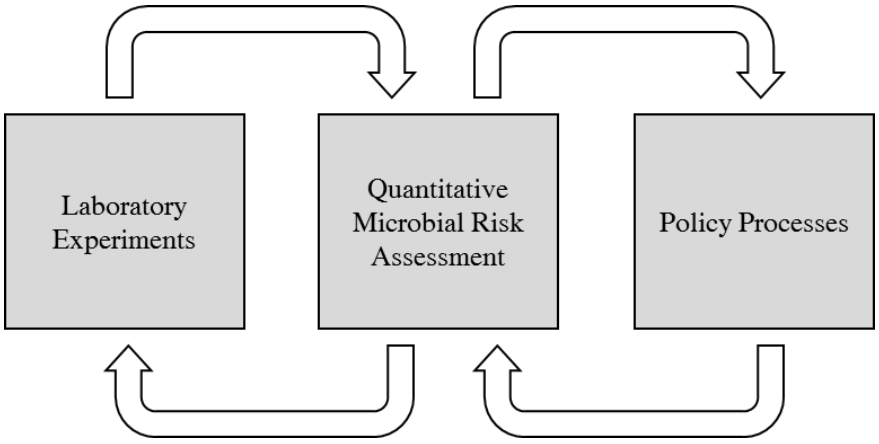
The research presented in this thesis directly relates to the FDA's proposed Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption. Yet, while public policy is to an extent informed by best available science, other factors play important roles in policy processes. Policy actors' principles and values necessarily guide policy formation (Lindblom and Woodhouse 1993). Likewise, institutions – the formal and informal mechanisms that structure individuals' or groups' actions – guide decision-making processes. Consideration of values, actors, institutions, and policy processes is necessary to understand the role for science in food safety policy. Chapter 4 will examine the policy relevance of the research provided in Chapter 3 by examining the history of food safety, providing general frameworks for food safety policy processes, and identifying specific stages in which research findings may be utilized.

1.5. Study Purpose and Specific Aims

The purpose of this thesis is to provide tools for the evaluation of human health impacts associated with apple consumption. The three tools provided here are reflected in a broader framework that links laboratory methods, risk assessment modeling, and policy processes

(Figure 1.2). In this framework, experimental studies provide data that can be used in risk assessment models. In turn, risk assessments can inform policy processes.

Figure 1.2 – Three Research Areas Associated with Specific Aims



The three specific aims for this thesis correlate to the three domains in Figure 1.2. Specifically, this thesis aims to:

Aim 1: Evaluate the efficacy of a novel surface elution method to quantify *E. coli* on apples.

Aim 2: Use quantitative microbial risk assessment models to:

Aim 2A: Generate estimates of the probability of gastrointestinal illness associated with apple consumption, under varying orchard and packing house conditions.

Aim 2B: Identify specific conditions or processes that likely have the most and least consequential impact on apple safety.

Aim 2C: Identify data gaps in the literature to guide future research; propose improvements to future iterations of apple QMRA models.

Aim 3: Examine the policy context of study findings and identify stages of food safety policy processes in which the findings could be used.

2. Evaluating a Novel Method for the Quantification of *E. coli* on Apples

2.1. Introduction

Proposed regulations relating to the growing and handling of produce have stimulated interest in conducting studies to evaluate the risks of foodborne illness associated with apples. Because new standards for the growing, harvesting, packing, and holding of apples have economic implications for apple growers, key stakeholders are increasingly interested in characterizing the foodborne illness risks associated with apple growing practices. As a first step towards determining the human health risks associated with apple production, reliable and efficient methods must be developed for enumerating microbiological contaminants on apples. Such methods can be used to generate data on bacterial growth and die-off rates during various growing, harvest, cleaning, packaging, and storage processes. This data can, in turn, be used in QMRA models to estimate the health risks associated with apple consumption. Current methods are time-consuming and labor intensive, presenting a barrier to laboratory and field studies.

While an array of microbes could cause foodborne illness, testing for numerous distinct pathogens is costly and time-consuming. Consequently, fecal coliforms and *E. coli* are typically used as indicators to signify the potential presence of intestinal pathogens (Geldreich 1966). Fecal coliforms are defined as facultatively anaerobic, rod-shaped, gram-negative non-sporulating bacteria that ferment lactose at 44 ± 0.5°C. Because fecal coliforms may not necessarily indicate the presence of feces, *E. coli* is more widely accepted as an indicator of fecal contamination (Doyle and Erickson 2006). The conventional method for detecting and quantifying fecal coliform or *E. coli* contamination in food samples is the FDA BAM Most Probable Number (BAM MPN) method. Under BAM protocols, a specified mass of food sample

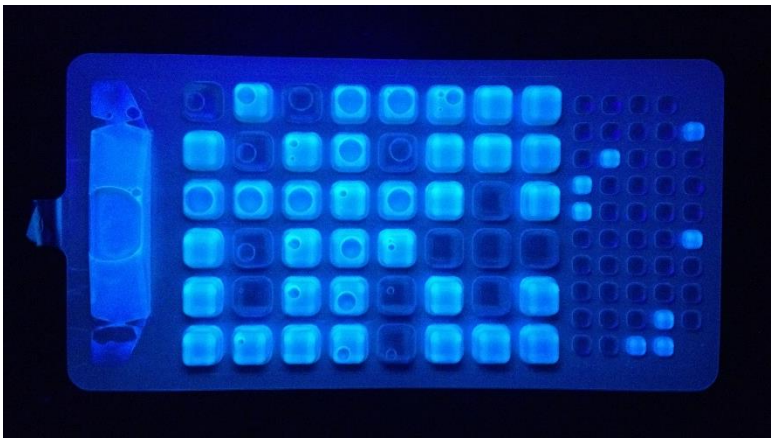
is first homogenized in a blender or stomacher. Then, a 15-tube dilution method is used to statistically estimate the concentration of target microorganisms (FDA 2002).

An efficient and easy-to-perform coliform quantification method, the IDEXX Colilert®-18 and Quanti-Tray® system, is widely used for the detection and quantification of total coliforms, fecal coliforms, and *E. coli* in water. This method is designed to estimate the Most Probable Number (MPN) of *E. coli* and coliform bacteria in drinking waters and other similar treated and untreated waters. Colilert-18® uses Defined Substrate Technology® nutrient indicators to detect coliforms and *E. coli* in water samples. The use of *β*-glucuronidase for the rapid detection of *E. coli* in environmental media was proposed by Kilian, M., & Bülow (1976), and since has become a widely utilized method for the identification of fecal bacteria (Rice et al. 1990). In the presence of the Colilert substrate, *E. coli* use *β*-glucuronidase to metabolize indicators and create fluorescence. Non-coliforms that are equipped with these enzymes are selectively suppressed by Colilert's specifically formulated matrix. Colilert-18® is used in conjunction with IDEXX's Quanti-Tray®/2000 (Figure 2.1), which is based on the same statistical model as the traditional 15-tube serial dilution. By dividing the sample into wells, Quanti-Tray® uses the Standard Method MPN approach to determine the number of bacteria in the original sample. The relatively large number of wells (49 large wells and 48 small wells) provides a high counting range and narrower 95% confidence intervals compared to the traditional 15-tube fermentation method. Both methods use the following equation to estimate the MPN (λ):

$$\sum_{j-1}^{k} \frac{g_j m_j}{1 - \exp(-\lambda m_j)} = \sum_{j-1}^{k} t_j m_j$$

Where k denotes the number of dilutions, g_j denotes the number of positive tubes in the *j*th dilution, m_j denotes the amount of the original sample in each tube in the *j*th dilution, and t_j is the number of tubes in the *j*th dilution.

Figure 2.1 – IDEXX Colilert®-18 and Quanti-Tray®/2000 under UV Light



The Colilert®-18/Quanti-Tray® method is an International Organization for Standardization (ISO) worldwide standard for detecting total coliforms and *E.coli* in water (ISO 9308-2:2012). Numerous validation studies have shown that IDEXX Colilert®-18, Quanti-Tray® produces results that are not significantly different from membrane filtration methods for enumerating coliforms and *E. coli* in drinking water (Boubetra et al. 2011), recreational waters (Kinzelman et al. 2005), municipal wastewater (Warden et al. 2011), and treated sewage sludge (Eccles et al. 2004). The U.S. Environmental Protection Agency (EPA) has approved the use of Colilert®-18 and Quanti-Tray® for testing wastewater treatment effluent. While Colilert®-18 and Quanti-tray®/2000 has been used to estimate the concentration of *E. coli* on lettuce and tomatoes (Leang 2013), the method has not been formally validated for testing fecal contamination of tree fruit. This study aims to evaluate the efficacy of a surface elution method developed to maximize the recovery of *E. coli* from contaminated apples, using the IDEXX system for quantification.

Specifically, this study will determine the rate at which *E. coli* are recovered off of inoculated apple surfaces.

2.2. Methods

Apple Preparation

Unwaxed Fuji, Red Delicious, Gala, and Honeycrisp apples were procured from apple growers in Washington State. Apples were stored at 4ºC. Prior to inoculation, apples were rinsed with water, dried, and disinfected with 70% ethanol solution and UV light.

Preparation of Inocula

Non-pathogenic strains of *E. coli* were recovered from irrigation water samples. Stock concentration of inoculum was prepared with Difco™ Nutrient Broth, incubated overnight at 37ºC with agitation. Three inocula were prepared using serial dilutions in sterile phosphate buffered saline (PBS); high, medium, and low inoculum concentrations were ~10^5, ~10^4, and ~10^3 CFU/ml, respectively. Concentrations of *E. coli* in the inocula were determined by plating serial dilutions on Difco™ Nutrient Agar with MUG.

Inoculation Procedure

Disinfected apples were spot inoculated with 100µl of inoculum. Approximate *E. coli* concentrations for the high, medium, and low inoculation levels were 10^4, 10^3, and 10^2 CFU/apple, respectively. Droplets of inoculum were deposited evenly over the apple surface, including the stem bowl and/or calyx. After inoculation, apples were dried in a biosafety cabinet with air flow for 1.5-2 hours, until droplets had visibly dried. Negative control apples were spot inoculated with 100µl of sterile PBS and dried under the conditions described above.

Recovery

Inoculated apples were placed in a 22-oz Whirl-Pak® bag with 100ml of sterile PBS. The bagged apples were placed on a shaker table for 10 minutes, and then massaged by hand for 3 minutes. Eluate from the Whirl-Pak bags was transferred to individual sterile Nalgene sample bottles. Bagged apples shown in Figure 2.2.

Figure 2.2 – Apples Being Transferred to Whirl-Pak® Bags

Enumeration

Eluate from the Whirl-Paks® was mixed with IDEXX Colilert®-18 reagent, then poured into Quanti-tray®/2000 and sealed in a Quanti-tray® sealer. After incubation at 37ºC for 18-24 hours, an MPN for *E. coli* was generated for each sample based on the number of positive wells (those that fluoresced under UV light).

Calculations

Percent recovery was determined by dividing the number of *E. coli* in the apple sample eluate by the number of *E. coli* inoculated on the apple, using the equation:

$$Percent\ Recovery = \frac{E.coli\ in\ eluate}{C_{inoculum} * V_{inoculum}} * 100$$

Where $C_{inoculum}$ is the concentration of *E. coli* in the inoculum and $V_{inoculum}$ is the volume of inoculum applied to the apple. The percent recovery value estimates the proportion of *E. coli* that were recovered off of the apple from the surface eluation process.

PCR Enumeration

Quantitative PCR was used to evaluate the effect of die-off during the inoculation process on recovery estimates. Three inocula were prepared as described above, with high, medium, and low inoculum concentrations of ~10^8, ~10^7, and ~10^6 CFU/ml, respectively. Apples were spot inoculated as described above; approximate high, medium, and low concentrations were 10^7, 10^6, and 10^5 CFU/apple, respectively. *E. coli* were recovered as described above. After the apples were massaged, 50ml of eluate was transferred from the Whirl-Pak® into sterile 50ml conicals. Conicals were centrifuged at 7000G for 15 minutes. The pellet was resuspended in 400µl of sterile PBS. DNA was extracted using UltraClean® Microbial DNA Kit (MoBio Laboratories). Primer and probe sequences and cycling conditions were taken from Frahm and Obst (2003).

2.3. Results

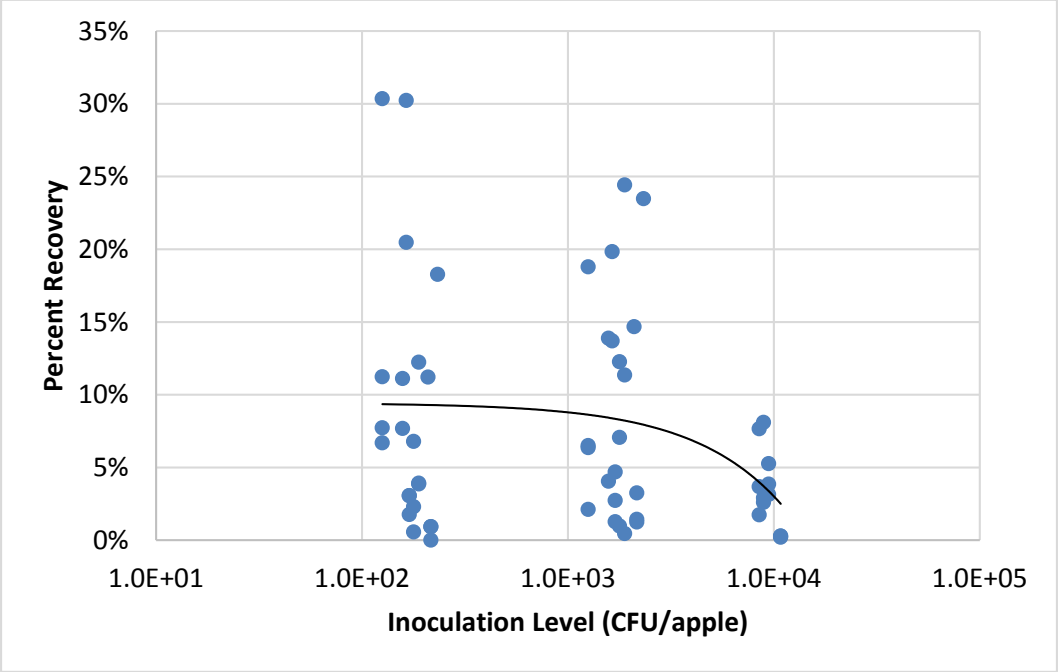
Sixty-seven apples were inoculated and analyzed for *E. coli* levels. Apple mass ranged from 112.1g to 198.5g. Of the inoculated apples, *E. coli* were recovered from all but one sample. *E. coli* were not recovered from any of the negative controls. Eleven apple samples exceeded the upper limit of the IDEXX assay (2419.6 MPN/apple), and therefore recovery rates could not be determined. Of the 56 apples for which the recovery rate could be calculated, the average

recovery was 7.65%. The mean recoveries for low, medium, and high levels of inoculation were 8.83%, 8.84%, and 3.31%, respectively. Mean, median, and the range of recovery rates are shown in Table 2.1. Figure 2.3 shows the correlation between the inoculation level and the percent recovery. The trend line suggests that higher inoculation levels are associated with lower rates of recovery.

Table 2.1 – Mean, Median, and Range of Percent Recoveries for Inoculated Apples

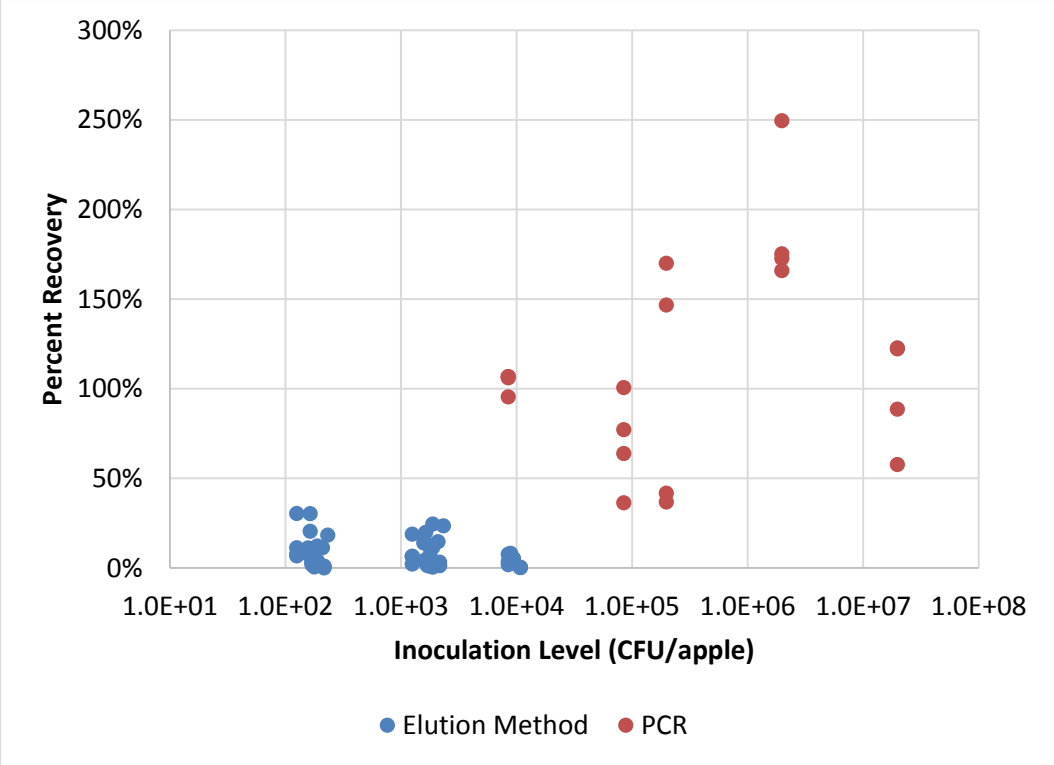
Inoculation Level	Mean	Median	Maximum	Minimum
High (n=12)	3.31	3.03	8.10	0.20
Medium (n=22)	8.84	6.43	24.42	0.45
Low (n=22)	8.83	6.74	30.35	0.00
All samples (n=56)	7.65	4.37	30.35	0.00

Figure 2.3 – Correlation Between Inoculation and Percent Recovery



Quantitative PCR was used to evaluate the effect of die-off during the inoculation process on recovery estimates. PCR results suggest a mean and median recovery rate of 112% and 106%, respectively (range: 36% - 249%). Figure 2.4 depicts the percent recoveries associated with both the IDEXX and PCR results. Unlike the IDEXX results, recovery estimations with PCR do not appear to have a correlation with inoculation level.

