ABSTRACT

Title of Document: QUANTITATIVE RISK ASSESSMENT FOR *ESCHERICHIA COLI* O157:H7 IN FRESH-CUT LETTUCE

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Directed By: Abani K. Pradhan, Ph.D., Department of Nutrition and Food Science

A farm-to-fork quantitative microbial risk assessment (QMRA) model was developed to estimate the risk of illnesses associated with *Escherichia coli* O157:H7 in fresh-cut lettuce, and to evaluate the effects of potential intervention strategies on reducing public health risks. Assuming a prevalence of 0.1% of lettuce entering the processing plant, the baseline model reflecting current industry practices predicted an average of 2,160 cases per year in the United States. For each of the additional intervention strategies evaluated, health risks were reduced by 11- to 18-fold. Treatment with ultrasound and organic acid combination was the most effective, reducing the mean number of cases by approximately 18-fold compared to baseline model. The developed risk model can be used to estimate the public health risk of *E. coli* O157:H7 from fresh-cut lettuce and to evaluate different potential intervention strategies to mitigate such risk.

QUANTITATIVE RISK ASSESSMENT FOR *ESCHERICHIA COLI* O157:H7 IN FRESH-CUT LETTUCE.

By

Hao Pang

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Advisory Committee:
Dr. Abani K. Pradhan, Chair
Dr. Robert L. Buchanan
Dr. Y. Martin Lo

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Chapter 1: Introduction

Leafy green vegetables, including lettuce, are of serious food safety concern, as those are recognized as vehicles for foodborne pathogens such as *Escherichia coli* O157:H7 that could cause human illnesses. Ready-to-eat packaged salad greens including fresh-cut lettuce have become increasingly popular over the past few decades due to its convenience (Buzby and Wells, 2007). However, fresh-cut lettuces are at greater risk for causing foodborne illnesses compared to other food products as they are in general not cooked before consumption.

Quantitative microbial risk assessment (QMRA) is being increasingly applied in recent years to identify and manage food safety risks. Development and application of QMRA models have been recognized as strong tools to identify and minimize potential risks associated with foodborne pathogens. QMRA has been applied to the production of microbiologically safe food products and to the development of effective and efficient risk-based food safety tools and programs. Evaluating the microbial safety of food products requires considerations of multiple factors that influence the prevalence and concentration of microbial pathogens in the product. The objective of this research was to develop a QMRA model to estimate the risk associated with fresh-cut lettuce potentially contaminated with *E. coli* O157:H7 in the United States and to evaluate the effects of different intervention strategies on public health risks due to consumption of fresh-cut lettuce.

Chapter 2: Literature Review

2.1 Burden of foodborne illnesses

Food safety is one of the top priorities in public health. Foodborne illnesses are a major food safety concern and many foodborne pathogens continue to cause significant public health burden in the U.S. and worldwide. The Centers for Disease Control and Prevention (CDC) recently reported that each year in the U.S., foodborne pathogens and a broad category of unspecified agents cause an estimated 48 million foodborne illnesses, 128,000 hospitalizations, and 3,000 deaths, which lead to an estimated economic loss of $77.7 billion per year (Scallan et al., 2011a,b; Scharff, 2011). Foodborne Diseases Active Surveillance Network (FoodNet) has conducted public health surveillance on six key food pathogens (*Campylobacter, Salmonella, Listeria,* Shiga-toxin producing *E. coli* O157 (STEC O157), *Vibrio, and Yersinia*) since 1996. The overall incidence of infections in 2012 caused by the aforementioned six key foodborne pathogens was 22% lower when compared with the data from 1996 surveillance (FoodNet, 2012). However, for *Campylobacter*, *Listeria*, STEC O157, *Shigella*, and *Yersinia*, the decline was mostly in the first few years and in recent years the decline was not significant (FoodNet, 2012). In addition, the overall incidence of *Salmonella* was unchanged and the incidence of *Vibrio* infection now is 116% higher than that in 1996 (FoodNet, 2012).

Some progresses have been made with the efforts from food safety regulatory agencies and food industry to prevent foodborne illnesses in recent years. In 2011, performance standards have been established for *Campylobacter* contamination in

young chickens and turkeys (USDA-FSIS, 2011). The Food and Drug Administration's (FDA) Food Safety Modernization Act (FSMA) was signed into law on January 4, 2011. FSMA aims to ensure the safety and security of the food supply to protect public health by establishing a new modern food safety system that not just respond to contamination but prevents food safety problems in the first place (FDA, 2011).

2.2 Lettuce and *E. coli* O157:H7

The amount of leafy green vegetables, including lettuce, available for consumption have dramatically increased over the past few decades in the U.S. This is attributable to both the growing popularity and convenience of ready-to-eat packaged salad greens, started in the late 1980s (Buzby and Wells, 2007) and the increasing interest in healthy diets to avoid overweight or other public health related issues. However, foodborne pathogens have been found in various kinds of fresh produce in the U.S. (Harris et al., 2003; Beuchat, 2006; Calvin, 2007; Elviss, 2009). According to the CDC, produce commodities accounted for the most foodborne illnesses (46%) during 1998-2008 in the U.S., out of which 22% were associated with leafy vegetables (Painter et al., 2013). Lettuce, which has an annual consumption of 13 pounds (5.9 Kg) per person, is the dominant type of leafy green vegetables in the U.S. (USDA-ERS, 2010a). Raw lettuces are generally considered to have a native non-pathogenic microflora, however, during the farm-to-fork supply chain of leafy vegetables including lettuce (production, harvest, processing, packaging, transportation, handling, retail, and home storage), microbial contamination can be introduced to vegetables from a variety of sources such as irrigation water, soil or

harvesting tools (FAO/WHO, 2008). Fresh-cut lettuce, which is pre-washed, shredded and packaged, has gained great popularities in recent years due to its convenience as ready-to-eat food. However, as no cooking process is needed for consumption, fresh-cut lettuce is at greater risks of causing foodborne illnesses compared to cooked food products.

Shiga toxin-producing *Escherichia coli* (STEC) are capable of causing human illnesses. In the U.S., most outbreaks of STEC have been caused by *E. coli* serotype O157:H7 (STEC O157). *E. coli* O157:H7 can cause acute gastrointestinal disease: hemorrhagic colitis, which is characterized by abdominal pain and bloody diarrhea (FDA, 2012a). *E. coli* O157:H7 can also cause hemolytic uremic syndrome (HUS), a potentially life threatening sequelae characterized by renal failure (CDC, 2012a). It is estimated that STEC O157 is annually responsible for 63,153 cases of foodborne illnesses, 2,138 hospitalizations, and 20 deaths in the U.S. (Scallan et al., 2011a). Traditionally, *E. coli* O157:H7 emerged as a human pathogen that has been linked to foods from animal origin; the consumption of undercooked meat product has been implicated in many foodborne outbreaks (Riley et al., 1983; Abdul-Raouf et al., 1993). More recently, *E. coli* O157:H7 outbreaks have implicated produce, especially leafy green vegetables (FDA, 2006a; FDA, 2007a; FDA, 2007b; Elviss, 2009; CDC, 2010; CDC, 2012a; CDC, 2012b) (Table 1). *E. coli* O157:H7 and leafy greens pathogen-commodity pair ranks the first in the risk-ranking of fresh produce in the U.S. (Anderson et al., 2011).

In 2006 and 2007, two multistate *E. coli* O157:H7 outbreaks, which caused a total of 152 illness cases including 79 hospitalizations, were attributable to shredded

iceberg lettuce served at the restaurants (FDA, 2006a; FDA, 2007b). In 2011, another multistate outbreak of *E. coli* O157:H7 caused 58 cases was linked to lettuce sold at grocery store (CDC, 2012b). The increasing number of lettuce related foodborne outbreaks has gained a lot of attention among government agencies, industries, and the public that resulting in national efforts to identify and implement preventive controls to address the risk associated with lettuce.

Table 1. Recent foodborne outbreaks associated with leafy greens

Year	Microorganism	Product	Cases	Reference
2006	*E. coli* O157:H7	Shredded iceberg lettuce	71	FDA, 2006a
2006	*E. coli* O157:H7	Spinach	205	FDA, 2007a
2007	*E. coli* O157:H7	Shredded iceberg lettuce	81	FDA, 2007b
2007	*S.* Senftenberg	Basil	51	Elviss, 2009
2010	*E. coli* O145	Shredded romaine lettuce	33	CDC, 2010
2011	*E. coli* O157:H7	Romaine lettuce	58	CDC, 2012b
2012	*E. coli* O157:H7	Organic spinach/spring mix	33	CDC, 2012c

2.2 Risk factors along the supply chain of lettuce

Main stages in a typical supply chain of fresh-cut lettuce include: infield production, processing, storage and consumption (Figure 1). Infield production includes two important steps, irrigation and harvesting. Sub-stages under processing include washing, shredding, and packaging of lettuce in processing plant. After processing, the storage of processed lettuce includes retail storage, transportation, and home storage.