Figure 2.4 – Percent Recoveries for IDEXX and PCR



2.4. Discussion

2.4.1. Method Development

A novel approach to rapid quantification of *E. coli* was developed using a surface elution method and IDEXX Colilert®-18 and Qunti-tray®/2000. The rationale for focusing on *E. coli*

quantification is three-fold. First, *E. coli* serve as a model organism for studies on the efficacy of cleaning and antimicrobial treatment processes. This method could be employed in such research. Second, fecal coliforms, and *E. coli* specifically, are used as indicator organisms to estimate levels of microbial contamination in environmental media. This method could therefore be employed to estimate the frequency and magnitude of microbial contamination on apples in orchards due to field contamination such as contact with irrigation water and fecal material. Finally, this method relates to concern over *E. coli* O157:H7 and other enterohemorrhagic *E. coli* as a cause of produce-related foodborne illness. While Colilert® does not expressly indicate presence of the O157:H7 serotype, the protocols presented here can be augmented to include serotyping of recovered *E. coli*.

Compared to the conventional BAM MPN method for quantifying *E. coli* in food samples, the proposed IDEXX method requires considerably less effort in the lab, and produces reliable results in a much shorter amount of time. Hands-on time with the kit (not including elution procedures) is less than three minutes per sample. Colilert®-18 can confirm the presence and concentration of fecal coliforms and *E. coli* after 18-24 hours. Conversely, the FDA BAM 3-tube MPN method takes a minimum of 120 hours (5 days) to confirm the presence of *E. coli*. The limit of detection for the BAM MPN method is <36 MPN/100ml (Downes and Ito 2001), while the IDEXX limit of detection is 1 MPN/100ml. The multiple steps involved in the BAM MPN method provide multiple opportunities for technical error; the IDEXX method is straight-forward and interpretation of the assay is less ambiguous. Additionally, the BAM MPN method may not be ideal for sampling apples. Unlike other food matrices, apple contamination is likely not distributed throughout the sample; the calyx, stem, and surface tend to have the highest microbial

contamination (Burnett et al. 2000). As such, fractioning the apple into the appropriate mass for the BAM MPN test may not provide a representative microbial sample of the apple.

2.4.2. Discussion of Results

The results of this study suggest that the proposed method is effective at recovering *E. coli* from the surface of apples. *E. coli* were recovered from all but one apple sample, and recovery was possible even at lower levels of inoculation. The relatively low estimates of recovery (an average of 7.65% overall) using the surface elution method is likely due to two factors (a) die-off of bacteria due to drying during the inoculation process, and (b) bacterial attachment to the apple surface or uptake into the apple that cannot be reversed in the elution process. Regarding the latter explanation, *E. coli* have been shown to preferentially attach to discontinuities in the apple surface and infiltrate the floral tube (Burnett et al. 2000), making removal by surface elution difficult or impossible.

Die-off of *E. coli* during the inoculation process was investigated using quantitative PCR. Since desiccation during the drying process may result in significant die-off of bacteria, low recovery rates may attributed not to elution inefficiency, but to inability of the enumeration method to show the presence of inactivated cells. Quantitative PCR was therefore employed to estimate the total number of recovered bacteria. The results indicate that recovery of *E. coli* is higher than estimated with the IDEXX system. The high estimates using PCR – which indicate above 100 percent recovery – were likely due to aggregations of *E. coli* in the apple sample eluate. Despite the high recovery estimates, the PCR results nevertheless provide insights into the actual elution process; because PCR is able to quantify both living and dead bacteria, the results provide an estimate of total recovered organisms regardless of die-off during inoculation. High recovery estimates from PCR suggest that the method is more effective at recovering *E.*

coli than the IDEXX method results suggest. While the exact rate of recovery cannot be derived from this study, we can confidently assert that the proposed surface elution method, combined with the IDEXX Colilert®-18 and Quanti-Tray®/2000, is effective at recovering *E. coli* off of apple surfaces. Low recovery rates, likely due to die-off of bacteria during the inoculation process, are consistent with findings from a study that examined the recovery of *E. coli* from the surface of inoculated ceramic tiles and stainless steel (Meschke et al. 2009). Further studies may elucidate the efficacy of this method compared to other methods.

2.4.3. Limitations and Directions for Future Research

One concern in this study is the discrepancy in units; inoculum concentrations were estimated in CFU, while the IDEXX method generated estimates in MPN. MPN values are estimates of bacteria based on a statistical model, while CFU are direct counts of colonies. While this would suggest that the values are not directly comparable, this analysis nevertheless provides useful information on the estimated recovery of *E. coli* from the apple surfaces. Differences in MPN and CFU are understood to result not from human error or laboratory procedure variability, but are recognized as a consequence of the probabilistic basis for calculating the MPN (Gronewold and Wolpert 2008). Conceptually, MPN can be viewed as a statistical estimate of CFU; under this assumption, this analysis can provide an approximate estimation of the efficacy of the novel surface elution method.

This method is not able to capture bacteria that may have penetrated the surface. Pathogen uptake into the apple interior has been investigated as a mechanism of contamination (Buchanan et al. 1999). Furthermore, apples are more susceptible to contamination in puncture wounds and damaged tissue (Fatemi et al. 2006). This method is not capable of extracting bacteria from the interior of the apples; thus, the results may not reflect the true level of apple

contamination. Nevertheless, the surface elution method can estimate the concentration of *E. coli* on the surface. This method would therefore be useful for evaluating apple cleaning processes; by comparing the microbial load of an untreated inoculated apple against the microbial load of an inoculated apple that has undergone a decontamination procedure, one may estimate the effectiveness of that cleaning process. This study provides information that may guide further validation studies. Future research may compare this method against other methods (namely, FDA BAM protocols) to formally validate this procedure. Furthermore, this method could be modified to test for other microbiological contaminants.

2.5. Conclusion

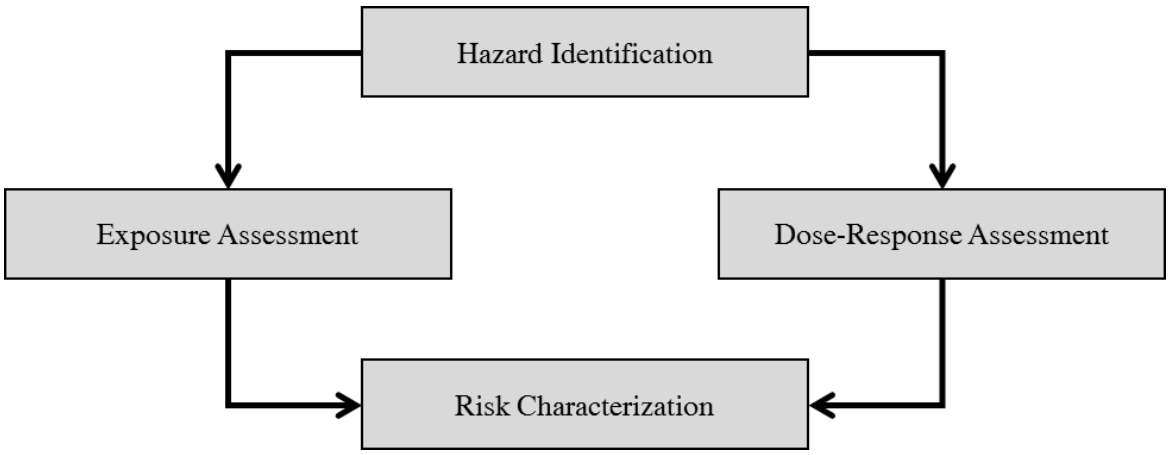
While this study is limited in its ability to draw conclusions about the efficacy of this method compared to other protocols, it nevertheless provides strong evidence to support the continued development and validation of the proposed method.

3. Estimating the Health Effects of Apple Contamination

3.1. Introduction

Risk assessment is the qualitative or quantitative characterization and estimation of potential adverse health effects associated with exposure to hazards (Haas et al. 1999). A framework for risk assessment is provided in Figure 3.1. The conventional risk assessment framework begins with hazard identification, followed by exposure assessment and dose-response assessment. Exposure assessment and dose-response assessment are together used to characterize the risk. (Nam et al., 2014)

Figure 3.1 – The Risk Assessment Framework



Hazard identification is the first step in risk assessment. This step aims to identify an environmental hazard – such as a pathogen – and describe the human health effects associated with the hazard. The exposure assessment determines the size and nature of the population exposed to the environmental hazard. This includes the route, concentration, frequency, and duration of exposure. The dose-response assessment characterizes the relationship between the amount of hazard (dose) to which an individual is exposed and the incidence of health outcome (response). Risk characterization integrates the exposure assessment and dose response

assessment to estimate the public health consequences of the given hazard. Risk characterization includes the evaluation of variability and uncertainty in the risk assessment model (Haas et al. 1999).

The process of conducting a risk assessment is useful not only in generating a quantitative estimate of risk, but can provide unique insights into factors that affect risk for a certain exposure pathway. The value of a risk assessment is not solely measured by the model outputs, but by qualitative information gained in identifying data gaps and recognizing the relative impact of various environmental conditions. This QMRA has three aims:

A) Provide quantitative estimates of the risk of foodborne illness associated with apple consumption, given varying orchard conditions and cleaning processes.
B) Identify specific conditions or processes that likely have the most and least consequential impact on apple safety.
C) Evaluate the reliability of the models' projections and identify data gaps in the literature that, if filled, would improve the risk assessment models.

3.1.1. Sources of Microbial Contamination

Waterborne Contamination from Evaporative Cooling

Irrigation water has been identified as a source of pathogenic bacteria on fresh produce (Steele and Odemeru 2004). An association between water quality and produce safety has been demonstrated by epidemiological studies where outbreak-implicated pathogens were recovered from both infected individuals and irrigation water. Contaminated irrigation water has been identified as a likely source of contamination of *E. coli* O157:H7 in outbreaks associated with lettuce (Wachtel et al. 2002; Ackers et al. 1998). While the literature is lacking similar reports

for fruit-associated outbreaks, field and experimental evidence has identified irrigation water as a vector for contamination (Espinoza-Medina et al. 2006).

In apple production, water is used for irrigation, mixing pesticides, evaporative cooling, and washing processes. Contaminated surface or groundwater used for any of these purposes may potentially contaminate apples with bacteria. Irrigation water used for evaporative cooling is of particular concern because it has a higher probability of direct fruit contact near harvest. Evaporative cooling has also been shown to improve color, firmness, fruit size, soluble solid concentration, and anthocyanin levels when applied to apple crops (Iglesias et al. 2005). Evaporative cooling is often employed to mitigate economic losses associated with sunburn of the fruit surface (Evans 2004).

The likelihood of fruit contamination from irrigation water necessarily depends on the quality of the water applied; irrigation water with high microbial concentrations has a greater probability of contaminating the fruit to which it is applied. Furthermore, the quality of irrigation water depends on the source. Groundwater tends to be of better microbial quality unless it is contaminated with surface run-off. Surface water is more susceptible to contamination of pathogenic microorganisms, and thus is of highly variable microbial quality depending on proximity to sources of contamination (e.g., sources of animal waste) (Steele and Odumeru 2004).

Direct Contact with Feces or Soil

Animal feces have been recognized as a likely source of microbial contamination on fresh produce; yet its relative impact on apples is not well characterized. Contact with feces can occur when apples drop to the ground, or through direct deposition by birds or insect vectors. Fecal material on the ground may be present in the form of manure, sewage sludges, or animal

droppings from wild or domestic animals. Fecal material on the ground poses a greater risk to produce grown in close proximity to the soil (e.g., lettuce, cantaloupes, and strawberries). While fecal contamination from the ground has been recognized as a source of microbial contamination on fresh fruits (FDA 1998), this route of pathogen introduction is generally recognized as lower risk for hand-picked apples. Studies have shown that dropped apples are more likely to become colonized by bacteria. Additionally, bruised apple tissue becomes more neutral in pH and sugar content is lowered, providing conditions that are more conducive to bacterial growth (Dingman 2000). Industry practices discourage or prohibit the use of apples that have come in contact with the ground. Yet, a survey of Wisconsin apple cider manufacturers indicated that 14% of producers used wind-fall apples to make cider (Uljas and Ingham 2000). Outbreaks associated with whole tree fruit have not been conclusively linked to fecal contamination; however, there is convincing evidence that feces of infected deer may have contaminated apples used for the manufacture of unpasteurized apple juice, leading to *E. coli* O157:H7 outbreaks (Garcia et al. 2006).

Apples may potentially be contaminated via direct deposition of bird feces on the fruit. Duffy and Schaffner (2002) examined bird feces as a possible source of apple contamination. Birds are capable of carrying and shedding *E. coli* O157:H7 (Wallace et al. 1997) and *Salmonella* (Fenlon 1981) when an infected food source is located nearby. *L. monocytogenes* carriage in birds has also been observed (Fenlon 1985), particularly in proximity to sewage sources (Fenlon et al. 1996). While contamination of apples from bird feces is possible, there is little evidence in the literature to support this as a key source of contamination on apple crops; no data currently exists on the frequency at which bird feces directly lands on apples. The analysis by Duffy and Schaffner (2002), utilizing a quantitative microbial risk assessment model,

estimated 3-4 log CFU contamination on 1000 hand-picked apples as a result of bird feces. However, among other limitations, their model assumed that all feces dropped by birds would land on the fruit.

Insects and other invertebrates have also been shown to serve as vectors of microbiological contamination for apples in experimental settings. Insects have been shown to carry and excrete human pathogens (Xu et al. 2003). Janisiewicz et al. (1999) demonstrated that infected fruit flies were capable of transferring E. coli O157:H7 to exposed apple tissues. However, little is known about the risks associated with transmission of human pathogens via invertebrate vectors in food production systems (Bach and Delaquis 2009).

Contamination During Harvest, Cleaning, Packing, and Storage

Apple harvest and cleaning process provide a number of mechanisms by which microbial contamination could occur. Apple contact with any surface during harvest and processing may be viewed as a potential source of contamination. Contaminated gloves or hands used to pick the apples could transfer bacteria from the harvester to the fruit. A survey of found that 16% of Wisconsin apple cider producers utilized apple harvest methods in which the apples in one storage box would contact the bottom of the storage box below (Uljas and Ingham 2000). Furthermore, if apples are removed from boxes by immersion, rather than dumping, soil particles from the boxes may be transferred to the wash water, and subsequently to apple surfaces.

3.1.2. Methods of Pathogen Reduction

Controls and cleaning processes in apple production are utilized to remove debris and reduce risk of microbial contamination. Methods of pathogen control on apples include source controls – which prevent initial contamination of the fruit – and use of antimicrobial substances or processes following harvest.

Source Control

Measures that prevent contamination are often preferred over decontamination methods because a) decontamination is typically associated with higher costs, and b) decontamination methods may fail to inactivate all microorganisms on the fruit. Source control methods include best management practices that prevent apple contact with surfaces or substances that may contaminate the fruit. Examples of these practices include monitoring to restrict wild or domestic animal access; the use of glove and hand hygiene practice during harvest; preventing the collection of apples that have dropped to the ground; and ensuring that collection containers are clean (Brackett 1999; Gravani 2009; Uljas and Ingham 2000). Some growers assess water quality for irrigation and evaporative cooling water.

Antimicrobial Processes

Antimicrobial substances or processes are used to inactivate microorganisms that may have affixed to the apple surface prior to or during harvest and prevent cross-contamination in water systems. Most fresh produce is washed after harvest, typically involving flume transport systems, batch tanks, or water sprays. Washing steps are important for removing soil and debris and improving appearance. Washing can also reduce the microbial load on apples by removing them from the surface (Annous et al. 2001). However, wash water may be reused, potentially increasing the risk of contamination; pathogens removed from one batch during cleaning may potentially contaminate the wash water and transfer microbes to subsequent batches of apples (Gil et al. 2009). While wash water has been recognized as a possible source of contamination, it has not been well characterized in industry settings.