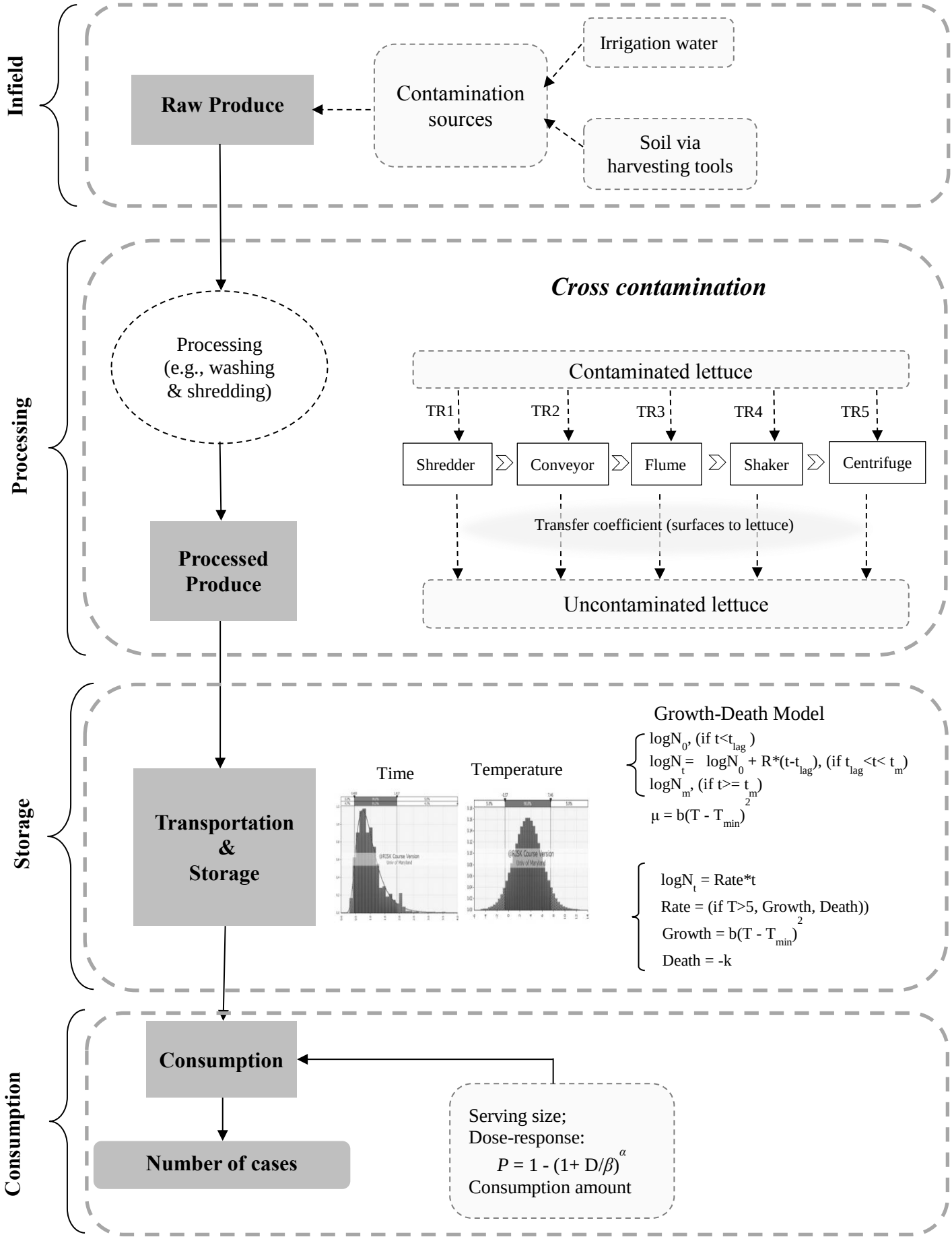

Figure 1. General framework of the QMRA model for *E. coli* O157:H7 in fresh-cut lettuce.

2.2.1 Infield production

Leafy vegetables, including lettuce, are grown in environments that have various potential sources of microbial hazards that may lead to contamination in produce (Beuchat, 2006; Brackett, 1999). Potential sources include animal or human activities and wastes, irrigation water, soil and soil amendments, seeds and plant stocks (FAO/WHO, 2008). Irrigation and harvesting are two important steps during infield production of lettuce that impact the contamination risk of lettuce. Production of lettuce is water intensive. Its requirements are met by irrigation with water from various sources such as rivers, lakes, rainwater, groundwater captured in wells, reclaimed wastewater or potable water sources (FAO/WHO, 2008). Contaminated water used in the production of lettuce can become a vector for transmission of pathogen to humans. Poor irrigation water quality indicated by elevated fecal coliform counts has long been known to correlate with the incidence of human pathogens in leafy vegetable crops (Norman and Kabler, 1953). Contamination of iceberg lettuce in a large outbreak caused by *E. coli* O157 in Sweden was linked to the use of contaminated irrigation water drawn from a small stream (Söderström et al., 2005). Thus, the microbiological quality of irrigation water could become a risk factor of *E. coli* O157:H7 contamination in lettuce.

Harvesting of lettuce could be done either by hand or machine. Recently, in food industry, coring-in-field (CIF) has been applied for harvesting lettuce for fresh-cut processing (Suslow et al., 2003). During the CIF process, the outer/wrapper leaves are removed and only the inner leaves are kept (NFPA/IFPA/United, 2001). However, CIF requires additional human handling using harvesting tools such as knives and

coring rings in the field, which may increase the risk of microbial contamination as the process involves considerable contacts between lettuce, soil and harvesting tools. *E. coli* O157:H7 have been found in several studies to have the ability to transfer from soil to lettuce through harvesting tools (Taormina et al., 2009; McEvoy et al., 2009; Yang et al., 2012).

2.2.2 Processing

During processing, lettuce is washed and shredded in flume tank and shredder. Then lettuce is conveyed to shaker and centrifuge to remove surface water. Chlorinated water is widely used as chlorination has been reported to have the ability to reduce the *E coli* O157:H7 contamination levels in lettuce (Stopforth et al., 2008; Keskinen et al., 2009; Zhang et al., 2009; Nou and Luo, 2010; Nou et al., 2011). However, cross contamination may occur during processing, which could spread *E. coli* O157:H7 on lettuce. Most commonly, cross contamination occurs during washing and shredding as the uncontaminated vegetables are washed, shredded, and conveyed together with the contaminated ones. During these processes, it is possible that *E. coli* O157:H7 cells transfer from lettuce to processing surfaces and then transfer from these surfaces to uncontaminated lettuce, which would affect the prevalence distribution and number of pathogen cells on lettuce after processing.

2.2.3 Storage

Storage of lettuce includes storage at retail, storage at home, and storage during transportation. Time and temperature during storage are the two most important factors that affect the microbiological safety of lettuce. Temperature of fresh produce should be maintained at low levels (<5 ℃) in order to suppress the growth of human

pathogens. If the temperature is not low enough, they may favor the growth of pathogens.

2.3 Quantitative microbial risk assessment

QMRA is being increasingly applied in recent years to identify and manage food safety risks. QMRA is defined by Codex Alimentarius Commission as a scientific based approach consisting of four parts: hazard identification, dose-response assessment, exposure assessment, and risk characterization with the aim to provide numerical expressions of risk and indication of the attendant uncertainties (Codex Alimentarius, 1999). There is an increasing interest in the application of QMRA in the production of microbiologically safe food products and the development of effective and efficient risk-based food safety tools and programs. In 2011, FSMA was signed into law, which further reinforced the importance of food safety. FSMA requires evaluation for known or potential hazards for each type of food manufactured, processed, packed or held at the facility and implementation of risk-based preventive controls that are adequate to reduce the hazards (FDA, 2011). QMRA, which generates estimates of risk from consumption of a certain food and evaluates reductions in risk by application of interventions, is highly valuable in terms of providing regulatory agencies and industries scientific evidence to make science-based food safety policies and decisions .

In recent years, several leafy green QMRA works have been published (Carrasco et al., 2010; Franz et al., 2011; Danyluk and Schaffner, 2011; Ottoson et al., 2011; Ding et al., 2013) (Table 2).

Table 2. Recent published QMRA models associated with leafy greens.

Year	Pathogen	Commodity	Regions	Reference
2010	*E. coli* O157:H7, *Salmonella, Listeria monocytogenes*	Leafy greens at salad bars	The Netherlands	Franz et al., 2010
2010	*Listeria monocytogenes*	RTE lettuce salads	Spain	Carrasco, 2010
2011	*E. coli* O157:H7	Leafy greens	United States	Danyluk and Schaffner, 2011
2011	*E. coli* O157	Lettuce	Sweden	Ottoson et al., 2011
2013	*Listeria monocytogenes*	Lettuce	Korea	Ding et al., 2013

However, no published QMRA models were targeted specifically for *E. coli* O157:H7 in fresh-cut lettuce. In addition, possibly because of lack of data and information, these studies did not include contamination from different environmental sources, such as irrigation water, soil or harvesting tools, which could affect the level of contamination in lettuce with *E. coli* O157:H7 during infield production. Besides, due to contacts with different facility surfaces in the processing line, fresh-cut lettuce is likely to be cross contaminated during processing. Therefore, a QMRA model incorporating contamination from different environmental sources in the field and cross contamination mechanism in the processing plant is needed for *E. coli* O157:H7 in fresh-cut lettuce to: (1) provide estimates of the expected current risks of *E. coli* O157:H7 illnesses from consumption of fresh-cut lettuce in the U.S.; (2) identify the most important factors affecting the frequency and growth of *E. coli* O157:H7 in fresh-cut lettuce and the number of cases; and (3) evaluate different pre-harvest, processing, and post processing interventions for their influence of public health risks

associated with consumption of fresh-cut lettuce contaminated with *E. coli* O157:H7 in the U.S.

Chapter 3: Research Goal and Objectives

The overall goal of this project is to assess public health risk due to consumption of fresh-cut lettuce potentially contaminated with foodborne pathogen *E. coli* O157:H7.

Specifically, the objectives of this study were to:

(i) Develop a QMRA model to estimate the risks of *E. coli* O157:H7 illnesses in the U.S. population due to consumption of fresh-cut lettuce.

(ii) Perform sensitivity analyses to identify important factors affecting the growth of *E. coli* O157:H7 in fresh-cut lettuce and number of cases to gain insights into possible mitigation strategies aimed at protecting consumers from *E. coli* O157:H7 illnesses arising from fresh-cut lettuce consumption.

(iii) Perform scenario analysis for different mitigation strategies to evaluate their effects on reducing the risk of human illnesses associated with fresh-cut lettuce.

Chapter 4: Materials and Methods

4.1 Model overview

The developed farm-to-fork QMRA model described lettuce contamination with *E. coli* O157:H7 in the field, the fate of such contamination along the fresh-cut production chain, and the risk of illness upon consumption. The model considered that *E. coli* O157:H7 pre-harvest contamination in lettuce can occur via two main routes: (1) irrigation water, and (2) soil via harvesting tools. Other possible contamination routes from environment such as animal activities or fertilizer were not considered in this QMRA model due to lack of available data. Production stages relevant to modeling *E. coli* O157:H7 levels in lettuce are shown in Figure 1. *E. coli* O157:H7 can be present in lettuce as a result of handling, contacts with contaminated equipment, and/or environmental surfaces. Irrigation and harvesting are two important steps during infield production where lettuce may get contaminated with *E. coli* O157:H7. Once transported to the processing plant, lettuce heads are washed, shredded, and packed in sealed bags. Washing could reduce the level of *E. coli* O157:H7 in lettuce. However, cross contamination from contaminated leaves and processing facilities to uncontaminated leaves may occur during washing and shredding, which may increase the prevalence of contamination. During the storage stages, temperature plays an important role. Under abusive temperatures growth of *E. coli* O157:H7 is likely to occur. Table 3 provides a summary of the variables and parameters considered in the QMRA model.

Table 3. Overview of variables, point-estimate values, statistical distributions, and formulas used in the QMRA model

Symbol	Variable	Distribution, Value or Formula	Unit	Source
	Irrigation			
C_w	*E. coli* concentration in irrigation water	=RiskUniform(1,235)	CFU/100 ml	LGMA, 2010
R_w	VTEC portion in irrigation water	=10^RiskNormal(-1.9,0.6,RiskTruncate(,0))[a]		Ottoson et al., 2011
W	Water holding	=RiskNormal(0.108,0.019,RiskTruncate(0,))[b]	ml/g	Ottoson et al., 2011
C_i	Concentration after irrigation	= (C_w /100)× R_w× W	CFU/g	Calculated[c]
	Inactivation during holding time			
t_{hold}	Holding time after irrigation	=RiskTriang(2,4,8)	days	FDA,2012b
d_{hold}	Log reduction during holding time	=-POWER((t_{hold} /(2.45/24)),0.3)	log CFU/g	Bezanson et al., 2012
C_{hold}	Log concentration after holding time	=LOG(C_i) + d_{hold}	log CFU/g	Calculated
	Harvesting			
C_s	Soil *E. coli* concentration	=10^RiskNormal(0.928,1.11,RiskTruncate(0,3.67))	CFU/g	Lenehan et al., 2005
R_s	VTEC portion in soil	=10^RiskNormal(-1.9,0.6,RiskTruncate(,0))		Ottoson et al., 2011
M	Attached soil on harvesting tools	10.22	g/blade	Yang et al., 2012
N_b	Number of *E. coli* O157:H7 cells per blade	=C_s × R_s × M	CFU/blade	Calculated
$R_{t\text{-}l}$	Transfer rate from harvesting tools to lettuce	0.0013		Yang et al., 2012
$C_{h\text{-}l}$	Transfer from harvesting tools to lettuce	= N_b × $R_{t\text{-}l}$ /1500'	CFU/g	Calculated
C_h	Concentration of *E. coli* O157:H7 after harvest	=10^ C_{hold} + $C_{h\text{-}l}$	CFU/g	Calculated
	Processing			
$Prev_0$	Initial prevalence	0.1	%	Danyluk & Schaffner, 2011
d_w	Log reduction by washing with water	= RiskPert(0.6,1,1.4)	log CFU/g	(Stopforth et al., 2008; Keskinen et al., 2009; Zhang et al., 2009; Nou and Luo, 2010)
C_{lw}	Log concentration after washing	= C_h - d_w	log CFU/g	Calculated
C_w	Concentration after washing	= 10^ C_{lw}	CFU/g	Calculated
N_{int}	CFU in unit batch after washing	= C_w × $Prev_0$	CFU/unit batch	Calculated
TR1	Transfer(%) from contaminated lettuce to flume	= RiskTriang(0,0.01,0.02)	%	Perez et al., 2011
TR2	Transfer(%) from contaminated lettuce to shredder	= RiskTriang(0,0.02,0.02)	%	Perez et al., 2011
TR3	Transfer (%) from contaminated lettuce to shaker	= RiskTriang(0,0.01,0.02)	%	Perez et al., 2011

$TR4$	Transfer (%) from contaminated lettuce to centrifuge	= RiskTriang(0.01,0.04,0.08)	%	Perez et al., 2011
$TR5$	Transfer (%) from contaminated lettuce to conveyor	= RiskTriang(0,0.1,0.24)	%	Perez et al., 2011
O_{fu}	Overall transfer coefficient (%) from facilities to uncontaminated lettuce	= RiskTriang(9.9,15.33,18.83)	%	Perez et al., 2011
N_{fac}	CFU transferred to facility surfaces	$= N_{int} \times (TR1 + TR2 + TR3 + TR4 + TR5)$	CFU/unit batch	Calculated
N_{tran}	CFU transferred from facility surfaces to uncontaminated lettuce	$= N_{fac} \times O_{fu}$		
N_{final}	CFU in unit batch after cross-contamination	$= N_{int} - N_{fac} + N_{tran}$	CFU/unit batch	Calculated
S	Spread of contamination due to processing	=RiskPert(1,1.2,2)		FDA, 2012b
$Prev_f$	Prevalence after cross contamination	$= Prev_0 \times S$	%	Calculated
C_p	Concentration of on processed lettuce	$= N_{final} / Prev_f$	CFU/g	Calculated
	Retail storage			
t_R	Retail storage time, t_R	=RiskTriang(0.5,4,7)×24	Hours	
T_R	Retail storage temperature, T_R	=RiskNormal(4.4441,2.9642,RiskTruncate(0,20.56))	°C	EcoSure, 2008
	Transportation - retail to home			
t_{Tran}	Transportation time, t_T	=RiskLognorm(1.421,0.46478,RiskTruncate(0.1833,3.8667),RiskShift(-0.24609))	Hours	EcoSure, 2008
T_{bH}	Temperature before putting in home refrigerator	=RiskNormal(8.386,3.831,RiskTruncate(0,20))	°C	EcoSure, 2008
T_{Tran}	Transportation temperature	$=1/2 \times (T_R + T_{bH})$	°C	
	Home storage			
t_f	Time to first (home storage)	=RiskWeibull(1.13,2.84)×24	Hours	Pouillot, 2010
t_l	Time to last (home storage)	=RiskWeibull(1.7,7.96)×24	Hours	Pouillot, 2010
t_H	Time selected-home storage, t_H	$=1/2 \times (t_f + t_l)$	Hours	Calculated
T_H	Home storage temperature, T_H	=RiskNormal(3.4517,2.4442,RiskTruncate(-5,17.22))	°C	EcoSure, 2008
	Growth/die-off parameter			
b	Growth model parameter	0.023		McKeller & Delaquis, 2011
T_{min}	Growth model parameter	$=1.335-5.766 \times b$	°C	McKeller & Delaquis, 2011

k	Die-off rate	=RiskLognorm(0.013,0.001,RiskShift(0.001))/ 2.303	log CFU/gh	McKeller & Delaquis, 2011
μ_R	Retail-growth rate	=(b×(T_R - T_{min}))^2/2.303	log CFU/gh	Calculated
μ_{Tran}	Transportation-growth rate, mu/2.303 = R, growth rate	=(b×(T_{Tran} - T_{min}))^2/2.303	log CFU/gh	Calculated
μ_H	Home-growth rate, mu/2.303 = R, growth rate	=(b×(T_H - T_{min}))^2/2.303	log CFU/gh	Calculated
	Growth/die-off calculation			
Q_R	Growth/die-off during retail storage?	=IF(T_R >5,1,0)		
G_R	Log change during retail storage	=IF(Q = 1, μ_R × t_R, -k× t_R)	log CFU/g	Calculated
C_{lR}	Log concentration after retail storage	=LOG C_p + G_R	log CFU/g	Calculated
Q_{Tran}	Growth/die-off during transportation?	=IF(T_{Tran} >5,1,0)		
G_{Tran}	Log change during transportation	=IF(Q = 1, μ_{Tran} × t_{Tran}, -k× t_{Tran})	log CFU/g	Calculated
C_{lTran}	Log concentration after transportation	= C_R + G_{Tran}	log CFU/g	Calculated
Q_H	Growth/die-off during home?	=IF(T_H >5,1,0)		
G_H	Log change during home	=IF(Q = 1, μ_H × t_H, -k× t_H)	log CFU/g	Calculated
C_{lH}	Log concentration after home storage	= C_{Tran} + G_H	log CFU/g	Calculated
L	Limit of level if >10^7 (7 log CFU/g)	=IF(C_H <7, C_H ,7)	log CFU/g	Calculated
C_H	Concentration after home storage in CFU/g	=POWER(10, L)	CFU/g	Calculated
	Serving			
Ser	Serving size	85	g	FDA,2002
D	Dose per serving (CFU/serving)	= C_H × Ser	CFU/serving	Calculated
	Dose response			
α	Dose response parameter	0.267		Cassin et al.,1998
β	Dose response parameter	229.2928		Cassin et al.,1998
P	Probability of illness per serving	=(1-(1+D/β)^-α)×$Prev_f$		Calculated
	Risk characterization			
N_{pop}	U.S. population	316,085,800		DOC-Census Bureau, 2013
A	Lettuce availability per capita adjusted for food loss	5887.6	g/year	USDA-ERS,2010
N_P	Consumed servings per person per year	= A /Ser		Calculated
N_{CS}	No. of servings consumed per year in U.S. population	= N_{pop} × N_P		Calculated
N_{cases}	Number of cases per year	= N_{CS} × P		Calculated

[a] RiskTruncate(,0)), distribution truncated at maximum value of 0.
[b] RiskTruncate(0,)), distribution truncated at minimum value of 0.
[c]Calculated, values that are calculated in this QMRA model.

4.2 Contamination routes: irrigation water

Various types of irrigation techniques based on how water is distributed to the field have been applied in lettuce production. In the U.S., three types of irrigation methods are used: gravity system (e.g., furrow irrigation, flood irrigation), sprinkler system (overhead irrigation), and low-flow irrigation (drip, trickle, or micro sprinklers) (FRIS, 2008). For irrigation with gravity systems and low-flow irrigation, water is delivered at or near the root area of lettuce and has no or little contact with the edible part of lettuce. However, in overhead irrigation, water is distributed through overhead sprinklers. Thus, during overhead irrigation, water has direct contact with the edible part of lettuce, which may lead to transfer of pathogens from irrigation water to lettuce. Therefore, overhead irrigation method should pose a relatively higher risk of contamination in lettuce comparing to other methods.