Sanitizing compounds are often added to wash water to prevent the transmission of microorganisms. Chlorine and peracetic acid are the most commonly used sanitizers or

disinfectants used in commercial apple production. Hydrogen peroxide has also been investigated as an effective disinfectant in the apple cleaning process (Liao and Sapers 2000; Sapers et al. 1999), but has not been adapted for commercial use to a large extent. Additionally, wax application has been shown to reduce bacterial concentrations on apples (Kenney and Beuchat 2002). The antimicrobial efficacy of these processes, however, relies upon numerous factors.

Factors that Influence Antimicrobial Efficiency

The efficacy of microbial reduction using chlorine, peracetic acid, and hydrogen peroxide depends on a variety of factors. Considerations when optimizing an antimicrobial washing system include concentration, temperature, pH, soil load on the produce, buildup of organic material in the wash water, characteristics of the target microbes, characteristics of the fruit, and level of produce contamination. Because wash water is generally reused, especially for dump tank or flume systems, organic matter accumulation will cause a decrease in the effective concentration of the antimicrobial, contributing to an increased risk of bacterial contamination (Herdt and Feng 2009).

- **Concentration:** antimicrobial wash efficacy is primarily determined by the concentration of the compound in solution. Typically, high antimicrobial concentrations correlate to high antibacterial activity when other factors (e.g., pH, temperature, and organic content) are held constant. However, high antimicrobial concentration may damage apple tissue, and could result in chemical residues on the fruit.
- **Time:** the duration of antimicrobial wash contact with the fruit influences the efficacy of microbial reduction. Generally, increased contact times will result in increased bacterial removal.

- **Temperature:** Generally, an increase in the temperature of an antimicrobial solution is associated with an increase in antimicrobial activity. However, higher temperatures are also associated with a decrease in produce quality; produce industry practices have generally shifted to cooling the washing plant to 4 ^{o}C. This temperature is additionally beneficial because the maximum solubility of chlorine in water occurs at about this temperature (Herdt and Feng 2009). However, apples may be cleaned under warmer conditions to facilitate wax application.
- **pH:** a solution's pH can substantially affect the antimicrobial activity. An increase in pH significantly decreases the inactivating activity of chlorine; low pH favors the formation of hypochlorous acid (HClO), the most active species of chlorine. This is generally true for acid sanitizers; the pH of the solution must be at or below the dissociation constant for the acid to be effective (Herdt and Feng 2009).
- **Location of microbes on the apple:** microbial entrapment in the calyx and stem of the apple is significantly more difficult to overcome during wash treatments than other parts of the apple. Sapers (2001) found that attachment of *E. coli* was greater in the stem and calyx areas, and *E. coli* survival rates after washing were greater in these areas than elsewhere on the apple.

Chlorine

Chlorine is the most widely used antimicrobial agent used in washes for fresh produce, due to its efficiency, low cost, and ease of operation (Nguyen-the and Carlin 1994). Chlorine is active against a wide spectrum of microorganisms, including viruses, acid-fast bacilli, non-acid-fast vegetative bacteria, bacterial spores, fungi, algae, and protozoa (Trueman 1971). Free available chlorine includes chlorine gas, hypochlorous acid, or hypochlorite ions. For raw fruits

and vegetables, treatments typically use chlorine concentrations around 200 mg/l at a pH of < 8.0 with a contact time of 1-2 minutes for processing (Beuchat 1996).

A study by Beuchat et al. (1998) examined the efficacy of chlorine spray application in reducing *Salmonella*, *E. coli* O157:H7, *L. monocytogenes*, and other microorganisms on whole apples. Inoculated produce was sprayed with solutions containing 200 or 2,000 ppm of chlorine for various lengths of time. Compared to control treatment (spray with water), pathogen reductions with chlorine ranged from 0.35 to 2.30 log CFU/cm^2 (Beuchat et al. 1998).

Peracetic Acid

Peracetic acid (peroxyacetic acid or PAA) has a stronger oxidizing potential than chlorine, chlorine dioxide, chlorous acid, and hydrogen peroxide (Herdt and Feng 2009). The primary mechanism of disinfection for peracetic acid is oxidation. Peracetic acid has also been thought to cause rupturing of outer cell walls by disrupting the sulfydryl and sulfur bonds in proteins and enzymes in microbes (Block 1991). In some instances, peracetic acid is preferential to other oxidizing agents because it has higher tolerance to organic materials; it can retain effectiveness with higher levels of soil and organic load in the water wash (Herdt et al. 2007).

Wright et al. (2000) found that apples inoculated with *E. coli* O157:H7, when treated with 80 ppm of peracetic acid for 2 minutes, reduced pathogen load by 2.6 logs. These findings were consistent with other studies that examined bacterial reductions on cantaloupes and honeydew melons (Park and Beuchat 1999). Using a 160 ppm concentration of peracetic acid for 15 minutes on whole apples, Wisniewsky et al. (2000) achieved a 5-log reduction in *E. coli* O157:H7. In another study, Rodgers et al. (2004) observed a 4.3-log reduction in *L. monocytogenes* and a 4.5-log reduction in *E. coli* O157:H7 when treating fresh produce with 80 ppm peracetic acid for 5 minutes.

Waxing

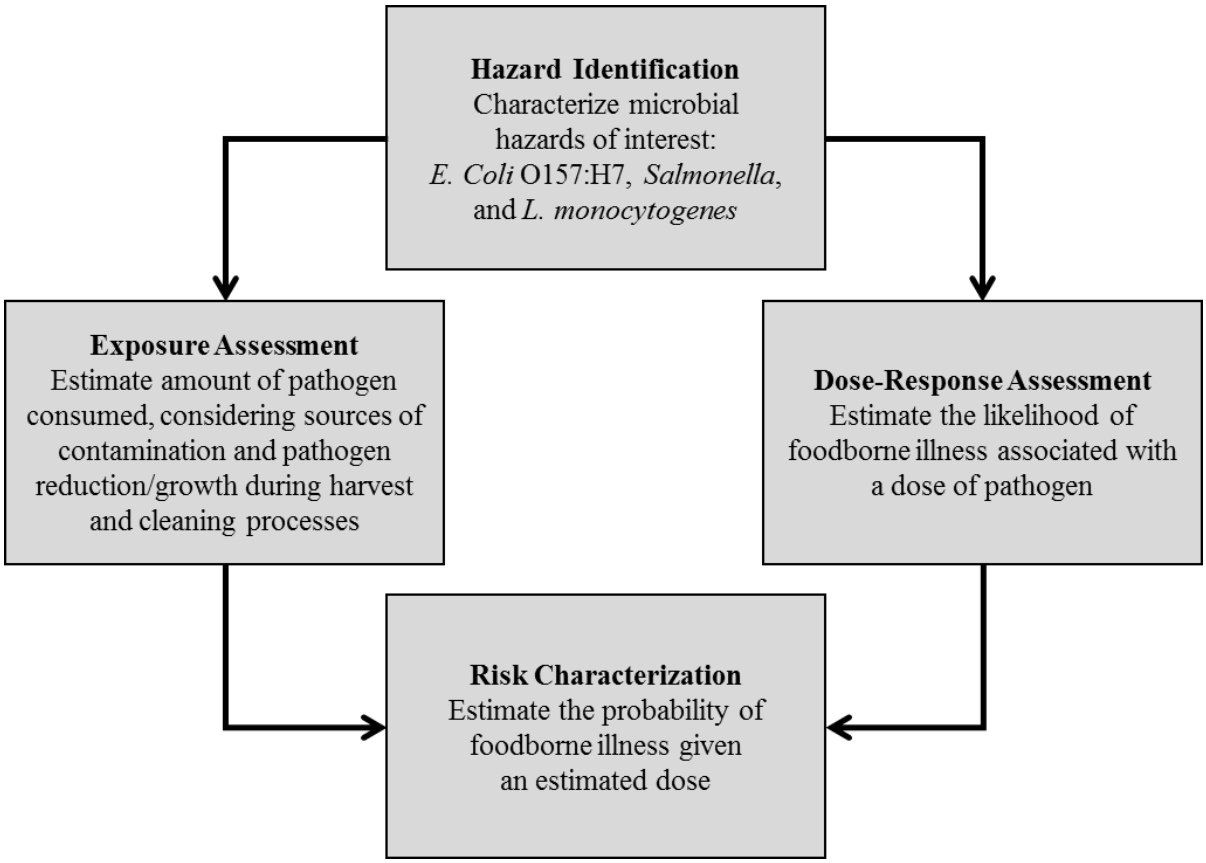
Wax is applied to fresh apples primarily to maintain the quality of the fruit. However, waxing has been demonstrated to aid in microbial reduction as well. Kenney and Beuchat (2002) investigated how wax chemical components affected the survival of *E. coli* O157:H7. Apples were inoculated with *E. coli* O157:H7, then waxed, dried, and stored at 2°C. After storage, the apples exhibited significant reductions in pathogen levels, with 3.05-log reductions after three weeks of storage and 4.95-log reduction after six weeks of storage. By comparison, unwaxed inoculated apples exhibited very little reduction in *E. coli* O157:H7 after 6 weeks of storage.

3.2. Methods

Three QMRA models were developed to estimate the health impacts associated with three pathogens of concern: *E. coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes*. Risk assessment models follow the conventional QMRA structure of hazard identification, exposure assessment, dose-response assessment, and risk characterization (shown in Figure 3.2, detailed explanations in subsequent sections). Exposure and dose-response assessments were populated with data from the literature. Exposure assessments in all three models include the same sources of contamination and cleaning processes. Models were created in Crystal Ball™ with 10,000 simulations per run.

For several contamination sources and reduction/growth steps, data could not be found in the literature to populate the model parameters. Where data was missing for given parameters, alterative data was used. For example, studies were not found on the reduction of *Salmonella* on apples during storage; proxy data from a study on the reduction of *Salmonella* on tomatoes during storage was used instead. While alternative data introduces uncertainty into the model, it was necessary to fully construct the models.

Figure 3.2 – Risk Assessment Framework for Provided Models



3.3. Hazard Identification

3.3.1. *E. coli* O157:H7

E. coli are Gram-negative, facultatively anaerobic and non-sporulating bacteria that reside in the lower intestinal tracts of mammals. They are rod-shaped and are approximately two microns in length and 0.5 micron in diameter. The Escherichia genus is a member of the Enterobacteriaceae family, which includes other pathogenic bacteria, such as *Salmonella*, *Yersinia pestis*, *Klebsiella* and *Shigella* (Garrity et al. 2005). *E. coli* O157:H7 is included in a group of bacteria called enterohemorrhagic *E. coli* (EHEC), shiga-like toxin-producing E. coli (STEC), hemolytic uremic syndrome–associated enterohemorrhagic *E. coli* (HUSEC) and verocytotoxin- or verotoxin-producing *E. coli* (VTEC) (Karch, Tarr and Bielaszewska 2005).

Infection with *E. coli* O157:H7 can be entirely asymptomatic or can result in a variety of clinical symptoms. The most common condition associated with infection is hemorrhagic colitis, which results in stomach cramps and acute bloody diarrhea. In one study, more than 90% of hospitalized patients with *E. coli* O157:H7 infection displayed these symptoms. The reported diarrhea was severe, with two thirds of patients having at least seven bowel movements per day (Besser et al. 1999). Although bloody diarrhea is the hallmark symptom of infection, non-bloody watery diarrhea is common. Vomiting and nausea is also observed in about half of infected patients (Meyers, Schulman and Kaplan 1998). The illness generally resolves in five to seven days with no obvious sequelae. However, in 5-10% of *E. coli* O157:H7 cases, infection causes hemolytic-uremic syndrome (HUS), which leads to additional complications. STEC toxin enters the bloodstream and causes damage to the renal glomerular endothelial cells, which express receptors for the toxin. HUS is characterized by hemolytic anemia (anemia caused by destruction of red blood cells), uremia (acute kidney failure), and thrombocytopenia (low platelet count) (Boyer and Niaudet 2011). Decreased urine output, increased pallor, and mild jaundice are indicative of HUS, which typically occurs 1 to 14 days after the onset of diarrhea. Young children constitute an overwhelming majority of HUS cases.

E. coli O157:H7 causes an estimated 73,000 illnesses in the United States annually (Mead et al. 1999). A large proportion of *E. coli* O157:H7 foodborne illness outbreaks have been linked to contaminated meat; from 1998 to 2008, 41 percent of *E. coli* O157:H7 foodborne outbreaks were associated with ground beef (Rangel et al. 2005). However, foodborne outbreaks of *E. coli* O157:H7 have also been linked to produce items, such as lettuce, salad, coleslaw, melons, sprouts, and grapes. Unpasteurized apple juice and cider has been implicated in several *E. coli* O157:H7 outbreaks (Hilborn et al. 2000; CDC 1996; CDC 1997; Besser et al. 1993).

3.3.2. *Salmonella*

Every year, *Salmonella* is estimated to cause about 1.2 million illnesses in the United States, with about 23,000 hospitalizations and 450 deaths (Scallan et al. 2011a). Most persons infected with *Salmonella* develop diarrhea, fever, and abdominal cramps 12 to 72 hours after infection. Since *Salmonella* has been implicated in produce-related foodborne outbreaks, this risk assessment will attempt to estimate the risk of illness associated with consumption of apples contaminated with *Salmonella*.

Salmonella is a genus of Gram-negative rod-shaped bacteria of the family Enterobacteriaceae. While there are only a few species of *Salmonella*, thousands of serovars have been identified. Salmonellae are found worldwide in both cold-blooded and warm-blooded organisms, and in the environment. Pathogenic *Salmonella* can be categorized as typhoidal and non-typhoidal serovars, latter of which are more common. Food poisoning by *Salmonella* occurs when the person ingests a dose of an infectious serovar with subsequent growth of the bacteria in the gastrointestinal tract. Nearby cells are poisoned with endotoxins released from the salmonellae, leading to enteritis and gastrointestinal disorder. Symptoms of *Salmonella*-induced acute gastrointestinal illness (AGI) include diarrhea, fever, vomiting, and abdominal cramps. Most cases resolve without treatment; however, severe cases may require hospitalization, typically related to dehydration (CDC 2014).

Outbreaks of salmonellosis are typically associated with contaminated meat and poultry. However, many outbreaks in the U.S. have been linked to fresh produce over the past five years, including: cucumbers (2013), mangoes (2012), cantaloupe (2012, 2011 and 2008), and alfalfa sprouts (2011, 2010, and 2009) (CDC 2014). While no outbreaks have been associated with fresh apples, irrigation water has been identified as a possible source of microbial contamination that

could expose consumers to pathogens. Studies show that *Salmonella* can survive and grow on plant surfaces (Lynch et al. 2009).

3.3.3. *L. monocytogenes*

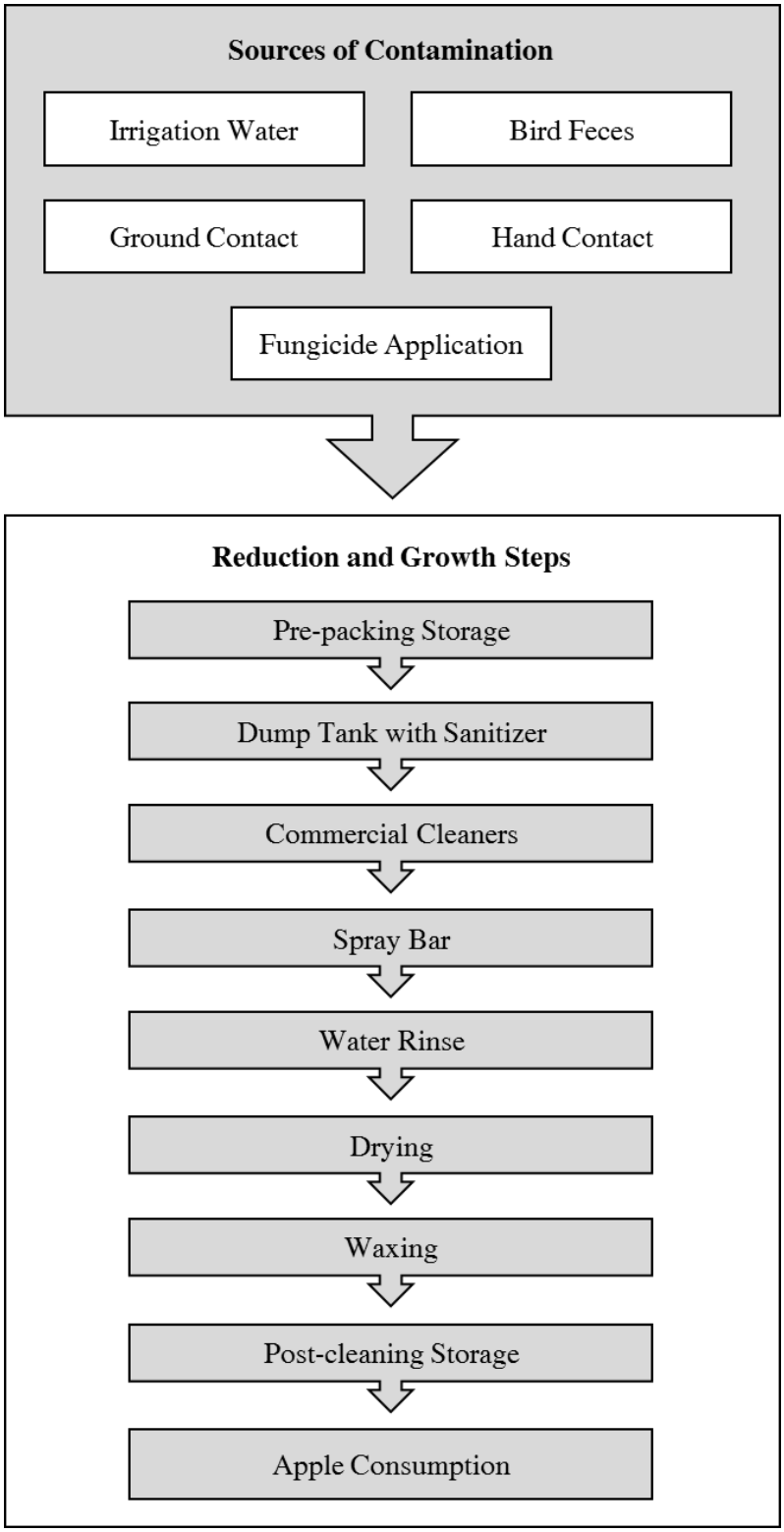
In the United States, *L. monocytogenes* is estimated to cause 19-28 percent of deaths due to foodborne illness annually (Scallan et al. 2011a; Mead et al. 1999). *L. monocytogenes* infections are associated with a 92.2% hospitalization rate and a 20% case fatality rate (Mead et al. 1999). The majority listeriosis cases occur in individuals with an underlying condition that leads to suppression of their T-cell-mediated immunity (Farber and Peterkin 1991). The clinical syndromes associated with adult listeriosis include mild diarrhea, meningitis, primary bacteremia, septicemia, central nervous system infections, and endocarditis (Farber and Peterkin 1991, Marth 1988). *L. monocytogenes* is thought to enter the host through the intestine, then actively multiply in the liver. Unrestricted proliferation of the pathogen in the liver may result in prolonged low-level bacteremia, potentially leading to invasion of secondary target organs (Vázquez-Boland et al. 2001)

L. monocytogenes is a Gram-positive, nonsporeforming, facultatively anaerobic bacterium. It is ubiquitous and is resistant to diverse environmental conditions including low pH and high NaCl concentrations (Rocourt et al. 2003). Additionally, *L. monocytogenes* is microaerophilic and psychrophilic. It grows between -0.4 and 50ºC (Junttila et al. 1988), and therefore can proliferate at food storage temperatures that typically inhibit microbial growth. Dairy products have been identified as a source of *L. monocytogenes*, but it has also been found in beef, pork, poultry, and seafood. In the United States, *L. monocytogenes* has been found on fresh produce such as cabbage, potatoes, cucumbers, celery, lettuce, onion, leeks, watercress, and radishes (Heisick et al 1989; Sizmur and Walker 1988).

3.4. Exposure Assessment

The purpose of the exposure assessment is to estimate the number of *E. coli*, *Salmonella*, or *L. monocytogenes* a person would ingest when consuming an average serving of apple. This estimated dose will factor possible sources of pathogen contamination, as well as growth and die-off associated with various cleaning and handling processes. Figure 3.3 is a flow chart depicting the sources of contamination and processes by which pathogen growth or die-off may occur. All three models include the same contamination sources and pathogen reduction/growth processes. The models are populated with microbe-specific data for environmental prevalence, cleaning step reduction, and growth. Where data for a particular parameter was unavailable, alternative data was used (as described in section 3.2). In some cases, it was necessary to utilize data from surrogate microbes (e.g., populating a Salmonella model parameter with data from an *E. coli* study) or proxy produce (e.g., populating a model parameter with data from a study that looked at microbial behavior on tomatoes). The models include switches that enable the user to turn modules "on" or "off" to customize the model configuration to include specific processes.

Figure 3.3 – Sources of Contamination and Reduction/Growth Processes



3.4.1. Sources of Contamination

Irrigation Water for Evaporative Cooling

This QMRA includes irrigation water – applied to apples during evaporative cooling – as a potential source of microbial contamination. Evaporative cooling involves spraying the apple orchards with water to prevent sun damage to the fruit prior to harvest. If the cooling water contains microbes, this process could introduce pathogens onto the apples' surface, potentially causing illness to consumers. The concentration of microbial contamination on a single apple is estimated by the equation:

$$C_{apple\_IW} = C_{IW} * \left(\frac{V_{IW}}{acre}\right) * \left(\frac{acre}{apples}\right) * F$$

Where C_{IW} is the concentration of bacteria in irrigation water, V_{IW} is the volume of irrigation water applied to an acre of orchard for evaporative cooling, and F is the fraction of applied irrigation water that lands on apples. The irrigation water component of the model allows the user to select between multiple levels of contamination. Pathogen concentration levels are based on data from the literature, field data, or values that reflect "worst case" scenarios. *E.coli* O157:H7 concentrations were derived from generic *E. coli* levels using a 1:100 ratio (FDA 2012). Distributions were applied to each value to account for variability or uncertainty. Table 3.1 provides bacterial concentration levels and respective data sources.

Table 3.1 – Microbial Concentration Parameters and Sources

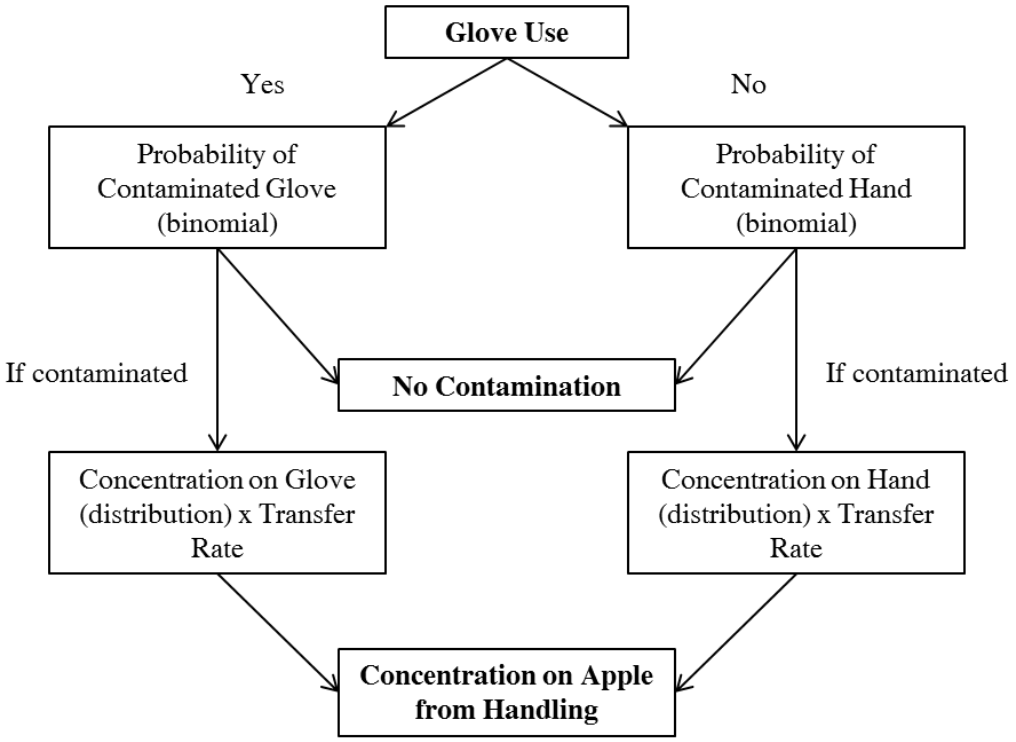
Model	Level	Mean Concentration (CFU/100ml)	Data source
E. coli O157:H7*	Most likely	46.13 (lognormal distribution; location = 0, mean = 46.13, standard deviation = 184.8)	Meschke et al. 2014; Irrigation water quality data, collected from agricultural surface waters in Washington State.
	High	1,575,897 (triangular distribution; likeliest = 1,575,897, min = 30,000, max = 6,200,000)	Payment et al (2001); concentration of *E. coli* in untreated wastewater
Salmonella	Most likely	18.2 (uniform distribution; min = 0, max = 36.3)	Haley et al. 2009; Salmonella densities estimated in waters in a rural watershed in Georgia.
	High	1,300 (normal distribution; mean = 1,300; standard deviation = 130)	Langeland 1982; Salmonella density in untreated wastewater in Oslo, Norway. 10-fold factor for a conservative estimate.
L. monocytogenes	Most likely	20 (normal distribution; mean = 20, standard deviation = 2)	Bernagozzi et al. 1994; Listeria densities in surface waters.
	High	700-18,000 (uniform distribution; min = 700, max = 18,000)	Bernagozzi et al. 1994; Listeria density in untreated wastewater

* concentrations in water are for generic *E. coli*; 1:100 ratio applied in model to estimate *E. coli* O157:H7 concentration.

Hand Contact

In the model, the user can choose between three options to incorporate contamination due to handling: glove use, no glove use, or unknown glove use. The first two options use the logic shown in Figure 3.4. A scenario with unknown glove use incorporates a probability into the model, based on the likelihood that gloves are used during harvest. While no literature was found describing glove use on apple orchards, a study by Pate and Nummer (2013) found that 66.6% of workers used gloves on USDA Good Agricultural Practice (GAP) certified farms.

Figure 3.4 – Flow Chart for Glove and Hand Contamination



No studies in the literature were found that examined the density of *E. coli* O157:H7, *Salmonella*, or *L. monocytogenes* on apple picker hands. Data was drawn from a study by Materon et al. (2007) that examined microbial loading on the hands of cantaloupe pickers in Mexico. Since melon harvest involves direct contact with soil, the values likely represent a highly conservative estimate of hand contamination in apple picking. Since data could not be found for glove contamination, the same data was used for both glove and hand contamination. Transfer rates between glove/hand and fruit were taken from a study that examined *Salmonella* transfer between gloves/hands and green bell peppers (Jimenez et al., 2007).

Bird Feces

Fecal contamination from birds has been discussed as a possible source of apple contamination (Beuchat and Ryu 1997). Even though there is limited evidence to demonstrate

that bird feces pose a high risk to the microbial quality of apple crops, this model incorporates a module that predicts the level of apple contamination due to bird feces. The level of contamination is the product of the pathogen density in feces and the mass of feces. The probability that a given apple is contaminated incorporates the number of birds per acre, defecations per bird per day, number of days that the apple is growing in the orchard, ratio of apple area to the acre of orchard, and a 10-fold factor (for the increased likelihood that a bird would perch on an apple tree).

Mass of feces and frequency of defecation was drawn from Duffy and Schaffner (2002). Their QMRA examined *E. coli* O157:H7 contamination on apples from bird droppings and ground manure. In their study, the mass of gull feces was derived from data on broiler chickens (Hermanson et al. 2001) and *E. coli* O157:H7 concentration in bird feces was estimated using data from cow feces (Zhao et al. 1995). Fecal *Salmonella* densities were taken from Fenlon (1981). The concentration of *L. monocytogenes* in feces was derived from ruminant shedding rates (Fenlon et al. 1996). Log decreases in *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* in the feces were taken from Himathongkham and Riemann (1999).

Ground Contact

While industry best management practices discourage collection of fallen apples, this model includes a module that introduces soil contact as a possible source of apple contamination. Transfer rates of bacteria from soil to apples were based off of a study by Guo et al. (2002) that examined tomato contamination from soil. Data from Guo et al. was used to generate a transfer parameter that estimates the concentration on a downed apple given a concentration in the soil. The models enable the user to select between two soil contamination levels: most likely and high. Most likely generic *E. coli* concentrations in soil were taken from Ishii et al. (2006) and

used to estimate *E. coli* O157:H7 concentration with the 1:100 ratio (FDA 2012). Most likely *L. monocytogenes* concentration in the soil was taken from Dowe (1997) and *Salmonella* soil concentration was taken from Trimble et al. 2013. High soil concentrations were derived from studies that examined sewage sludge. These high levels simulate worst-case scenarios in which soils are heavily contaminated. Worst-case generic *E. coli* concentrations were taken from Dudley et al. 1980. Krzyzanowski et al. (2014) found a maximum of 12 MPN/g of *Salmonella* in sewage sludge; to provide a more conservative worst-case scenario, high *Salmonella* soil concentrations were estimated to be the same as wastewater (Langeland 1982).

Fungicide Drench Application

After apples are picked, the collection bins may be drenched in fungicide. If mixed with contaminated water, the fungicide may serve as a source of pathogenic bacteria. The QMRA models include a module for fungicide application that incorporates either (a) the bacterial concentration specified in the irrigation water module, or (b) an alternative water contamination level. The alternate concentration may be utilized if water for the fungicide is drawn from a separate source (e.g., groundwater). It was assumed that 10ml of fungicide would coat the apple.

3.4.2. Cleaning Processes

Modules for apple storage, cleaning, and waxing processes were included in the models. Data on log reductions or growth was pulled from the literature and used to populate the models. Table 3.2 lists the reduction or growth values and respective sources associated with each cleaning step. In many cases, pathogen-specific data was not available; it was necessary to assume that *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* would exhibit similar responses to treatment processes. Many studies on the efficacy of decontamination treatments show similar

log reductions in *Salmonella*, *Listeria,* and *E. coli* when subject to uniform treatment conditions (Neal et al. 2012; Ruiz-Cruz et al. 2007; Tapp III et al. 2013; Yang et al 2009).

Table 3.2 – Distributions and Sources of Model Parameters for Cleaning Process

Cleaning Step	**Model**	**Log Reduction or Growth (log cfu)**	**Source and Notes**
Pre-cleaning storage (reduction)	*E. coli* O157:H7	Triangular distribution (likeliest = 0.13 per day, min = 0.08 per day, max = 0.16 per day)	Janes et al. 2002: examined the reduction of *E. coli* O157:H7 on inoculated apples stored at 4°C
	Salmonella	Triangular distribution (likeliest = 0.28 per day, min = 0.00 per day, max = 1.00 per day)	Guo et al. 2002: examined the survival of *Salmonella* on tomatoes stored at 20°C
	L. monocytogenes	No reduction	Conway et al. 2000: found no significant reduction of *L. monocytogenes* on inoculated apple slices
Pre-cleaning storage (growth)	*E. coli* O157:H7 and *Salmonella*	Triangular distribution (likeliest = 0.83 per day, min per day = 0, max per day= 1.16)	Janisiewicz et al. 1999: examined the growth of *E. coli* O157:H7 in damaged apple tissue at 24°C
	L. monocytogenes	No growth	Conway et al. 2000: found no significant growth of *L. monocytogenes* on inoculated apple slices
Chlorine dunk tank (reduction)	All models	Triangular distribution (likeliest = 3.0, min = 0.5, max = 4.9)	Sapers et al. 1999, Wisniewsky et al. 2000, Rodgers et al. 2004: studies investigated rates of pathogen reduction on inoculated apples in 200 ppm chlorine water
Commercial cleaners (reduction)	All models	Toggle between two cleaners (0.5 probability each). Cleaner A: triangle distribution (likeliest = 0.27, min = 0, max = 0.85); Cleaner B: triangle distribution (likeliest = 0.47, min = 0, max = 0.99)	Annous et al. 2001: examined a variety of washing agents and their efficacy at removing *E. coli* from inoculated apples

Cleaning Step	Model	Log Reduction or Growth (log cfu)	Source and Notes
PAA spray bar (reduction)	All models	Triangular distribution (likeliest = 1.12, min = 0.76, max = 1.42)	Wang et al. 2006: examined the *E. coli* O157:H7 population reduction on fresh-cut apples after treatment with PAA
Chlorine spray bar (reduction)	*E. coli* O157:H7	Triangular distribution (likeliest = 0.75, min = 0.68, max = 0.83)	Beuchat et al 1998: examined the efficacy of chlorinated water spray in killing *E. coli* O157:H7 on apples.
	Salmonella	Triangular distribution (likeliest = 0.75, min = 0.38, max = 1.15)	Beuchat et al 1998: examined the efficacy of chlorinated water spray in killing *Salmonella* on apples.
	L. monocytogenes	Triangular distribution (likeliest = 0.84, min = 0.73, max = 0.95)	Beuchat et al 1998: examined the efficacy of chlorinated water spray in killing *L. monocytogenes* on apples.
Water rinse (reduction)	All models	Triangular distribution (likeliest = 0.57, min = 0.11, max = 0.99)	Annous et al. 2001: examined a variety of washing agents and their efficacy at removing *E. coli* from inoculated apples
Drying (reduction)	All models	Triangular distribution (likeliest = 0.27, min = 0.19, max = 0.30)	Laboratory data from method evaluation in Chapter 2. Reductions due to drying were assumed to be half of difference between inoculation and recovery.
Waxing (reduction)	*E. coli* O157:H7 and *L. monocytogenes*	Triangular distribution (likeliest = 0.32, min = -0.28, max = 1.22)	Kenney and Beuchat 2002: examined the effect of waxing on *Salmonella*-inoculated apples
	Salmonella	Triangular distribution (likeliest = 0.40, min = -0.11, max = 0.92)	Kenney and Beuchat 2002: examined the effect of waxing on *Salmonella*-inoculated apples
Post-cleaning storage (reduction)	*E. coli* O157:H7	Triangular distribution (likeliest = 0.13 per day, min = 0.08 per day, max = 0.16 per day)	Janes et al. 2002: examined the reduction of *E. coli* O157:H7 on inoculated apples stored at 4°C
	Salmonella	Triangular distribution (likeliest = 0.28 per day, min = 0.00 per day, max = 1.00 per day)	Guo et al. 2002: examined the survival of Salmonella on tomatoes stored at 20°C

Cleaning Step	Model	Log Reduction or Growth (log cfu)	Source and Notes
	L. monocytogenes	No reduction	Conway et al. 2000: found no significant reduction of *L. monocytogenes* on inoculated apple slices
Post-cleaning storage (growth)	*E. coli* O157:H7 and *Salmonella*	Triangular distribution (likeliest = 0.83 per day, min per day = 0, max per day= 1.16)	Janisiewicz et al. 1999: examined the growth of *E. coli* O157:H7 in damaged apple tissue at 24°C
	L. monocytogenes	No growth	Conway et al. 2000: found no significant growth of *L. monocytogenes* on inoculated apple slices

3.5. Dose-Response Assessment

This dose-response assessment was conducted to describe the relationship between the number of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* consumed and the likelihood of gastrointestinal illness. A dose-response model is, in essence, a mathematical function where the input is a dose of contaminant and the output is the probability of particular adverse health effect. Two key features differentiate microbial hazards from other health risks such as chemicals. First, a population of humans exposed to microbial hazards will necessarily receive a distribution of doses. Second, infectious microorganisms are able to propagate within a host. Given these factors, Haas et al. (1999) posit that there are two biologically-plausible dose-response models for microbiological hazards: exponential and beta-Poisson.