In this study, it was assumed that overhead sprinkler is the primary method used and the main source of contamination in lettuce farms during irrigation. California Leafy Green Products Handler Marketing Agreement (LGMA) recommended that concentration of *E. coli* in leafy green irrigation water should not exceed 235 CFU/100 ml (LGMA, 2010). As very limited data are available on the overall quality of irrigation water, a uniform distribution (min=1 CFU/100 ml, max=235 CFU/ml) was used in this QMRA model. Distribution parameter value min=1 CFU/100 ml represents the detection limit in irrigation water and max=235 CFU/100 ml represents the upper limit in LGMA recommendation. By using this distribution, it was assumed that the irrigation water quality for all lettuce farms considered in this QMRA model is in compliance with LGMA recommendation. To estimate the probability of

illnesses and number of cases for scenarios where irrigation water quality exceeded 235 CFU/100 ml, several scenario analyses were performed, which is discussed later in this document. To estimate the number of *E. coli* O157:H7 in irrigation water, Ottoson et al. (2011) calculated and described the ratio of verotoxin-producing *E. coli* (VTEC) to generic *E. coli* using a lognormal distribution (parameter values shown in Table 3) based on data from Muniesa et al. (2006) and Hutchison et al. (2004). This lognormal distribution by Ottoson et al. (2011) was used in this study to model the ratio of *E. coli* O157:H7 to generic *E. coli*. To determine the transfer of pathogen cells from irrigation water to lettuce following overhead irrigation, it was assumed that all pathogens in the irrigation water captured on the plant after irrigation will attach to it. This conservative assumption has been used in previous risk assessment works (Petterson, 2001; Hamilton et al., 2006). For lettuce, there is very limited data on volume of water that gets in contact with or remains on lettuce as a result of overhead irrigation. Shuval et al. (1997) reported an estimate of 0.018 ml/g for lettuce based on experiment where 12 head of lettuces were completely immersed in water. This estimate (0.018 ml/g) for irrigation water attachment has been used in some previous QMRAs (Shuval et al., 1997; Petterson, 2001). Hamilton et al. (2006) assigned a normal distribution to this parameter (μ=0.108 ml/g, σ=0.019 ml/g) based on the estimate made by Shuval et al. to account for variability. In this current study, normal distribution (μ=0.108 ml/g, σ=0.019 ml/g) from Hamilton et al. (2006) was used to describe the volume of water remaining on lettuce heads after overhead irrigation. However, further experiments are needed to verify whether the estimate of Shuval et al. based on total immersion experiments represents the situation of

overhead irrigation. The concentration of *E. coli* O157:H7 on lettuce after irrigation was calculated by multiplying the concentration in irrigation water by the volume of water retained on lettuce.

4.3 Pre-harvest holding time

During infield production of lettuce, there is usually a holding time between the last irrigation and harvest. Such irrigation withdrawal is applied as a risk-reducing strategy as pathogenic bacteria can be reduced by UV radiation, desiccation, or competition with microorganisms during the holding time. The rate of pathogen inactivation in the field during holding time was expressed using a Weibull survival function, i.e., $\log N_t/N_0 = -(t/\delta)^p$, with parameter values derived by Bezanson et al. (2012) in their study of *E. coli* O157:H7 survival on romaine lettuce. According to estimates from the FDA, the last overhead irrigation is usually applied 3-5 days before harvesting, and in some cases the holding time is as short as 2 days or as long as 8 days (FDA, 2012b). Thus, in this study a triangular distribution with minimum of 2 day, maximum of 8 days, and most likely value (mode) of 4 days was used to describe the number of days that lettuce remains in the field after the last irrigation but prior to harvest.

4.4 Contamination routes: contamination from soil via harvesting tools

In this study, possible contamination in lettuce with *E. coli* O157:H7 during harvesting was considered. Pathogens could transfer to lettuce during harvesting because harvesting tools, such as knives and coring rings, get in contact with soil, and subsequently get in contact with lettuce. Soil *E. coli* concentration data based on a 7

month experiment from the study by Lenehan et al. (2005) was used in this QMRA study. In this QMRA, *E. coli* levels were described by a normal distribution with μ= 0.93 log CFU/g and σ = 1.11 log CFU/g from Lenehan et al. (2005) data. All superficial soil was assumed to be contaminated at the same level, i.e., with a prevalence of 100%. The ratio of *E. coli* O157:H7 to generic *E. coli* was described using the same distribution that was used for the ratio in irrigation water, as there is no data specific to soil. The transfer of pathogens from soil to harvesting tools depends on the amount of soil that attaches to harvesting tools (i.e., attachment rate). The attachment rate of soil is affected by water content of soil. Yang et al. (2012) reported an average of 10.22 g attaching to the blade for soil with a water content of 20%, when mimicking harvest procedures. This value for attachment rate was used in the QMRA described in this article to calculate concentration of *E. coli* O157:H7 on the blade by multiplying with concentration of *E. coli* O157:H7 in soil. Yang et al. (2012) determined the transfer from harvesting blades and coring rings to lettuce and their data (0.0013) were used to define the transfer rate from harvesting tools (coring rings and blades) to lettuce in this QMRA model. Based on information from Yang et al (2012), it was assumed in this QMRA that each contaminated blade will transfer pathogens to three consecutive heads of lettuce and the pathogen cells on the blade will evenly transferred to each of the three lettuce head. The average weight of lettuce head was determined as 450 g in a previous study (Carrasco, 2010), and weights of 24 heads carton range from 25 to 40 lbs. (Meister, 2004), which yields a weight of 471-755 g per head of lettuce. In this QMRA model the average weight of one lettuce

head was set as 500 g, and transfer of *E. coli* O157:H7 from blade to lettuce was calculated by dividing 1500 g (500 g × 3) from the number of cells in each blade.

4.5 Exposure assessment: washing

After harvesting, lettuce heads are delivered to the processing plant. In lettuce processing, washing is an important step to reduce the level of *E. coli* O157:H7 in lettuce entering the processing line. Several studies have investigated the effect of washing with water on reduction of *E coli* O157:H7 (Stopforth et al., 2008; Keskinen et al., 2009; Zhang et al., 2009; Nou and Luo, 2010). The reported log reduction ranged from 0.6 to 1.4 log CFU/g. Data on log reduction due to washing were extracted from these studies and were summarized with a PERT distribution (min=0.6 log CFU/g, most likely=1 log CFU/g, max=1.4 log CFU/g).

4.6 Exposure assessment: cross contamination

Cross contamination plays an important role of spreading *E. coli* O157:H7 on lettuce during processing. During lettuce processing, lettuce heads are washed, shredded and then conveyed to shaker and centrifuge via conveyor belts to remove surface water. During these processes, uncontaminated lettuce and contaminated lettuce are washed, shredded, and conveyed together. Thus, the pathogen cells in contaminated lettuce may transfer to facilities such as shredder, conveyor belts, flume tank, shaker or centrifuge. Then the pathogens on these facility surfaces may transfer to uncontaminated lettuce. In this study, the transfer of *E. coli* O157:H7 from contaminated lettuce to different processing surfaces (shredder, conveyor belts, flume tank, shaker and centrifuge) were determined using the transfer rates described by

Perez-Rodriguez et al. (2011). The transfer of pathogen cells from processing surfaces to uncontaminated lettuce was determined using the overall transfer coefficient (*Ofu*) described by a triangular distribution (min=9.9%, most likely=15.33%, max=18.83%) based on the study by Perez-Rodriguez et al. (2011). After washing and shredding, as some of the initially uncontaminated lettuces become contaminated from initially contaminated lettuce through contact with processing surfaces, the prevalence of contamination will increase. According to the expert panel estimate in FDA's risk assessment for *E. coli* O157:H7 on lettuce (FDA, 2012b), the prevalence will increase by 1- to 2-fold (most likely 1.2-fold) due to cross contamination during washing. Thus in the QMRA model described in this article a triangular distribution (min=1, most likely=1.2, max=2) was used to describe the fold increase of prevalence after processing of lettuce. Calculation of concentration of *E. coli* O157:H7 on lettuce after processing was described in detail in Table 3.

When estimating cell number partitioning between contaminated and uncontaminated lettuce, and processing surfaces, the cell mass balance was checked to ensure that the overall cell number on a lettuce batch after cross contamination was not higher than before.

4.7 Exposure assessment: microbial kinetics

The prevalence of contaminated lettuce is considered to remain the same during post processing steps (retail storage, transportation, home storage) of the supply chain as they have been packaged individually and thus are separated from each other. However, the concentration of *E. coli* O157:H7 on lettuce are subject to change during different storage conditions. They could either be growing under abusive

temperatures or declining under refrigerator temperatures (McKellar and Delaquis, 2011). A dynamic growth-death model for *Escherichia coli* O157:H7 in minimally processed leafy green vegetables (lettuce and spinach) under variable temperature conditions developed by McKellar and Delaquis (2011) was used to model the change of *E. coli* O157:H7 concentration on lettuce in this QMRA model. Numerous studies have been conducted to determine the minimum growth temperature of *E. coli* O157:H7, which seems to be in between 5 ℃ and 6 ℃ (Nauta and Dufrenne, 1999; Palumbo et al., 1995; Rajkowski and Marmer, 1995; Tamplin et al., 2005). Tamplin et al. (2005) reported that levels of *E. coli* O157:H7 decreased at 5 ℃ on ground beef. Abdul-Raouf et al. (1993) and Luo et al. (2010) reported that number of *E. coli* O157:H7 cells on cut lettuce decreased at 5 ℃. However, in the study conducted by Koseki and Isobe (2005), no decline of *E. coli* O157:H7 cells on cut lettuce was found at 5 ℃ temperature. These conflicting experimental outcomes indicated that it is unclear what the exact threshold temperature is for growth of *E. coli* O157:H7 in lettuce since many factors could have influenced the conclusions drawn from individual investigations, such as differences in experimental design, test strains, raw materials and packaging conditions (McKellar and Delaquis, 2011). The combined model for pathogen growth and death by McKellar and Delaquis (2011), which excludes both the lag and maximum population density (MPD), was used in this QMRA study, which calculates bacterial growth or die-off depending on the cut-off temperature of 5 ℃. At temperatures exceeding 5 ℃, the increase in number of *E. coli* O157:H7 cells was determined by growth model, while at temperatures below 5 ℃, the decline of pathogen cells was determined by a die-off model. For growth model, it

includes a primary (Buchanan et al., 1997) and a secondary (Ratkowsky et al., 1982) model. The three-phase log-linear primary model consists of a lag phase, an exponential phase, and a stationary phase was expressed as follows:

$$\log N_t = \begin{cases} \log N_0, (\text{if } t < t_{lag}) \\ \log N_0 + R \times (t - t_{lag}), (\text{if } t_{lag} < t < t_m) \\ \log N_m, (\text{if } t >= t_m) \end{cases} \quad (1)$$

$$R = (\log N_m - \mathrm{Log} N_0) / (t_m - t_L) \quad (2)$$

where N_t is the concentration at time t (CFU/g), R is the growth rate (log CFU g^{-1} h^{-1}), t_L is the lag time, N_0 is the concentration at 0 time (CFU/g), N_m is the Maximum Population Density (MPD; CFU/g), t_m is time at which the MPD is reached.

The secondary model is Ratkowsky's square root model:

$$\mu = b(T - T_{min})^2$$

where b is the temperature coefficient, T is the temperature (℃) and T_{min} is the theoretical minimum growth temperature. This model was used to describe the change of growth rate (μ) as a function of storage temperature.

The decline in *E. coli* O157:H7 levels below 5℃ was described by die-off model using the following equation:

$$\log (N_t / N_0) = -k \times t; \quad (3)$$

where k is the death rate (log CFU g^{-1} h^{-1}).

These equations were fitted to growth or death data by McKellar and Delaquis (2011) to acquire model parameters. In this QMRA, parameter values from McKellar

and Delaquis was used, where b and T_{min} were 0.023 and 1.2023 respectively; death rate k was described by a lognormal distribution (μ=0.013 CFU g^{-1} h^{-1}, σ=0.0010 CFU g^{-1} h^{-1}) (McKellar and Delaquis, 2011). It is worth mentioning that lag phase was not included in the final combined model for pathogen growth and death by McKellar and Delaquis (2011) because according to these authors only 6 of the available 62 relevant data sets for growth showed a lag phase, thus limiting the calculation of lag phase in their growth model. Lag phase has also been not included in a number of previous QMRA studies related to lettuce (Carrasco et al., 2010; Franz et al., 2010; Danyluk and Schaffner, 2011) because of unavailability of reasonable estimates for this parameter in this product-pathogen combination. This assumption represents a conservative approach which may overestimate the growth of pathogen cells on lettuce during storage.

4.8 Exposure assessment: storage conditions

For retail storage, data for all refrigerated food products were extracted from the EcoSure Cold Temperature Report (2007) and a normal distribution (μ=4.4441 ℃, σ=2.9642 ℃) was used to represent retail storage temperature for fresh-cut lettuce. This normal distribution was truncated at 0 ℃, as refrigerator at retail store seldom falls below 0 ℃, and 20.56 ℃, which is the maximum temperature reported in EcoSure report. Similarly, a normal distribution (μ=3.4517 ℃, σ=2.4442 ℃) truncated at -5 ℃ and 17.22 ℃ for minimum and maximum reported temperature was used to describe the temperature during home storage based on data from the EcoSure Cold Temperature Report (EcoSure, 2008). Data from the study of Pouillot et al. (2010) for

bagged salads were used as substitute data to model home storage time as no data were found for fresh-cut lettuce.

During storage at retail, transport, and storage at home, the number of *E. coli* O157:H7 cells changed depending on the time and temperature in these stages. Because of data availability, transportation between retail to home was considered in the model. Temperature during transportation from retail to home was described by considering both retail storage temperature and temperature before putting in home refrigerator by following the procedures from the risk assessment study by Latorre et al. (2011). Data on temperature at the end of retail-home transport and just before being put in the home refrigerator was extracted from the EcoSure report, and fitted to a normal distribution (μ=8.3858 ℃, σ=3.8314 ℃), truncated at 0 ℃ and 20 ℃. The EcoSure data for all refrigerated food products were analyzed for calculating the temperature of products before putting in the home refrigerator. In addition, transportation time (hours) for all refrigerated commodities were extracted from EcoSure report and fitted to a lognormal distribution (μ=1.421 h, σ=0.46478 h), truncated at 0.1833 h and 3.8667 h. Figure 2 shows the result of data fitting for transport time from retail to home.

The possible correlation between storage time and temperature was not considered in this model in accordance with similar studies reported previously (Danyluk and Schaffner, 2011). In a study of storage time and temperature for different ready to eat foods in the U.S. by Pouillot et al. (2010), the linkage between storage time in the household and home refrigerator temperature was only significant for 1 of the 10 categories of foods considered (sliced deli meat to order). Danyluk and

Schaffner (2011) also reported that correlation or linkage between storage time and temperature for ready to eat foods may be rare.

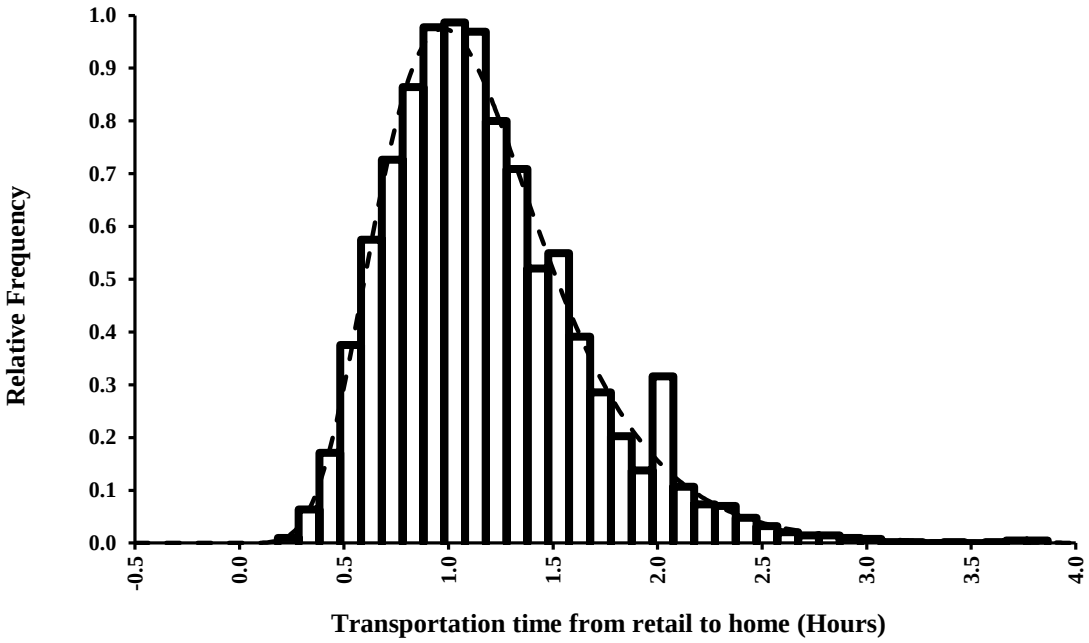

Figure 2. Transportation time from retail to home by fitting 2007 EcoSure data (EcoSure, 2008) for all refrigerated products to represent retail to home transportation time for fresh-cut lettuce. Data were fitted by a lognormal distribution (μ=1.4210, σ=0.46478) with a shift of -0.24609.

4.9 Dose response relationship and risk characterization

Serving size was set in this model to be 85 grams based on a U.S. Food and Drug Administration's (FDA) report "Reference amounts customarily consumed per eating occasion" (FDA, 2002). Concentration after storage was multiplied by serving size to calculate dose (i.e., number of *E. coli* O157:H7 cells) ingested per serving of fresh-cut lettuce. The dose-response model in this study was the Beta-Poisson model first reported by Haas et al. (1983) with parameter values from Cassin et al. (1998)

and subsequently used in different risk assessments including the QMRA for *E. coli* O157: H7 in leafy greens by Danyluk and Schaffner (2011):

$$P = 1 - (1+ D/\beta)^{\alpha}$$

Where P is the probability of illness from estimated ingested dose. D is the number of organisms ingested (i.e., dose) per serving, and α and β are model parameters. The values for α and β from the study by Cassin et al. (1998) was used to determine the probability of illness from exposure to *E. coli* O157:H7 in lettuce. Probability of illness per serving was calculated as the product of contamination prevalence and the probability of illness from estimated ingested dose.

According to Food Availability (Per Capita) Data System, annual lettuce consumption amount per person was 26.8 lbs. (12.16 Kg) in the U.S. (USDA-ERS, 2010b). When food loss (loss of the edible amount of food that is available for human consumption but is not consumed for any reason) accounted for, the adjusted annual consumption of lettuce per person is 13 lbs. (5.9 Kg) in the U.S. (USDA-ERS, 2010a). Number of servings of lettuce per person per year in the U.S. was estimated to be 69 servings/person as calculated through annual consumption per person (5.9 kg) divided by serving size (85 g). The U.S. population was 316,085,800 by June 20, 2013, according to the statistic on U.S Department of Commerce - Census Bureau (DOC-Census Bureau, 2013). The annual number of servings consumed in the U.S. population is 2.19×10^{10} servings/year calculated as the product of number of servings consumed per person per year and the U.S. population. An estimate of the annual number of cases in the U.S. was calculated as the product of the probability of illness

per serving and the annual consumed number of lettuce servings in the U.S. population.

4.10 Scenario analysis for different intervention strategies

In this QMRA model, a total of five different intervention scenarios were analyzed to evaluate the effects of washing with chlorine during processing, ultrasound and organic acid, gamma radiation, *E. coli* O157:H7-specific bacteriophages, and decontamination by consumer washing, on their influence of the estimated probability of illnesses and number of illnesses due to fresh-cut lettuce consumption potentially contaminated with *E. coli* O157:H7.