The exponential dose-response model is the simplest dose-response model. This model assumes that (a) the distribution of organisms between doses is random, (b) each organism has an independent and identical probability of survival, and (c) that one organism is sufficient to cause infection (Haas et al. 1999). If the probability of survival is represented by parameter k, the probability of response is given by:

$$P(response) = 1 - \exp(-k * dose)$$

The exponential model assumes that the pathogen-host survival probability is constant. The beta-Poisson model instead assumes non-constant survival and infection probabilities, where the probabilities of survival, *k*, are given by the beta distribution. Given a median infectious dose, N_{50}, and a slope parameter of the equation, α, the probability of response in the beta-Poisson model is:

$$P(response) = 1 - \left[1 + \frac{dose}{N_{50}}\left(2^{1/\alpha} - 1\right)\right]^{-\alpha}$$

or $\quad P(response) = 1 - \left[1 + \frac{dose}{\beta}\right]^{-\alpha}$ where $\quad \beta = \frac{N_{50}}{2^{1/\alpha} - 1}$

In most studies, the maximum likelihood estimation (MLE) method is used to fit dose response data to the models described above by minimizing the deviance, as described by various authors (Haas 1983; Haas et al. 1993; Regli et al. 1991; Teunis et al. 1996). Data sources for dose-response models include human feeding studies, animal experiments, and outbreak data. Each of these sources presents unique challenges to dose-response modelling. For example, epidemiological outbreak data introduces a high level of uncertainty, while animal studies may not reflect the pathogen-host interactions that occur in humans. To provide more robust estimates of illness given a dose of bacteria, multiple dose-response models are used for each risk assessment model.

3.5.1. *E. coli* O157:H7

Five previously described dose response models were utilized to estimate the likelihood of gastrointestinal illness given ingestion of *E. coli* O157:H7. Dose-response model parameters from *E. coli* O157:H7 models are provided in Table 3.3; model curves are depicted in Figure 3.5. The first model was taken from Haas et al. (2000). Since there are no known human dose-

response studies for *E. coli* O157:H7, the authors analyzed animal dose-response data. Pai et al. (1986) examined diarrheal disease in infant rabbits after oral inoculation of *E. coli* O157:H7. Using the MLE method, Haas et al. (2000) generated parameters for both exponential and beta-Poisson models. Using a goodness of fit test, the authors found that the beta-Poisson model provided an acceptable fit to the data, while the exponential model did not. For validation, the model was compared against data from two human E. coli O157:H7 outbreaks. In both outbreaks, the predicted concentrations came close to the observed concentrations.

The second *E. coli* O157:H7 dose-response model is taken from Powell et al. (2000). Because there are no human feeding studies for *E. coli* O157:H7, Powell et al. utilized data from two enteropathogenic *E. coli* (EPEC) human feeding studies. The pooled data from Bieber et al. (1998) and Levine et al. (1978) yield a beta-Poisson model that is not statistically different from the fits provided by the individual models.

The third dose-response model is taken from Teunis et al. (2004). In 1996, a foodborne outbreak occurred at an elementary school in Morioka city, Japan. Stool samples from pupils and teachers implicated *E. coli* O157:H7 as the infectious agent. Teunis et al. used data from this outbreak to generate a dose-response model. A Markov chain Monte Carlo method (Metropolis-Hastings algorithm) was used to generate posterior mode parameter values. The proposed beta-Poisson model was compared to other *E. coli* O157:H7 dose-response models using a likelihood ratio test.

The fourth *E. coli* O157:H7 dose-response model was taken from Strachan et al. (2005). This analysis pulled data from eight different *E. coli* O157:H7 outbreaks, linked to both foodborne and environmental sources. Using the MLE method to fit the data, the authors generated exponential, and beta-Poisson dose-response models. The overall best fit was the exact

beta-Poisson with beta-binomial likelihood. While the fit is not statistically significant, the authors attribute this to the high variation in data obtained from outbreaks, compared to controlled feeding studies. Strachan et al. found that previous *E. coli* O157:H7 dose-response models – which used *Shigella* as a surrogate – were contained within confidence intervals of the proposed model.

The final *E. coli* O157:H7 dose-response model is taken from Danyluk and Schaffner (2011), which is based on a model by Cassin et al. (1998). With the assumption that *Shigella dysenteriae* exhibits similar virulence to *E. coli* O157:H7, Cassin et al. used data from three *S. dysenteriae* human feeding studies to generate a beta-binomial dose-response model. Danyluk and Schaffner (2011) converted the beta-binomial model into a simplified beta-poisson model.

Figure 3.5 – *E. coli* O157:H7 Dose-Response Model Curves

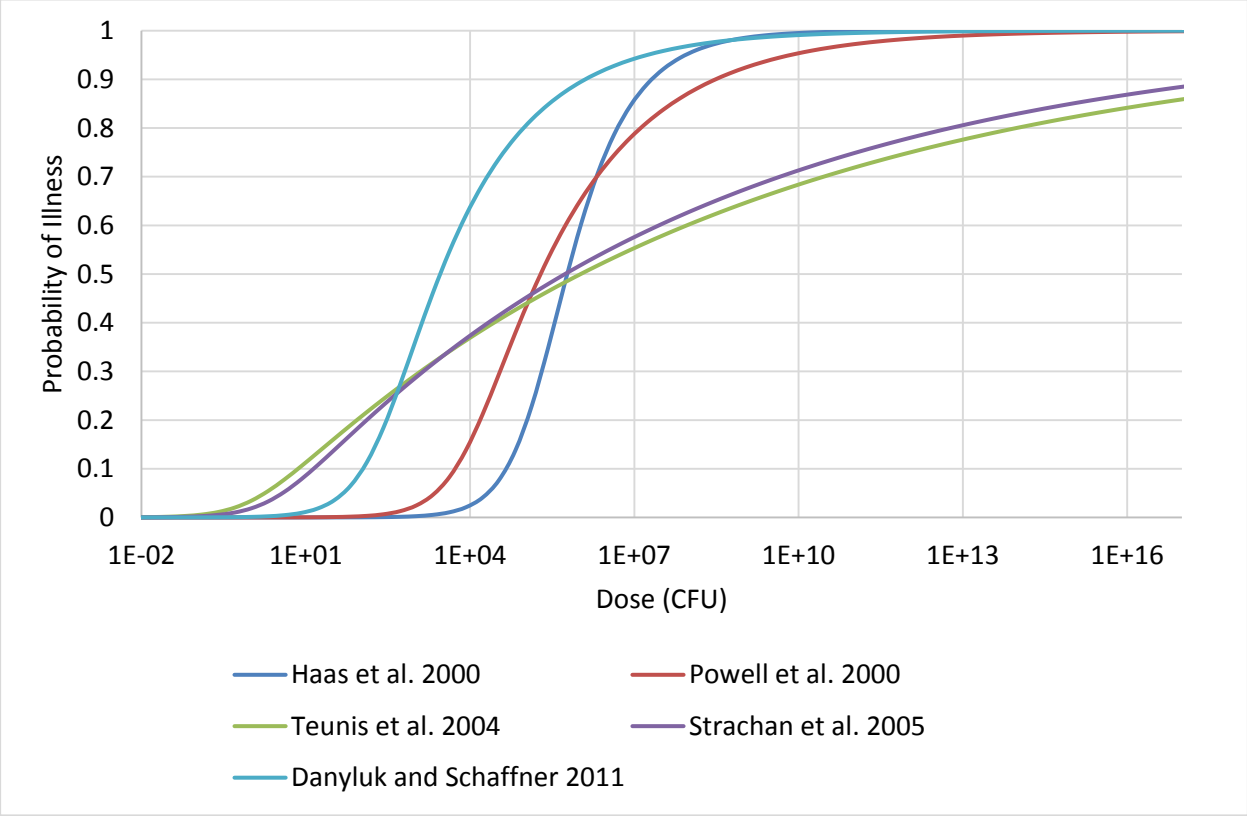


Table 3.3 – *E. coli* O157:H7 Dose-Response Model Parameters

Study	**Model**	**α**	**β**
Haas et al. 2000	Beta-Poisson	0.49	1.91×10^{5}
Powell et al. 2000	Beta-Poisson	0.22	8.7×10^{3}
Teunis et al. 2004	Beta-Poisson	0.050	1.001
Strachan et al. 2005	Beta-Poisson	0.0565	2.5487
Danyluk and Schaffner 2011	Beta-Poisson	0.267	229.2928

3.5.2. *Salmonella* spp.

Four previously described dose-response models were utilized to estimate the likelihood of illness due to ingestion of *Salmonella* on contaminated apples. Dose-response model parameters from *Salmonella* models are provided in Table 3.4; model curves are depicted in Figure 3.6. Fazil (1996) analyzed human feeding trial data and fit beta-Poisson, lognormal, and exponential dose-response models. Feeding trial data was taken from McCullough and Eisele (1951a, 1951b), in which healthy males from a penal institution were fed non-typhoid *Salmonella*. Five different serotypes were used, including *S.* Anatum, *S.* Meleagridis, *S.* Newport, *S.* Bareilly, and *S.* Derby. Fazil (1996) proposed that the beta-Poisson model best described the *Salmonella* dose-response relationship. Furthermore, it was reported that all the serotypes could be adequately described using a single beta-Poisson dose-response curve.

FAO/WHO (2002) posit a modified beta-Poisson model based on the work of Fazil (1996). Since the original model did not consider the effect of multiple feedings on the dose-response relationship, FAO/WHO used only naïve subject data. Subjects from McCullough and Eisele (1951a, 1951b) who received multiple doses were dropped from the analysis. Using the MLE method to fit the data, a beta-Poisson model was proposed.

The third dose-response model employed in this QMRA is the USDA (1998) model for *Salmonella* Enteritidis. USDA initially fitted a beta-Poisson model to data from *Salmonella*

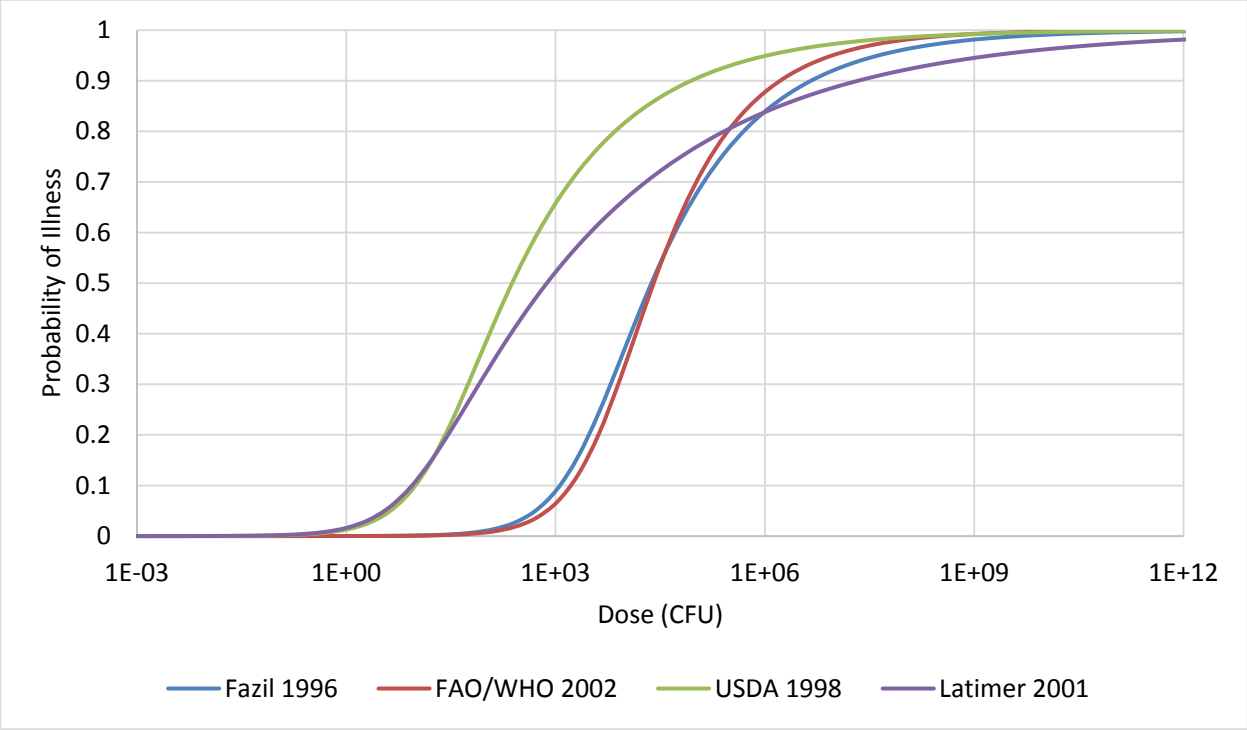
feeding trails in McCullough and Eisele (1951a, 1951b). However, this model did not reflect the higher attack rate and low doses observed in *S.* Enteritidis epidemiological outbreak data. USDA instead based their dose-response model on *Shigella dysenteriae*, using human feeding trial data from Levine et al. (1973). A beta-Poisson model was fit to the data, and comparison to *Salmonella* epidemiological outbreak data showed that the *S. dysenteriae* dose-response model was preferable to the prior model based on *Salmonella* feeding studies. To introduce uncertainty in the model, USDA characterized the beta parameter as a normal distribution. While models were created for both susceptible and normal populations, only the model for the normal population is utilized here.

The final dose-response model is drawn from Latimer et al. (2001). This study proposed a weighted composite dose-response model which incorporated three pathogenicity-specific models to account for uncertainty in *Salmonella* virulence. Lack of human feeding data at low dose levels led Latimer et al. to use *S. dysenteriae* 1 as a proxy for low-dose *Salmonella* strains. Exponential, two-subpopulation exponential, and beta-Poisson models were proposed at each pathogenicity level (high, moderate, and low). The beta-Poisson models fit well for all three pathogenicity levels. While Latimer et al. incorporated multiple models into the weighted composite dose-response model, only the high pathogenicity beta-Poisson model is utilized here.