4.10.1 Washing with chlorine

In lettuce processing, chlorinated water is widely used as chlorination has been reported to reduce the pathogen levels in lettuce. A number of studies have determined the effect of different concentration of chlorine on reduction of *E. coli* O157:H7 (Stopforth et al., 2008; Keskinen et al., 2009; Zhang et al., 2009; Nou and Luo, 2010). The reported log reduction data were extracted from the aforementioned literature and were fitted to a range of statistical distributions by using BestFit software (Palisade Corp., Ithaca, NY). The log reduction of *E. coli* O157:H7 achieved by washing with chlorine was described by a triangular distribution (min=0.68 log CFU/g, most likely=0.68 log CFU/g, max=4.0347 log CFU/g) truncated at 3.6 log CFU/g. In this QMRA model, chlorine was assumed to be applied on the lettuce during washing process.

4.10.2 Ultrasound and organic acid

Ultrasound has a variety of applications in food processing. Ultrasound with frequencies in the range of 20–100 kHz is widely applied to generate a powerful cavitation phenomenon which can lead to destruction and detachment of microorganisms from the surfaces of fresh produce (Scouten and Beuchat, 2002; Seymour et al., 2002). Sagong et al. (2011) hypothesized that ultrasound might help aqueous sanitizers to better penetrate, which may increase the effectiveness of aqueous sanitizers. The same authors determined the effectiveness of combining ultrasound and organic acids on reducing *E. coli* O157:H7 on fresh lettuce. The results show that the combined treatment could provide up to 2.75 log reduction on concentration of *E. coli* O157:H7 on lettuce (Sagong et al., 2011). Based on these data, log reduction on *E. coli* O157:H7 by ultrasound and organic acid was described by a uniform (min=0.89 log CFU/g, max=2.75 log CFU/g) distribution in this QMRA model. The decontamination of ultrasound and organic acid was assumed to be applied after the washing process.

4.10.3 Ionizing radiation

Ionizing radiation, such as gamma ray and electron beam radiation, is a penetrating non-thermal step that has been previously assessed as an effective way to inactivate foodborne pathogens such as *E. coli* O157:H7, *Salmonella*, and *Listeria monocytogenes* on various vegetables (Bidawid et al., 2000; Foley et al., 2002; Niemira, 2002). In 2008, the FDA permitted the use of ionizing radiation at a dose of up to 4 kGy in vegetables to control foodborne pathogens and to ensure quality (FDA, 2008). Studies have demonstrated that the sensory characteristics in irradiated

vegetables could be affected at irradiation levels above 0.5 kGy (Niemira et al., 2002; Foley et al., 2002; Niemira, 2008). The log reduction R achieved by ionizing radiation was calculated by: R=D/ D_{10}, where D is the radiation dose, and D_{10} is the radiation dose needed to achieve 1-log reduction. Niemira et al. (2002) reported a D_{10} value of 0.136 kGy. In this model, this D_{10}-value was used and the radiation dose was described using a uniform distribution (min=0.04 kGy, max=0.5 kGy) based on studies by Niemira et al. (2002), and Foley et al. (2002). The ionizing radiation was assumed to be applied after the lettuce was packaged.

4.10.4 E. coli-specific bacteriophage

Bacteriophages are viruses that have the ability to invade bacterial cells and cause cell lysis. Bacteriophages specific to foodborne pathogens could be applied during processing to reduce certain pathogens on food products. Many studies have reported that bacteriophages were effective in reducing bacterial pathogens in fresh produce, including lettuce (Sharma et al., 2009; Guenther et al., 2009), tomatoes (Ye et al., 2009), and broccoli (Abuladze et al., 2008). Bacteriophages specific for *L. monocytogenes* have been approved for use in deli meats and are considered ‘‘generally recognized as safe’’ by the U.S. FDA (FDA, 2006b). Thus, bacteriophages have the potential of targeted use in lettuce to reduce the contamination level of foodborne bacteria, such as *E. coli* O157:H7. Sharma et al. (2009) examined the effectiveness of bacteriophages specific for *E. coli* O157:H7 in reducing populations on fresh-cut lettuce. Their results show that by spraying bacteriophages on lettuce, the *E. coli* O157:H7 counts on lettuce were reduced by 1.92 log units. Viazis et al. (2011) reported that by applying different levels of a *E.*

coli O157:H7-specific bacteriophage cocktail on baby romaine lettuce, the concentration of *E. coli* O157:H7 was reduced by 0.65 to 2.05 log CFU/g under temperature range from 4℃ to 23℃. In this QMRA study, treatment with *E. coli* O157:H7-specific bacteriophage was assumed to be applied after lettuce is washed and shredded. The log reduction by bacteriophage treatment was described by a PERT distribution (min=0.65, most likely= 1.92 log CFU/g, max= 2.05 log CFU/g).

4.10.5 Consumer washing

Fresh-cut lettuce is washed during processing. However, the effectiveness of washing to remove pathogen cells from the surface of lettuce is limited, which may result in the presence of pathogen in lettuce at the moment of consumption. In the household, consumers may choose to wash lettuce again before consumptions, either due to a perceived increase in product cleanliness, or to refresh the product before consumption. Hence, this QMRA model includes a washing step carried out by the consumers in their kitchen. Fishburn et al. (2012) determined the efficacy of consumer washing at home with running tap water, and their results indicate that washing with running tap water provided an average reduction of 1.69 log CFU/g for *E. coli* O157:H7 on the surface of lettuce, which was used in this QMRA model. The reduction by consumer washing at home was included after home storage and before consumption.

4.11 Irrigation water quality scenarios

In this QMRA study, the model was used for eight risk scenarios represent situations where irrigation water is of different microbial quality. In the baseline

model, it was assumed that the microbial quality of irrigation water used in lettuce farms was in compliance with LGMA recommendation (maximum *E. coli* limit: 235 CFU/100ml) and *E. coli* concentration in irrigation water was described by a uniform distribution (min=1 CFU/100 ml, max=235 CFU/100 ml). Different distributions describing irrigation water quality that exceeded the 235 CFU/100 ml limit used in irrigation water quality scenario analysis were listed as following:

Uniform distribution (1 CFU/100 ml, 500 CFU/100 ml);

Uniform distribution (1 CFU/100 ml, 1000 CFU/100 ml);

Uniform distribution (1 CFU/100 ml, 5000 CFU/100 ml);

Uniform distribution (1 CFU/100 ml, 10000 CFU/100 ml);

Uniform distribution (235 CFU/100 ml, 500 CFU/100 ml);

Uniform distribution (500 CFU/100 ml, 1000 CFU/100 ml);

Uniform distribution (1000 CFU/100 ml, 5000 CFU/100 ml);

Uniform distribution (5000 CFU/100 ml, 10000 CFU/100 ml).

All scenarios with different irrigation water microbial quality were simulated to evaluate their influence on the number of cases per year due to consumption of fresh-cut lettuce.

4.12 Model simulations and analysis

The risk model was developed by integrating relevant data, information, statistical distributions, and formulas as detailed in Table 3. The risk models for all scenarios were simulated with the Monte Carlo simulation technique by using risk

modeling software @Risk 6.1 (Palisade Corp., Ithaca, NY). All models were simulated for 100,000 iterations based on previously published reports relevant to this current QMRA study (Danyluk and Schaffner, 2011; Latorre et al., 2011). Some previously published reports relevant to this QMRA study also utilized lower number of iterations (10,000 iterations) during model simulations than that was used in the study described herein (Danyluk et al., 2006; Carrasco et al., 2010; Franz et al., 2011; Ottoson et al., 2011; Ding et al., 2013). To sample different values for input parameters and variables, Latin Hypercube sampling method was used. Sensitivity analyses were performed to identify important parameters affecting public health risk of *E. coli* O157:H7 illnesses from fresh-cut lettuce consumption. Spearman's correlation coefficients were used for sensitivity analyses to determine the effect of input variables on the probability of illnesses per serving and the number of illnesses in the U.S. population per year.

Chapter 5: Results and Discussion

5.1 Probability of illnesses per serving and number of cases per year

The ingestion dose was integrated with the dose-response model to calculate the probability of illness associated with consumption of a serving of fresh-cut lettuce. By using a Beta-Poisson dose-response model, the average probability of illness per serving of fresh-cut lettuce was 9.87×10^{-8} (1^{st} & 99^{th} percentiles: 6.45×10^{-14} & 1.74×10^{-7}) (Table 4).

Table 4. Probability of illness per serving of fresh-cut lettuce for the baseline model and five intervention scenarios.

Scenarios	Probability of illness per serving					
	Mean	1^{st} percentile	5^{th} percentile	Median	95^{th} percentile	99^{th} percentile
Baseline	9.9×10^{-9}	6.5×10^{-14}	2.9×10^{-13}	2.5×10^{-11}	8.8×10^{-9}	1.7×10^{-7}
Chlorine	7.8×10^{-9}	2.5×10^{-16}	1.9×10^{-15}	4.7×10^{-13}	2.8×10^{-10}	5.8×10^{-9}
Ultrasound and organic acid	5.5×10^{-9}	5.0×10^{-16}	2.7×10^{-15}	3.9×10^{-13}	1.9×10^{-10}	3.8×10^{-9}
Irradiation	8.2×10^{-9}	9.9×10^{-17}	7.1×10^{-16}	2.6×10^{-13}	2.2×10^{-10}	5.2×10^{-9}
Bacteriophage	6.9×10^{-9}	1.5×10^{-15}	7.1×10^{-15}	7.2×10^{-13}	2.9×10^{-10}	5.6×10^{-9}
Consumer washing	8.7×10^{-9}	1.3×10^{-15}	6.0×10^{-15}	5.1×10^{-13}	1.8×10^{-10}	3.6×10^{-9}

The number of cases per year was based on probability of illness per serving and consumption data. The average number of cases per year predicted by baseline model was 2,160 (1^{st} & 99^{th} percentiles: 0.001 & 3,815) in the U.S. (Table 5).

Table 5. Number of cases per year due to consumption of fresh-cut lettuce in the U.S. population for the baseline model and five intervention scenarios.

Scenarios	Number of Cases per Year						
	Mean	Fold change*	1st percentile	5th percentile	Median	95th percentile	99th percentile
Baseline	2,160	-	1.4×10^{-3}	6.4×10^{-3}	0.55	191.5	3,816
Chlorine	170	12.7	5.4×10^{-6}	4.2×10^{-5}	1.0×10^{-2}	6.2	127
Ultrasound and organic acid	121	17.9	1.1×10^{-5}	6.0×10^{-5}	8.4×10^{-3}	4.3	84
Irradiation	180	12.0	2.2×10^{-6}	1.6×10^{-5}	5.7×10^{-3}	4.9	114
Bacteriophage	151	14.3	3.3×10^{-5}	1.6×10^{-4}	1.6×10^{-2}	6.2	122
Consumer washing	189	11.4	2.9×10^{-5}	1.3×10^{-4}	1.1×10^{-2}	3.9	78

*Fold changes were calculated by comparing mean values of five intervention scenarios with the mean value of the baseline model.

5.2 Scenario analysis for intervention strategies

Intervention strategies were evaluated on their influence of relative risks compared to the baseline model. All interventions reduced the average probability of illness per serving and the number of cases per year (Table 4 & Table 5). The application of chlorine washing, ultrasound and organic acid, ionizing radiation, *E. coli* O157:H7-specific bacteriophage and consumer washing reduced the mean number of cases per year in the U.S. by 12.7-, 17.9-, 12.0-, 14.3-, and 11.4-fold, respectively, relative to the baseline model (i.e., no intervention strategy). To better demonstrate the difference between intervention scenarios and the baseline model, the cumulative density functions (CDF) of probability of illness per serving and number of cases per year for the baseline model and each of the intervention scenarios are

provided in Figure 3 & Figure 4. These functions are plotted on a logarithmic scale as a convenient representation of probability of illness per serving and number of cases per year, which are concentrated near zero and not comparable under non-log scale. The CDF provided an indication of the degree of differences in risks when different intervention strategies were applied.

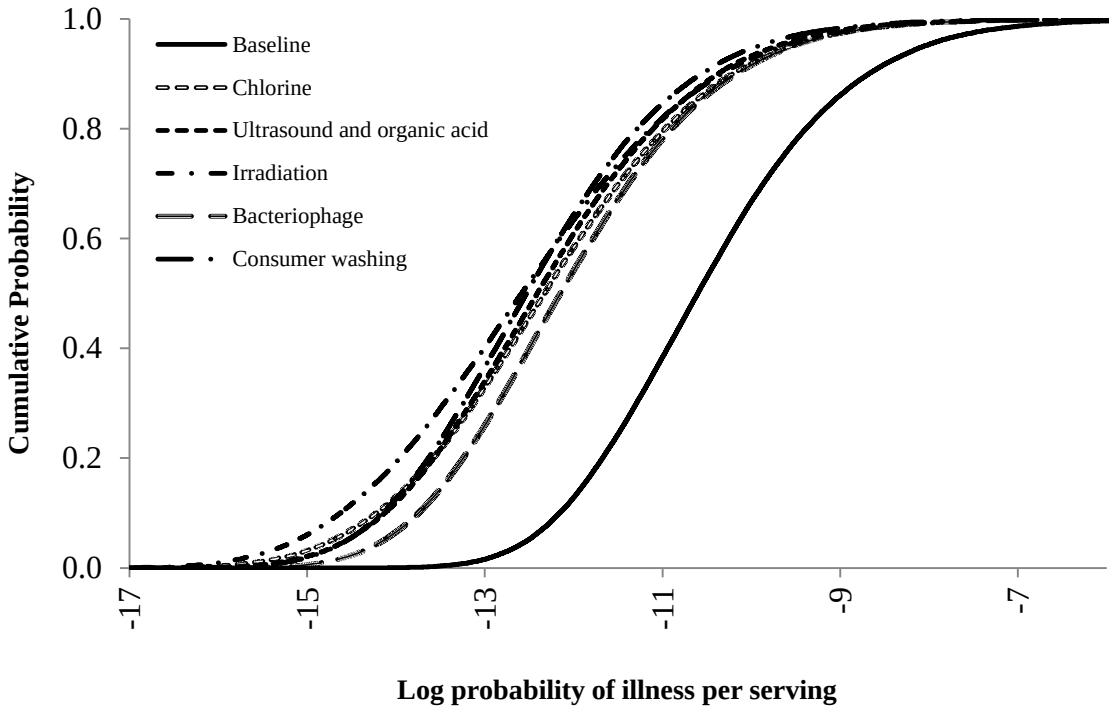

Figure 3. Cumulative density functions of probability of illness per serving due to consumption of fresh-cut lettuce for the baseline model and five intervention scenarios. Distributions are shown on a logarithmic scale.

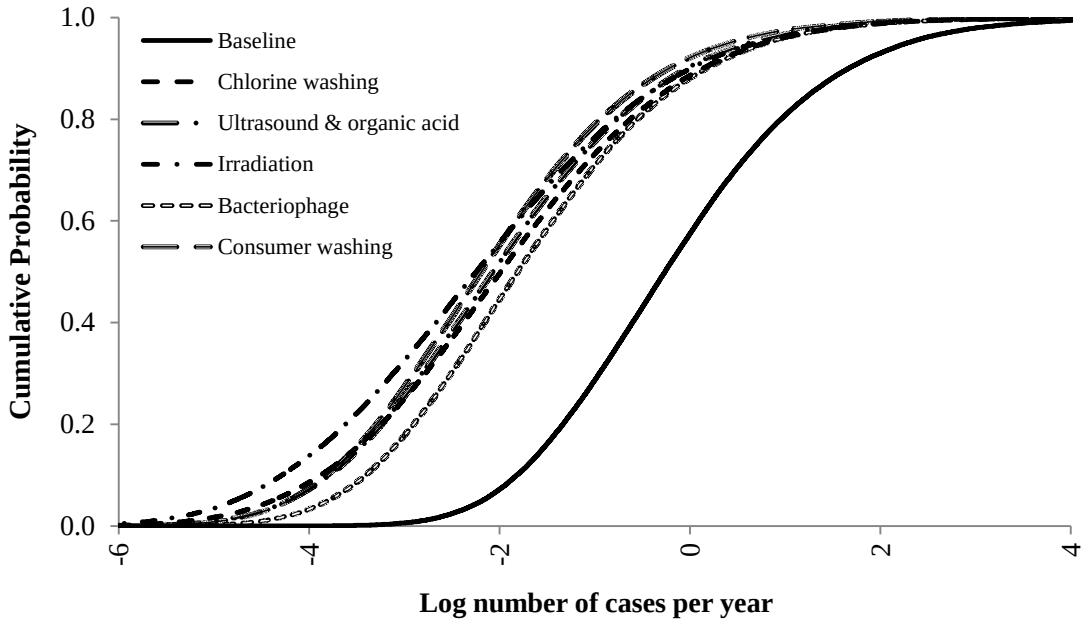

Figure 4. Cumulative density functions of number of cases per year due to consumption of fresh-cut lettuce for the baseline model and five intervention scenarios. Distributions are shown on a logarithmic scale.

Combined treatment of ultrasound and organic acid was the most effective in reducing the public health risks among the five intervention strategies which reduce the number of cases per year by approximately 18-fold comparing to the baseline model.

5.3 Scenario analysis for different irrigation water quality

Scenarios with different irrigation water quality that exceeds the LGMA 235 log CFU/100 ml limit were evaluated on their influence of relative risks compared to the baseline model. The predicted number of cases per year increased by 1.1-fold to 4.3-fold when *E. coli* concentration in irrigation water increases from 1 - 235 CFU/100 ml to 5000 - 10,000 CFU/100 ml (Table 6).

Table 6. Number of cases per year due to consumption of fresh-cut lettuce in the U.S. population for the baseline model and different irrigation water quality.

Irrigation water quality (CFU/100 ml)	Number of cases per year						
	Mean	Fold change*	1st percentile	5th percentile	Median	95th percentile	99th percentile
1-235	2,160	-	1.4×10^{-3}	6.4×10^{-3}	0.6	192	3,815
1-500	2,433	1.1	2.1×10^{-3}	9.4×10^{-3}	0.8	245	4,836
1-1000	2,859	1.3	3.1×10^{-3}	1.4×10^{-2}	1.1	333	6,218
1-5000	5,182	2.4	8.3×10^{-3}	4.1×10^{-2}	3.2	894	15,875
1-10000	7,226	3.3	1.3×10^{-2}	6.7×10^{-2}	5.5	1,517	27,929
235-500	2,658	1.2	3.4×10^{-3}	1.5×10^{-2}	1.0	296	5,705
500-1000	3,274	1.5	5.5×10^{-3}	2.4×10^{-2}	1.6	434	7,680
1000-5000	5,759	2.7	1.4×10^{-2}	6.1×10^{-2}	4.1	1,0775	18,687
5000-10000	9,320	4.3	3.3×10^{-2}	0.1	9.1	2,315	41,140

*Fold changes were calculated by comparing mean values of each of the irrigation water quality scenarios with the mean value of the baseline model.

5.4 Sensitivity analysis

The sensitivity of number of cases per year to input values was determined using Spearman's rank order correlation. Number of cases per year was most sensitive to the following inputs (Figure 5): retail storage temperature (0.50), home storage temperature (0.38), soil *E. coli* concentration (0.37), VTEC proportion in soil (0.25), VTEC proportion in irrigation water (0.18), time until last consumption (-0.12), log reduction by washing with water (-0.10), irrigation water quality (0.09), and holding time after irrigation (-0.07).

In this study, retail and home storage temperatures are the most important factors affecting the estimated number of cases per year. This result indicated that temperature control is critical during post processing storage stages in order to suppress the growth of *E. coli* O157:H7 on lettuce. Soil *E. coli* concentration and

VTEC proportion in soil are also important risk factors. Soil contaminated by pathogens from feces could be a source of contamination of *E. coli* O157:H7 in lettuce. This highlights the need for pre-harvest intervention strategies of lowering the level of *E. coli* O157:H7 concentration in the soil around lettuce field to reduce introduction of pathogen cells into lettuce production environment.