Table 3.4 – *Salmonella* Dose-Response Model Parameters

Study	**Model**	**α**	**β**
Fazil 1996	Beta-Poisson	0.3126	2885
FAO/WHO 2002	Beta-Poisson	0.4047	5587
USDA 1998	Beta-Poisson	0.2767	Normal(μ:21.159, σ:20, min:0, max:60)
Latimer et al. 2001	Beta-Poisson	0.157	9.17

Figure 3.6 – *Salmonella* Dose-Response Model Curves



3.5.3. *L. monocytogenes*

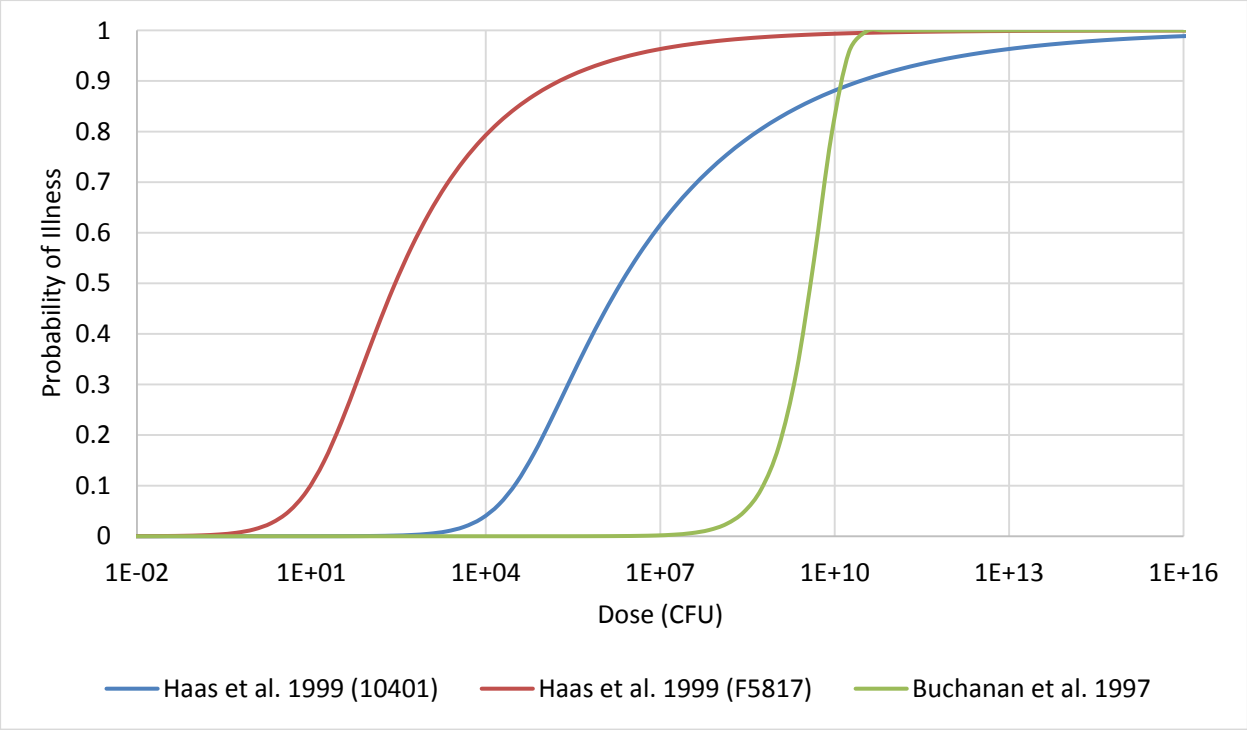
Dose-response models for *L. monocytogenes* were taken from Haas et al. (1999) and Buchanan et al. (1997). The former study utilized animal data, in which Swiss conventional female OF1 mice were exposed to *L. monocytogenes* 10401 (Audurier et al. 1979) and C57B1/6J mice were exposed to *L. monocytogenes* F5817 (Golnazarian et al. 1989). In both animal studies, infection was verified through detection of viable bacteria in the liver, lungs, spleen, brain, or kidneys. Data for *L. monocytogenes* strains were pooled together and analyzed using the MLE method. The beta-Poisson model was determined to be a better fit. In the case of the pooled F5817 dose-response model, pooled trial data was shown to improve the model. Buchanan et al. (1997) proposed a dose-response model based on epidemiologic data. Examining consumption rates of smoked fish, observed levels of *L. monocytogenes* in samples of the food matrix, and incidence of listeriosis in consumers, Buchanan et al. derived an exponential dose-response

model. Dose-response model parameters from *L. monocytogenes* models are provided in Table 3.5; model curves are depicted in Figure 3.7.

Table 3.5 – *L. monocytogenes* Dose-Response Model Parameters

Study	**Model**	**α**	**N50**	**k**
Haas et al. 1999 (10401)	Beta-Poisson	0.17	$2.1x10^{6}$	-
Haas et al. 1999 (F5817)	Beta-Poisson	0.25	$2.76x10^{2}$	-
Buchanan et al 1997	Exponential	-	-	$1.79x10^{-10}$

Figure 3.7 – *L. monocytogenes* Dose-Response Model Curves



3.6. Results

3.6.1. Model Simulations

Models were run under four scenarios (A-D), each with increasing likelihood of contamination and/or ineffective pathogen reduction processes. Scenarios are described in Table 3.6. Scenario A is an expected situation for hand-picked apples. The ground contamination

module was turned off, as best management practices discourage the harvest of fallen apples. Concentrations of pathogens in irrigation water and fungicide were set at "most likely" levels. PAA, rather than chlorine, was used in the spray bar. Since pathogen growth during storage is associated with tissue damage or puncture on the apples, this module was turned off with the assumption that damaged apples would be removed during sorting processes. Scenario B included soil contact as a source of contamination, where the probability that an apple is retrieved from the ground is 1:100. This scenario also included pathogen growth during storage and chlorine, instead of PAA, in the spray bar.

Table 3.6 – Model Components Utilized in Four Scenarios

Module	Scenario A Best case	Scenario B Good case	Scenario C Worst case: high contamination	Scenario D Worst case: no cleaning
Irrigation water	YES (low)	YES (low)	YES (high)	YES (low)
Ground contact	NO	YES (low)	YES (high)	YES (low)
Hand/glove contact	YES	YES	YES	YES
Bird feces	NO	YES	YES	YES
Fungicide application	YES (low)	YES (low)	YES (high)	YES (low)
Pre-cleaning storage (reduction)	YES	YES	YES	NO
Pre-cleaning storage (growth)	NO	YES	YES	YES
Dump tank	YES	YES	YES	NO
Commercial Cleaners	YES	NO	YES	NO
PAA Spray Bar	YES	NO	YES	NO
Chlorine Spray Bar	NO	YES	NO	NO
Water rinse	YES	YES	YES	NO
Drying	YES	YES	YES	NO
Waxing	YES	YES	YES	NO
Post-cleaning storage (reduction)	YES	YES	YES	NO
Post-cleaning storage (growth)	NO	NO	YES	YES

Scenario C and D represent "worst case" scenarios. Scenario C is a situation in which high levels of contamination occur through both irrigation water and soil. Pathogen concentrations were based on untreated wastewater and sewage sludge for water and soil, respectively. Cleaning steps were turned on in Scenario C. In scenario D, pathogen concentrations in soil and water were set at lower levels, but all reduction steps were turned off. Table 3.6 illustrates scenario module components.

3.6.2. Risk Characterization

The risk characterization incorporates the exposure assessment and dose-response components of the model to estimate the risks of illness associated with the consumption of an apple. This calculates the probability of illness, given a dose of infectious agent, using the beta-Poisson or exponential dose-response model given in Section 3.5. All models were run with a toggle between all dose-response models. Tables 3.7, 3.8, and 3.9 provide the model outputs for *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes*, respectively.

Table 3.7 – *E. coli* O157:H7 Model Outputs Under Four Scenarios

		Scenario A	Scenario B	Scenario C	Scenario D
Dose (CFU/serving)	Mean	5.21×10^{-8}	1.13×10^{-6}	58.3	1,286
	Median	6.04×10^{-11}	6.99×10^{-10}	0.0177	0.0861
	5^{th} Percentile	1.37×10^{-13}	1.31×10^{-12}	2.66×10^{-5}	1.14×10^{-4}
	95^{th} Percentile	7.85×10^{-8}	1.15×10^{-6}	24.9	343
Probability of Illness	Mean	6.82×10^{-10}	2.29×10^{-8}	0.0104	0.0262
	Median	4.68×10^{-14}	4.99×10^{-13}	1.39×10^{-5}	7.32×10^{-5}
	5^{th} Percentile	0	0	1.05×10^{-9}	4.27×10^{-9}
	95^{th} Percentile	4.67×10^{-14}	5.30×10^{-9}	0.0665	0.18

Table 3.8 – *Salmonella* Model Outputs Under Four Scenarios

		Scenario A	Scenario B	Scenario C	Scenario D
Dose (CFU/serving)	Mean	9.79×10^{-5}	3.60×10^{-3}	0.951	4.88×10^{6}
	Median	1.44×10^{-7}	3.99×10^{-6}	3.27×10^{-4}	633.0
	5th Percentile	1.58×10^{-10}	4.94×10^{-9}	1.48×10^{-6}	4.39
	95th Percentile	6.30×10^{-5}	1.60×10^{-3}	0.343	1.13×10^{6}
Probability of Illness	Mean	1.34×10^{-6}	3.29×10^{-5}	1.91×10^{-3}	0.370
	Median	1.57×10^{-10}	4.34×10^{-9}	4.40×10^{-7}	0.314
	5th Percentile	6.41×10^{-14}	1.99×10^{-12}	3.80×10^{-10}	7.98×10^{-4}
	95th Percentile	2.54×10^{-7}	6.83×10^{-6}	1.19×10^{-3}	0.908

Table 3.9 – *L. monocytogenes* Model Outputs Under Four Scenarios

		Scenario A	Scenario B	Scenario C	Scenario D
Dose (CFU/serving)	Mean	6.14×10^{-6}	0.0421	0.0116	53.1
	Median	2.80×10^{-7}	4.50×10^{-7}	1.42×10^{-5}	3.20×10^{-3}
	5th Percentile	6.57×10^{-9}	6.99×10^{-9}	7.73×10^{-8}	1.05×10^{-3}
	95th Percentile	1.89×10^{-5}	0.0539	0.0147	430.9
Probability of Illness	Mean	1.88×10^{-9}	1.14×10^{-5}	3.29×10^{-6}	7.10×10^{-3}
	Median	2.31×10^{-14}	8.57×10^{-14}	1.86×10^{-12}	3.26×10^{-10}
	5th Percentile	0	0	1.11×10^{-16}	2.65×10^{-13}
	95th Percentile	3.94×10^{-9}	5.13×10^{-8}	6.31×10^{-7}	6.41×10^{-5}

3.6.3. Sensitivity Analysis

A tornado analysis was performed to determine which parameters in each model had the greatest impact on the outcome projection (probability of illness). This analysis utilized built-in features of Crystal Ball™ to calculate sample correlation coefficients. Sample correlation coefficients provide a measure of linear association between two variables, and are used in sensitivity analysis to evaluate the relative effect of model input variables on outcomes. A tornado analysis was performed on the *E. coli* O157:H7 model. Graphical outputs of the analysis are shown in Appendix A. From this analysis, the most influential input variables were the log increase per day in storage and log reduction from dump tank in the *E. coli* O157:H7 model. The

most sensitive variable of the *Listeria* model was the concentration of *Listeria* in bird feces. Indeed, it was assumed that *Listeria* levels in feces – unlike *E. coli* or *Salmonella* – would not decrease over time (Himathongkham and Riemann 1999).

3.7. Discussion

3.7.1. Estimation of Foodborne Illness Risks

Under the best case scenario, the median risk of foodborne illness associated with apple consumption was estimated at 4.68×10^{-14}, 1.57×10^{-10}, and 2.31×10^{-14} for *E. coli* O157:H7, Salmonella, and *L. monocytogenes*, respectively. Mean probabilities of foodborne illness were estimated at 5.21×10^{-8} for *E. coli* O157:H7, 1.34×10^{-6} for *Salmonella*, and 1.88×10^{-9} for *L. monocytogenes*. These estimates suggest that there is a low risk of foodborne illness for healthy adults when consuming apples. This is consistent with the low occurrence of foodborne outbreaks associated with whole apples. The true probability of illness may even be lower, as the models were intentionally developed to be health protective (e.g., assuming that all irrigation water applied for evaporative cooling would have some pathogen load). The large differences between mean and median probabilities of illness suggest a highly skewed distribution of model outputs. When estimating the probability of illness associated with a single consumption event, it may be appropriate to use the median of the distribution. Alternatively, when considering the population risk, the mean may be a more appropriate value to examine.

Under worst-case scenarios, the risk of foodborne illness was estimated to be considerably greater. However, these worst-case scenarios are unlikely to occur in reality; assumptions in the worst-case models included contamination via numerous sources and/or significant failure of cleaning processes to remove pathogens from apple surfaces. Contamination levels such as those used in the worst case scenarios (e.g., irrigation water with

contamination equal to untreated wastewater) are extremely unlikely to occur in apple orchards. However, the worst case scenarios provide valuable insights into the relative impact of contamination sources and cleaning steps on the probability of illness. The model outputs for scenario C (high contamination with cleaning steps on) were greater than model outputs for scenario D (low contamination with cleaning steps off). For *Salmonella* and *L. monocytogenes*, the probability of illness estimated under scenario C and scenario D differed by several orders of magnitude. This suggests that the effectiveness of cleaning steps has a greater impact on the microbial quality of apples than orchard conditions. This finding has potential implications for the selection of appropriate policies to address microbial contamination of apples.

3.7.2. Limitations and Directions for Future Research

The models provided here provide insight into the sources of apple contamination and the cleaning processes used to reduce pathogen loads. However, there are several assumptions and limitations that challenge the certainty of the models' estimates. The models are largely limited by missing data; more robust datasets could provide more reliable risk estimates. In several instances, model parameters were populated with data from surrogate microorganisms, alternative environmental conditions, and studies that examined other types of produce. Minimal data was found that adequately characterized orchard conditions, and alternative data was used. For example, while birds are observed in apple orchards, the literature lacks data that characterizes bird behaviors, frequencies of defecation, and carriage of pathogenic microbes. Studies that survey the microbiological ecology of water, soil, and air in and around apple orchards would enhance these models.

While the literature is robust with studies that provide data on the efficacy of various antimicrobial processes for apples, experimental studies that examine these processes may not

reflect the actual microbial reduction and growth rates observed in packinghouses. Future research may seek to better understand the sources of contamination and cleaning processes that inform the microbial safety of fresh apples. Furthermore, as not all packinghouses utilize the cleaning steps included in the model, robust industry data on the use of these processes would enhance the reliability of model outputs.

4. Food Safety Policy: Values, Processes, and the Role of Science

Scientific research in the area of food safety both guides – and is guided by – policy decisions. Yet, allusions to policy implications in scientific publications are typically brief and lack substantive discussions about the specific policy processes in which the research findings may apply. Using broad frameworks, I aim to deconstruct United States food safety policy processes to answer the question: what is the role for this research in public policy? Specifically, this chapter will:

a) Review the history of food safety policy in the United States
b) Examine the values, institutions, and processes that guide food safety policy
c) Posit ways that study findings from previous chapters may be employed in policy processes

4.1. United States Food Safety Policy: Past and Present

While current food safety policies are implemented at the international, national, state, and local levels, the broadest and farthest-reaching policies in the United States have historically been implemented by the Federal Government. A timeline of federal food safety legislation and noteworthy agency changes is provided in Appendix B. Federal regulation of food safety originated in the Department of Agriculture (USDA), which was formed in 1862 with a mission to aid and promote American agriculture (Merrill and Francer 2000). The earliest food safety legislation, which aimed to prevent the importation of adulterated teas, went into effect in 1883 (Wilson 1942). The first statute aimed at regulation of domestic food was passed in 1886; it taxed margarine and sought to regulate cheese and butter imitations. While seemingly intended to protect consumers, the statute was largely a form of economic protectionism; the legislation was designed to protect dairy farmers from the growing threat of margarine producers (Merrill and Francer 2000).

The next significant food safety laws in the United States were the Pure Food and Drug Act (PFDA) and the Meat Inspection Act (MIA), both passed in 1906. The PFDA aimed to address the introduction of adulterated foods into interstate commerce. The MIA established a program of systematic inspection of meat processing facilities. The passage of both pieces of legislation was aided significantly by the publication of Upton Sinclair's *The Jungle*, which exposed the alarming practices of the Chicago meat packing industry. Furthermore, a coalition that included the American Medical Association, the American Public Health Association, labor unions, and consumer groups formed to support the legislation, which was opposed by food producers (Wiley 1929). Implementation of the PFDA and MIA was delegated to the USDA's Bureau of Chemistry and Bureau of Animal Industry, respectively (Herrick 1944).

Over the next 80 years, several other pieces of legislation were passed in the United States. These included the Grain Standards Act of 1916, the Filled Milk Act of 1923, the Food Drug, and Cosmetic Act of 1938, The Fungicide, Insecticide, and Rodenticide Act of 1947, the Poultry Products Inspection Act of 1957, the Fair Packaging and Labeling Act of 1967, the Egg Products Inspection Act of 1970, and the Toxic Substances Control Act of 1976 (Johnson 2014). Throughout this period, numerous changes in the federal bureaucracy occurred. The Food, Drug, and Insecticide Administration (FDIA), created within the USDA in 1927, became the Food and Drug Administration (FDA) in 1930 and was transferred to the Federal Security Agency in 1940. The FDA was later transferred to the Department of Health, Education, and Welfare – the predecessor of the current Department of Health and Human Services. Food safety policy in the 20th century therefore represents not only an increasing role of federal government in food safety, but a continually-changing landscape of institutions and ideologies.

Currently, United States food safety policies are implemented at the national, state, and local level. In the Federal Government, responsibilities for food safety fall primarily on the Food and Drug Administration (FDA) and the U.S. Department of Agriculture (USDA). The FDA is charged with ensuring the safety of all domestic and imported food products, with the notable exceptions of most meats and poultry. The USDA regulates most meat and poultry products, as well as some egg products. The Government Accountability Office has identified as many as 15 different federal agencies that collectively administer at least 30 food safety laws (Johnson 2014). Additionally, state and local authorities are responsible for various food safety functions, including the inspection of food establishments.

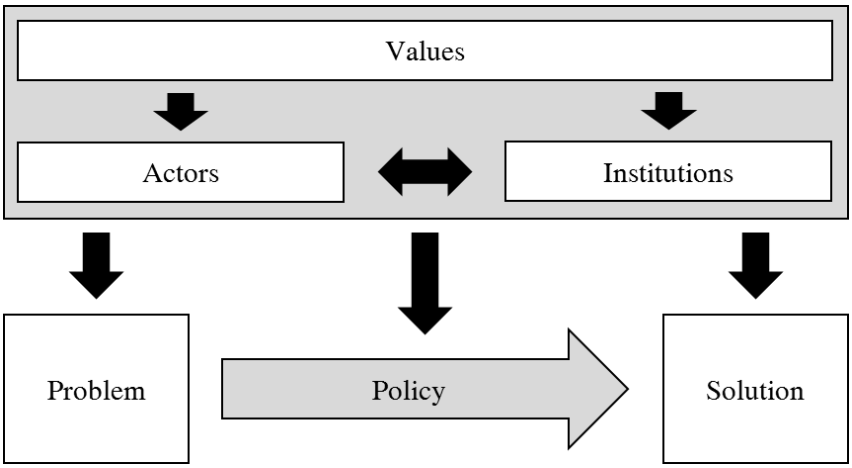
The piece of legislation most pertaining to apple production is the FDA Food Safety Modernization Act (FSMA), which was signed into law by President Obama in January 2011. FSMA is intended to protect human health by enabling the FDA to focus on prevention of food safety issues rather than taking a reactionary approach to foodborne outbreaks (FDA 2014). The law provides the FDA with enforcement authorities intended to achieve higher compliance with regulatory standards, using a risk-based approach to food safety. It also gives the FDA tools to hold imported foods to the same standards as domestic foods, and "directs FDA to build an integrated national food safety system in partnership with state and local authorities" (FDA 2014). Proposed rules under FSMA – namely Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption – have implications for apple growers in the US.

4.2. Values, Actors, Institutions, and Policy Processes in Food Safety

Figure 4.1 illustrates a basic framework for conceptualizing public policy. A policy can be defined as a relatively stable, purposive course of action that guides actors or institutions in

dealing with a problem. In a public policy context, a problem can be defined as a situation that causes people distress or dissatisfaction, for which relief is sought through policy. The identification of a problem, desired outcome, and policy design are informed by values, actors, and institutions.

Figure 4.1 – A Basic Policy Framework



For this analysis, values can be defined as the principles that guide an individual's behavior. Values necessarily inform the actions – or inactions – of policy actors. Policy actors are individuals or groups that seek to influence the creation and implementation of policies to address public problems. Institutions are formal or informal mechanisms that persist over time and structure the behavior of actors by constraining, enabling or encouraging behaviors. Policy actors can create, alter, or dismantle institutions; conversely, institutions may influence the behavior of actors.

4.2.1. Values in Food Safety

Policy scholars have long realized that principles and values play a vital role in policy formulation. As Lindblom and Woodhouse (1993) suggest, a rational person's first step after

identifying a problem is identifying and ranking values. To understand the food safety policy process, one must first understand the values that guide decision-making. Values inform the behavior of actors and the construction of institutions which, together, inform the design and implementation of public policies. An individual's values are not static; interactions between actors and institutions result in a dynamic system of values that evolves over time.

Under the assumption that prevailing values guide decision-making, an examination of past policies should reveal the dominant principles that guide food safety policy in the United States. I posit that two core American values have shaped the trajectory of US food safety policy: community and self-determination. Policy actors' positions on food safety policy – both for and against government intervention – have been informed by these principles.

Community

The notion of community is foundational to the American identity. The religious ideologies that guided early European settlers placed tremendous value on community. In his famous thesis, John Winthrop articulates "that every man might have need of others, and from hence they might be all knit more nearly together in the bonds of brotherly affection… every man afford his help to another in every want or distress" (1630). The Declaration of Independence concludes with the statement, "we mutually pledge to each other our lives, our fortunes and our sacred honor." Early Americans' sense of obligation to community informed a collective American identity which, while respecting the rights of the individual, recognizes a commitment to support fellow citizens.

In the early 20th Century, the value of community manifested in government programs that sought to promote the maximization of social welfare. Drawing from values of community, the Progressive Era saw introduction of antitrust laws, which aimed to improve competition by

breaking up large monopolistic corporations. Theorists and politicians of the early 20th century – including Herbert Croly, Theodore Roosevelt, and Woodrow Wilson – argued that social and economic inequalities justified government intervention to protect the welfare of American citizens. As President Wilson explained in his campaign speech, "You know that it was Jefferson who said that the best government is that which does as little governing as possible, which exercises its power as little as possible… But that time is past. America is not now, and cannot in the future be, a place for unrestricted individual enterprise" (1912). In *The Promise of American Life*, Herbert Croly argued that the founding fathers' envisioned government was insufficient to protect the people (1909). Progressives felt that unrestricted corporate entities contributed to adverse social conditions; government intervention was therefore warranted to improve the quality of life in the United States.

The Federal Government's role in food safety has strong ties to ideologies of the Progressive Era. It was during this time that growing concern about the quality of food, water, and medicine led to the passage of consumer safety legislation, including the PFDA and MIA in 1906 (Law 2003). This legislature set a precedent for federal regulation in industries whose products affected public health. Although the U.S. Constitution does not guarantee rights to the protection of health (Zietlow 2011), there is an implicit contract between individuals and society that places health-related functions above other social services (Butler 1989). Regulation relating to food safety, although not solely motivated by health concerns, carries with it an inherent recognition of wellbeing. For example, the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) established a system of pesticide regulation for the protection of human health and the environment.

In addition to the health and wellbeing of US citizens, food safety policies address the economic welfare of the nation. The burden of healthcare costs associated with foodborne illness is, among other factors, a driver behind the development of food safety regulation. Lost producer profits due to foodborne outbreaks – in the form of food recalls, lost sales, and legal fees – may provide further incentive for government intervention in food safety. Specifically, policies that focus on the prevention of produce contamination could reduce the costs associated with outbreaks and recalls. FSMA is federal legislation aimed at protecting not only consumers of food, but producers as well. This collectivist and utilitarian ideology stems from a deep-rooted value of community.

Self-Determination

Self-determination, or individual liberty, is a core American value that has shaped the policy terrain in the United States. Moral claims of individual liberty are intrinsically tied to claims about the role of the federal government. Self-determination is a principle employed to challenge food safety policies. Opponents of food safety policies have invoked moral claims on individual liberty, drawing heavily from Jeffersonian notions of democracy. A foundational value in the American identity, liberty is explicitly listed in the Declaration of Independence as an unalienable right: "…that among these are life, liberty, and the pursuit of happiness." Rhetoric used to challenge government intervention in food safety reflects anti-federalist sentiments that originated during America's formative years, and have persisted throughout U.S. history. The Letters from the Federal Farmer to the Republican asserted that a federal government could not adequately address the "unalienable and fundamental rights" of the people (1787). In the Fort Hill Address, John Calhoun states, "so numerous and diversified are the interest of our country, that they could not be fairly represented in a single government" (1831).

Policy actors who oppose food safety policy may invoke arguments that restrictions or regulations on food production represent overexertion of government power. These challenges to food safety are not necessarily based on the real risk that food production imposes on society, but rather the underlying principles that government intervention appears to violate.

The notion of individual liberty has also been used to support food safety policy. Under the view that the role of federal government is to protect the freedoms and rights of individuals, food safety could be claimed as a responsibility of the government. If one asserted that US citizens are bestowed with a right to consume unadulterated foods, it would be the job of the government to intervene and protect citizens from food contamination – not on a basis of social welfare, but out of a duty to protect the individual liberties of citizens.

Other Values

The distillation of food safety values into two tenets – individual liberty and community – perhaps oversimplifies the landscape of values that guide food safety policy. Numerous other values inform the policy processes relating to food safety, such as equity, enterprise, progress, and efficiency. Moreover, specific policies may invoke arguments based on particular values and principles. For example, FSMA is guided by values that place prevention of – rather than response to – foodborne outbreaks as a priority in food safety. I posit that it is less important to characterize all of the possible values involved in food safety policy, and more important to recognize that policy decisions are necessarily informed by individuals' values and principles. Community and self-determination are simply two far-reaching values that are foundational to the American identity and function as underlying justifications for and against federal food safety policy.

4.2.2. Actors and Institutions in Food Safety

Policy actors are individuals or groups of individuals that are directly or indirectly affiliated with or affected by the policy process. Policy actors may include individuals, governments, businesses, interest groups, social organizations, and communities. Individuals and groups are integral to policy processes; indeed, policies are created by people to address problems that affect people. In the realm of food safety, prominent policy actors include federal government agencies, researchers, and interest groups of both consumers and producers. Institutions are formal or informal mechanisms that persist over time and structure the behavior of actors by constraining, enabling or encouraging behaviors. Informal institutions are unwritten behavioral models, such as social norms or codes of conduct. Conversely, formal institutions are those for which a prescribed structure exists. Examples of formal institutions include government entities (e.g., agencies), laws, and regulations. The distinction between actors and formal institutions is not always clear; indeed, many entities could be regarded as both institution and actor. For example, the US Congress is both a group of people that influence policy and a formal mechanism that guides behaviors by enabling and constraining actors; Congress could therefore be considered both a group of actors and a formal institution. For this analysis, the distinction between actors and institutions is less important than the recognition that policy processes are facilitated by people through socially constructed entities.

Both formal and informal institutions guide food safety in the United States. Informal institutions include social norms that, while implicit, guide the actions of food producers and consumers. For example: even in the absence of federal regulation, farmers would not deliberately contaminate their crops with pathogens, given that social norms preclude them from such an activity that would adversely affect the health of consumers. Formal institutions related

to food safety include the laws, government agencies, and prescribed practices that relate to food safety. Select examples of formal food safety institutions are provided in Table 4.1.

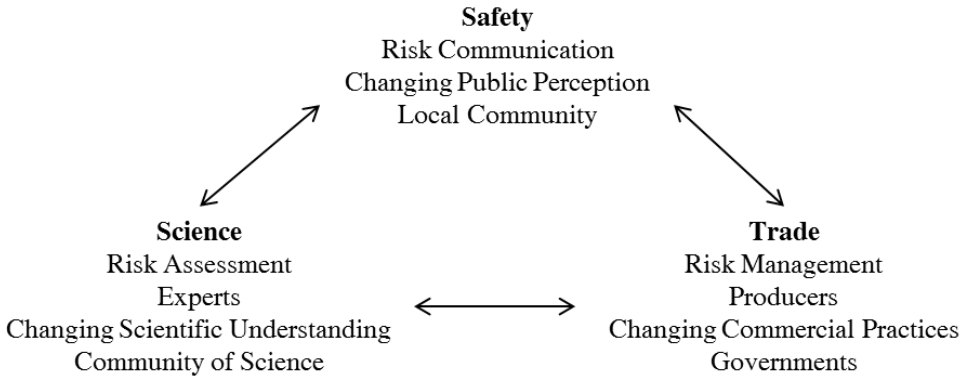
Table 4.1 – Select Examples of Formal Food Safety Institutions

Institution	**Enables, constrains, or structures behaviors by…**
U.S. Food and Drug Administration	establishing policies that require food producers to meet regulatory standards and/or implement best management practices.
Food Quality Protection Act	requiring practices by food producers and enables food consumers by mandating health-based standards for pesticides used in foods.
Nutrition Labeling and Education Act	providing consumers with nutrition information to aid in decision-making (Balasubramanian and Cole 2002).
Food Safety Modernization Act	enabling the FDA to implement additional rules that reduce the risk of foodborne illness outbreaks.
The media	deciding what events and information are disseminated to the public, thus shaping public opinion.
WTO SPS Agreement	requiring that regulatory measures be scientifically justified at the national and trans-national levels (Peel 2004).
International Association for Food Protection	facilitating cross-disciplinary networking of food safety professionals and scientists; publishing research in academic journals.

Phillips and Wolfe (2001) propose a conceptualization of the food safety system that consists of three interacting domains: science, safety, and trade (Figure 4.2). This framework reflects three different groups of actors (experts, citizens/consumers, and producers) who utilize different modes of reasoning (risk assessment, risk communication, and risk management) in the areas of science, safety, and trade, respectively. The authors, to their credit, admit that this is an oversimplification of the food safety system. Yet, it is a useful framework that reflects the ways in which both actors and institutions interact in food safety policy. In this framework, policy actors, formal institutions, and informal institutions are grouped into domains based on their

perceived positions with regard to food safety. The interaction between these three domains informs problem definition, policy development, and policy implementation.

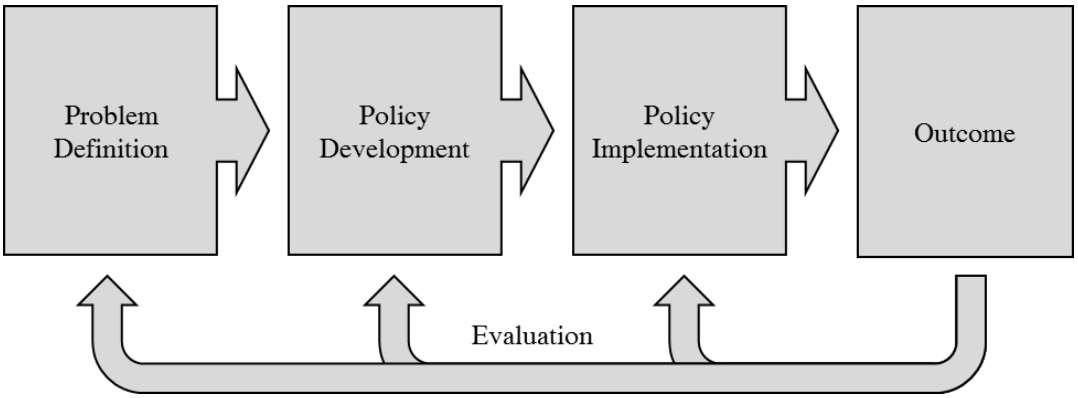
Figure 4.2 – Elements of the Food Safety System (Phillips and Wolfe 2001)



4.2.3. Policy Processes

Policy scholars have proposed numerous frameworks and theories for understanding the policy process. I propose here a framework for conceptualizing the policy process, particularly as it pertains to food safety (Figure 4.3). In this framework, problems are first identified and prioritized. Once a problem has been identified, a policy development process begins, whereby various policy options are considered. Once a specific policy is selected, the policy is implemented. After implementation, evaluation typically occurs; this evaluation allows implementers of the policy to determine whether alterations or modifications must be made to improve the policy. Values, actors, and institutions are integral to these stages in the policy processes. The process is necessarily political; politics are, after all the practice of influencing other people in the area of governance.

Figure 4.3 – A Policy Process Framework



As with all frameworks, this policy process framework is a generalization of the processes by which problems are identified and policies are implemented. The staggering complexity of the policy process requires simplification of the situation to facilitate understanding; "one simply cannot look for, and see, everything" (Sabatier 2007). In practice, stages of the policy process seldom occur sequentially and without conflict or reiteration. Furthermore, the complex interactions between actors and institutions make it difficult to tease out who, when, and how incremental decisions are made. Nevertheless, this framework serves as a useful tool for understanding not only how food safety policies come into existence, but for identifying places in the policy process that scientific studies, such as this one, may be employed in the design of policy.

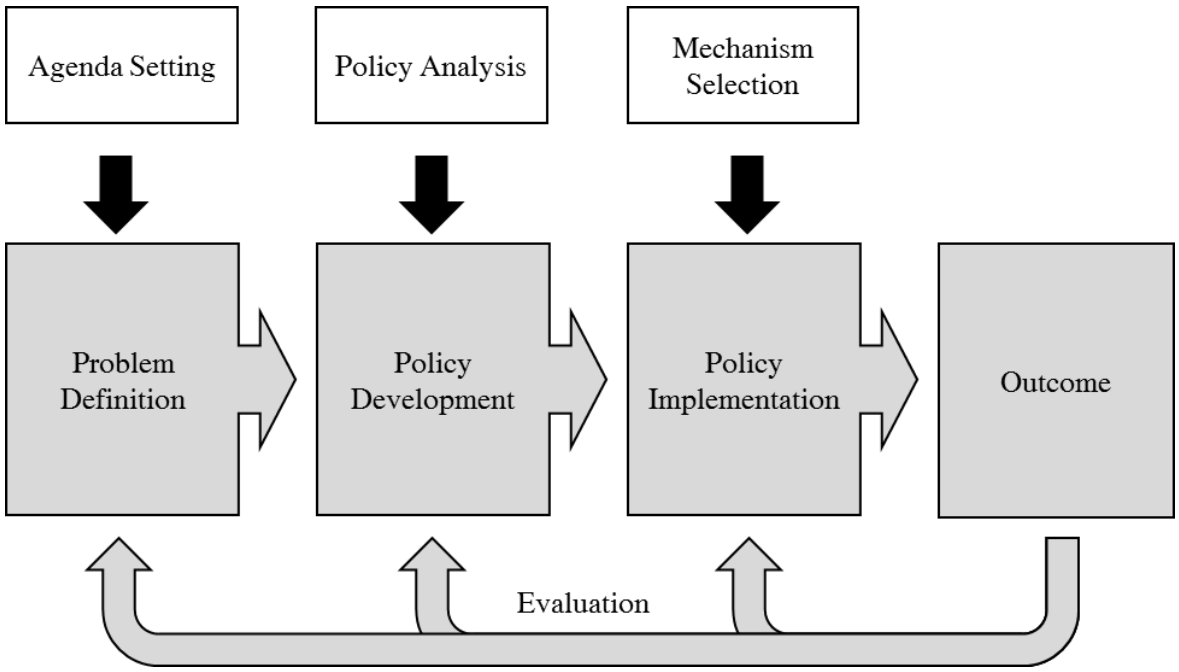
4.3. Research Findings: Implications for Policy

Considering the policy process frameworks provided here, the question remains: what role might the research findings in previous chapters play in public policy? Specifically, who might utilize this research, where in the policy process might it be employed, and to what end? I

posit that the tools developed in this study – namely the quantitative microbial risk assessment models – could be used in three key areas of the food safety policy process (see Figure 4.4):

1) Advocating for and prioritizing issues (agenda setting)
2) Choosing between policy options (policy analysis)
3) Selecting an appropriate mechanism by which the policy is implemented

Figure 4.4 – Uses for Study Findings in Public Policy Processes



4.3.1. Agenda Setting

Agenda setting is a process by which public issues are prioritized by policy actors and institutions. The public agenda is a "political barometer" that reflects the most sensitive issues that have come to the attention of the public and policy makers (Gerston 2014). Issues may be promoted by an array of policy actors, including citizens, interest groups, the media, and other policy institutions. In the context of food safety, agenda setting involves the prioritization of food

safety issues and the determination of which issues to address in policy-making. For example, interest groups advocating for public health in the early 1900s – such as the American Medical Association and American Public Health Association – pushed for the passage of PFDA and MIA (Wiley 1929), raising the issue of food safety on the public agenda.

Interest groups could use this research to place – or remove – the issue of produce safety. Sarah Pralle (2006) argues that advocacy groups make conscious decisions about issue framing, forming alliances, and selecting institutional venues in order to expand or contract policy issues. The research findings from this study suggest that current cleaning processes significantly minimize the risk of foodborne illness outbreaks associated with apples. Apple producers might use this information to suggest that produce contamination is not a problem that warrants government intervention. This argument could assert that other food safety issues – or perhaps other public problems entirely – deserve higher priority with regard to policy-making. Alternatively, proponents of enhanced food safety regulation could point to the data gaps in the risk assessment models and suggest that, given uncertainty in the scientific evidence, precautionary approaches should be taken to ensure consumer safety.

From a federal government perspective, research such as this could be used to prioritize various public problems. If the findings of this study suggest that apple contamination poses a higher risk to society when compared to other food safety issues (e.g., pesticides residues or microbial hazards on other fruits), then addressing apple contamination may be a priority. If other food safety issues are associated with a higher risk of illness – or risk of more severe illnesses – perhaps other problems should take priority. Indeed, government agencies – including the Food and Drug Administration – use risk assessment to determine what issues need to be addressed (Haas et al. 1999; FDA 2011).

4.3.2. Policy Analysis

Once a public issue is identified for policy intervention, policy analysis is a tool that enables policy-makers to select a policy from a pool of options. Specifically, policy analysis provides advice on policy options, given identification of the problem, analysis of stakeholders, consideration of alternatives, and examination of the implications associated with policies (Weimer and Vining 2005). Policy analysis may evaluate policy options on parameters such as: impact, efficiency, cost-benefit analysis, equity, and political feasibility.

Government agencies use analytic tools – such as cost-benefit analysis – to determine what policy options are best suited to address a public problem. Risk assessment is one potential tool at the disposal of decision-makers to decide between policy options. For example, if foodborne illness from fresh produce has been identified as a public problem, several policy options (or combinations of policy options) may be proposed to address it: command-and-control regulations, market-based policies, or transparency-based governance. An agency might conduct an analysis to determine which policy alternative will have the greatest impact on foodborne illness.

Risk assessment models, like the one presented in this study, may be used to select between policy options. The results of the risk assessment models suggest that, even with high levels of field contamination, there is a low risk of foodborne illness from apples. This would suggest that command-and-control systems of governance during apple production may not have a large impact on apple contamination. An alternative policy might focus on preparation of apples for eating (i.e., how apples are treated after they leave the cleaning and packing facility). Conversely, the uncertainty of this model might motivate decision-makers to pursue a policy that

provides additional protections. This is consistent with the proposed FSMA rules, which would mandate irrigation water quality standards to match those of recreational water.

Policy actors like interest groups may advocate for a specific policy option that is consistent with their values and desired outcome. Given that a problem has been identified, a particular course of action may be more desirable to policy actors than proposed alternatives. Policy advocates may feel that a particular policy option is best suited to achieve their goals, and pressure decision-makers to select their preferred course of action.

4.3.3. Selecting Policy Mechanisms

Selection of policy mechanisms here refers to the specific rules, protocols, and programs used to implement a policy. While laws provide direction for a government agency to address a public problem, specific rules are set to achieve a desired goal. For example, FSMA is a policy that directs the FDA to improve food safety; irrigation water quality standards set by the FDA are specific mechanisms for achieving the policy goal of lowering foodborne illness risk. Risk assessment models, like the ones provided in Chapter 3, can elucidate the specific mechanisms that are most likely to achieve the desired result.

The results from this study could be used to choose what media, processes, or technologies are subject to regulatory standards, and to what level. The results suggest that even high levels of bacteria in irrigation water – well above recreational water quality standards – are associated with low risks of foodborne illness. This might suggest that irrigation water standards are not an effective means of reducing microbial contamination of apples. With this information, alternative mechanisms – such as mandating certain disinfection protocols – might be pursued in policy implementation. The FDA may propose that apple growers use certain types of disinfectants at specified concentrations for a given duration of time to ensure decontamination.

Alternatively, storage or transport standards may be set to ensure ample time for bacterial reduction during other phases of apple harvest and processing.

As with problem definition and policy development, policy implementation is subject to input from other policy actors. Comment periods for proposed rules are opportunities for interest groups to voice opinions on the mechanisms by which polices are implemented. An association of farmers may, using the results of a risk assessment, argue for different regulatory standards than those proposed. Alternatively, stakeholders may advocate for an entirely different form of governance than command-and-control regulation. Policy mechanisms like certification programs and self-regulation have been proposed and utilized as alternatives to traditional government regulation paradigms (Caswell and Johnson 1991). Even within the realm of command-and-control regulation, mechanisms may allow producers a level of autonomy in determining what specific protocols or procedures are used to achieve a set goal. For example, HACCP (Hazard Analysis & Critical Control Points) is a management system that addresses food safety through the analysis and control of biological, chemical, and physical hazards in the production system (Mortimore and Wallace 2013). In response to juice-associate outbreaks, the FDA implemented a juice-HACCP rule that requires juice producers to apply interventions capable of achieving a 5-log reduction of pathogens, but leaves the choice of methods to the producer (Vojdani et al. 2008). The QMRA findings support a flexible management-based system like HACCP to address potential contamination of apples; the model predicts similar outcomes for various configurations of treatment processes, suggesting that producer autonomy in apple handling is justified given that end-of-process criteria are met.

4.4. Conclusion

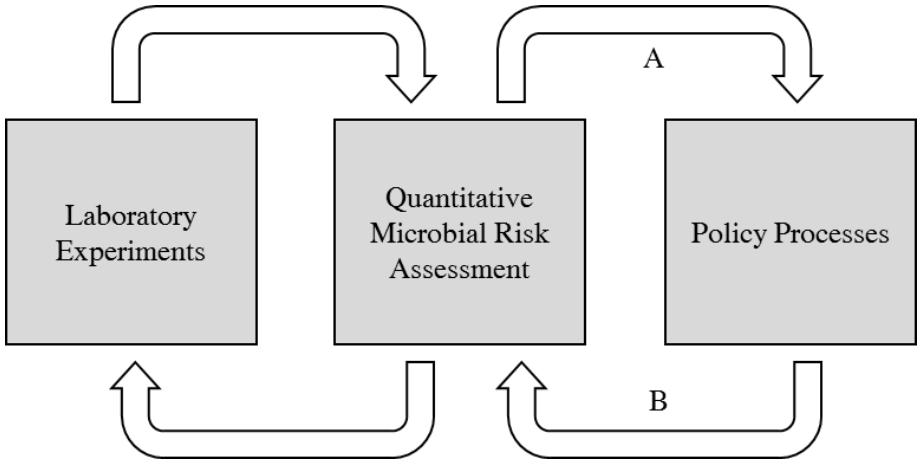
This chapter attempted to contextualize previous chapters' research findings in food safety policy processes, with the purpose of identifying places where study results could be utilized. Three stages of the policy process – agenda setting, policy analysis, and policy mechanism selection – were identified as places where QMRAs may be used to inform food safety policy. While the provided conceptual frameworks provide insight into policy processes and the relevance of this research, they fail to account for the complex interactions that guide problem formation, policy development, and policy implementation. Food safety policy outcomes are the result of complex trade-offs between competing demands of different interest groups that may be affected (Henson and Caswell 1999). The history of food safety policy in the United States has been shaped by dynamic political, social, and economic forces that change over time, and one might argue that oversimplification of these intricacies distorts the reality of food safety governance systems. I posit that the exercise of examining the policy process is valuable nevertheless; an analysis such as this affords academics and policy-makers an opportunity to reflect on the relationship between science and public policy.

Risk assessment is a discipline that is nestled in-between the realms of "science" and "public policy." In a traditional paradigm, the interaction between science and policy is a one-way relationship: scientists first discover facts, then policy-makers decide what to do with them (Haller and Gerrie 2007). This view is blurred by the integration of science directly into decision-making processes, such as the use of risk assessment by federal agencies. Furthermore, risk assessments are, perhaps to a greater extent than other scientific studies, informed by the investigators' values; "risk assessment is inherently subjective and represents a blending of science and judgement with important psychological, social, cultural, and political factors"

(Slovic 1999). Without opening a proverbial "Pandora's box" of discussion around the "objectivity" of science, my intention is merely to suggest that a risk assessment ought to involve discussion of the broader social context in which it applies.

This chapter was an attempt to explore the values, institutions, and policy processes that surround food safety, and situate the QMRA models within a broader policy framework. Figure 1.2 (copied below as Figure 4.5) illustrated a framework that links laboratory methods, risk assessment, and policy processes. In addition to identifying ways that QMRA results could be used in policy processes (Figure 4.5, arrow A), the analysis provided in this chapter serves the purpose of identifying the social and political forces that may inform the risk assessment paradigm (Figure 4.5, arrow B). Recognition of the values, institutions, and policy processes that inform risk assessment studies may lead toward directed and transparent research that has positive implications for human health.

Figure 4.5 – Thesis Research Areas



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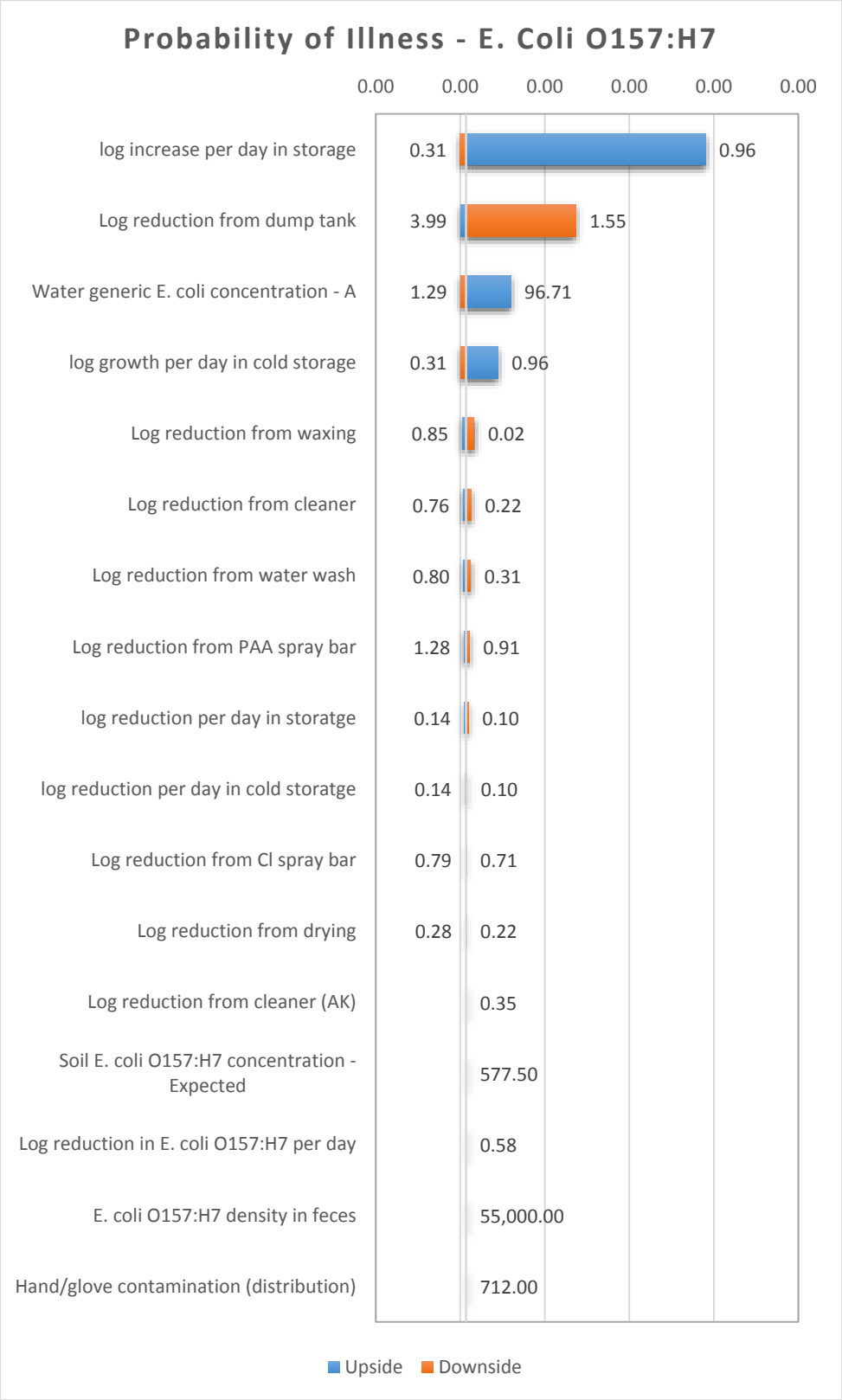
APPENDIX A – Tornado Analysis Output



APPENDIX B – Significant Events in US Federal Food Safety Policy*

Year	Event
1862	USDA established; Division of Chemistry established
1883	Legislation to prevent the importation of adulterated teas
1897	Tea Importation Act (superseded 1883 tea legislation)
1901	Division of Chemistry becomes Bureau of Chemistry
1906	Federal Food and Drugs Act (Pure Food and Drug Act)
1906	Federal Meat Inspection Act
1914	Federal Trade Commission Act
1916	US Grain Standards Act
1923	Filled Milk Act
1927	Food, Drug, and Insecticide Administration (FDIA) created within USDA
1927	Federal Import Milk Act
1930	FDIA becomes Food and Drug Administration
1938	Federal Food, Drug, and Cosmetic Act
1940	FDA transferred from USDA to the Federal Security Agency (FSA)
1946	Agricultural Marketing Act
1947	Federal Fungicide, Insecticide, and Rodenticide Act (FIFRA)
1953	FDA transferred to Department of Health, Education, and Welfare (HEW)
1957	Poultry Products Inspection Act
1958	Food Additives Amendment
1967	Fair Packaging and Labeling Act
1967	Wholesome Meat Act (amended Federal Meat Inspection Act)
1970	Egg Products Inspection Act
1970	Environmental Protection Agency established (took over FIFRA)
1971	Animal and Plant Health Inspection Service established (APHIS)
1976	Toxic Substances Control Act
1979	HEW becomes Department of Health and Human Services
1981	Food Safety and Inspection Service established within USDA
1990	Nutrition Labeling and Education Act (NLEA)
1990	Sanitary Food Transportation Act
1994	Dietary Supplement Health and Education Act (DSHEA)
1996	Food Quality Protection Act
1996	Federal Tea Tasters Repeal Act
1997	FDA Modernization Act
2002	Public Health Security and Bioterrorism Preparedness and Response Act
2007	FDA Amendments Act of 2007
2011	FDA Food Safety Modernization Act

*Adapted from Johnson 2014, Merrill and Francer 2000