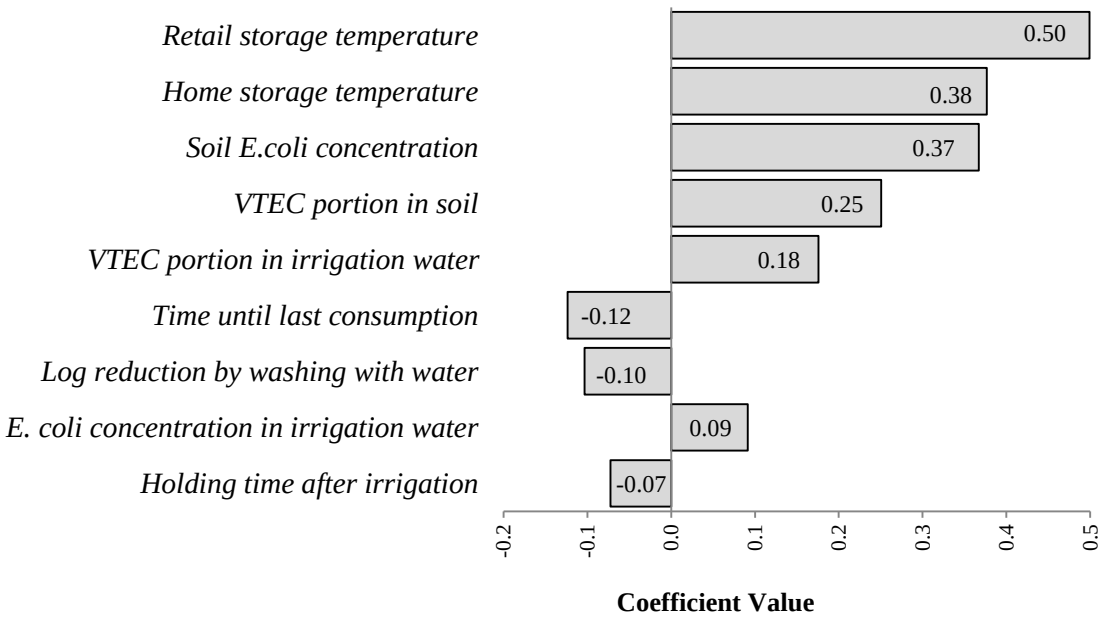

Figure 5. Tornado graph showing the most important parameters and variables affecting the estimated number of cases per year. Spearman correlation coefficients were obtained from @Risk sensitivity analysis and were shown next to each bar.

VTEC proportion in irrigation water and *E. coli* concentration in irrigation water are also factors affecting the estimated number of cases per year. Thus, irrigation water quality was considered as a risk factor. The sensitivity analysis also suggested that time until last consumption, log reduction by washing with water, and holding time after irrigation have effects on the number of cases per year.

5.5 Discussion

This study provided a QMRA for *E. coli* O157: H7 in fresh-cut lettuce in the U.S. Several QMRAs for leafy green vegetables have been reported (Franz, et al., 2010; Carrasco et al., 2010; Danyluk and Schaffner, 2011; Ottoson et al., 2011; Ding et al., 2013), some study has included a whole coverage of the production-to-consumption supply chain of leafy greens to relate the 2006 spinach outbreak (Danyluk and Schaffner, 2011), while other studies have focused only on specific infield inactivation factors aimed at comparing the effects of different mitigation strategies (Ottoson et al., 2011) or examined in detail only the stages during transportation and storage (Franz et al., 2010). There is a lack of data for determining the effects of environmental factors that could affect the concentration and prevalence of *E. coli* O157:H7 before harvesting of lettuce. In the study by Ottoson et al. (2011), effects of irrigation water quality and holding time between last irrigation and harvest were evaluated to compare their influence on reducing public health risks in their screening level QMRA. Danyluk and Schaffner (2011) used an inactivation rate to determine the change on produce contamination level during infield production expressed as a triangular distribution based on a previously published study. However, in other studies, stages before lettuce harvesting or processing were not considered as it was assumed that there are no controllable factors that impact the contamination level of pathogens in leafy green vegetables (Carrasco et al., 2010; Ding et al., 2013). In this current QMRA model contamination from irrigation water and from soil via harvesting tools were modeled as contamination routes to determine their effects on lettuce contamination with *E. coli* O157:H7 during infield production. Important

factors including irrigation water quality, ratio of VTEC to generic *E. coli*, and water attachment rate were quantified using different distributions based on available data. Pathogen inactivation during holding time between last irrigation and harvest was also modeled using a Weibull survival function. Factors including soil *E. coli* concentration, soil attachment on harvesting tools, and transfer rate from harvesting tools to lettuce were quantified to determine the contamination from soil via harvesting tools. The risk model in this study provided a mathematical description of the lettuce chain during infield production and could be used to evaluate the impact of potential pre-harvest interventions when relevant data are available. In previously published QMRA studies, Ding et al. (2013) considered the cross contamination of pathogen in lettuce caused by handling mistakes (e.g., unwashed cutting boards, unwashed kitchen tools); Danyluk and Schaffner (2011) modeled the cross contamination in leafy green processing based on extrapolation from one single study. In this QMRA study, a cross contamination model describing the transfer rates from contaminated lettuce to processing facilities and the subsequent transfer from facilities to uncontaminated lettuce during processing of lettuce was used. This approach used in the QMRA model described cross contamination in a more explicit way which enabled better characterization of the importance of cross contamination. During the transportation and storage, lettuce is subjected to varying temperatures, thus predictive models should be able to respond to the changing conditions (McKeller and Delaquis, 2011). In this QMRA study, a predictive model that combined growth and die-off models was applied. The combined model was able to

simulate the increase or decline of pathogen cells based on temperature conditions (threshold temperature: 5 ℃).

The QMRA provided an estimate of the risks associated with *E. coli* O157:H7 from fresh-cut lettuce in the U.S. In the QMRA study by Danyluk and Schaffner (2011), predicted mean number of cases per year ranges from 2,010 to 10,903 for different prevalence of positive incoming servings and initial concentration of *E. coli* O157:H7. In this QMRA study, the predicted mean number of cases per year (2,160) from the baseline model falls within the range of the QMRA study by Danyluk and Schaffner (2011) (2,010 to 10,903 average cases of illnesses). In this QMRA, irrigation water quality was described using a uniform distribution (1 CFU/100 ml, 235 CFU/100 ml) in the baseline model. To test the effects of using different distributions in describing irrigation water quality, a lognormal distribution (μ=126 CFU/100 ml, σ=20 CFU/100 ml) was used. By performing 100,000 iterations in @Risk software, the same number of iterations used in this study, for the lognormal distribution described here, the minimum, mean, and the maximum values obtained were 58, 126, and 247 CFU/100 ml, respectively. Using the lognormal distribution in the baseline model, the estimated mean number of cases per year was 2,173 compared to 2,160 when uniform distribution was used in the baseline model for the irrigation water quality, which indicates that there was no considerable difference in the average number of estimated cases per year when lognormal distribution (μ=126 CFU/100 ml, σ=20 CFU/100 ml) was used in place of uniform distribution (1 CFU/100 ml, 235 CFU/100 ml) for irrigation water quality in the baseline model. The QMRA provided was also used to evaluate the relative impacts of different potential

interventions on public health risks from consumption of *E. coli* O157:H7 in fresh-cut lettuce. By applying different pre-harvest, processing and post-processing interventions have shown the potential of reducing risks. However, many other factors including feasibility, cost, potential influence on quality, and must be taken into consideration when interpreting the effectiveness of interventions. In addition, data and distributions used for quantifying the effects of interventions on contamination levels of *E. coli* O157:H7 in lettuce are based on various previous studies with different study design. Many of the studies were conducted with high level of inoculated dose in order to measure the survival of *E. coli* O157:H7. Thus, the results of some studies might not adequately represent realistic contamination situation where levels of *E. coli* O157:H7 are generally low, and may overestimate the impact of interventions.

The combination treatment of ultrasound and organic acid was most effective in reducing number of cases per year associated with *E. coli* O157:H7 on fresh-cut lettuce. Ultrasound has bactericidal effect and has been reported to promote decontamination of raw vegetables (Seymour et al., 2002). Organic acids are effective under a wide temperature range and can be applied to inactivate foodborne pathogens on organic fresh produce (Sagong et al., 2011). Besides, the combined treatment of ultrasound and organic acid can be applied to reduce pathogens on lettuce surfaces without affecting the quality of organic produce (Sagong et al., 2011). However, organic acids are odorous and corrosive (Marriott and Gravani, 2006), which may limit their use in fresh produce industry. In addition, the efficiency of ultrasound can be affected by a number of factors such as organic matter, water hardness, and

dissolved gases (Sagong et al., 2011). Therefore, more knowledge and information will be needed for industrial application of ultrasound and organic acids.

Application of *E. coli* O157:H7-specific bacteriophage treatment to fresh-cut lettuce also provides great reduction on number of cases per year from consumption of fresh-cut lettuce. Such result in decrease indicated the potential benefits to reduce the public health and economic burden in the U.S. Using specific bacteriophage in foods could be an effective way to reduce level of contamination of foodborne pathogens. Bacteriophages are ubiquitous in nature, and they can be isolated from aquatic environment (e.g., sewage or waste water) and various foods (e.g., ground beef or chicken) (Campbell, 2003; Brussow, 2005; Greer, 2005). Besides, bacteriophages use in agriculture is not likely to select resistant species in untargeted bacteria as they are highly specific (Viazis et al., 2011). However, the potential negative effects of bacteriophages on the quality and appearance of produce commodities including lettuce are unknown. In addition, bacteriophage as virus may bring concerns from consumers which may affect the public acceptance on their application in food industry.

Chlorine washing also dramatically reduced the risk of *E. coli* O157:H7 associated with fresh-cut lettuce. Efficacy of chlorine against bacterial pathogens has been determined and proven in a variety of studies (Keskinen et al., 2009; Zhang et al., 2009; Nou et al., 2010; Nou et al., 2011). Washing with chlorine has widely been used as common practice in industries for fresh-cut produce to reduce microbial load. However, studies have reported that use of chlorine in produce processing could have adverse effects, such as formation of trihalomethanes (Richardson, 1998). In addition,

washing with sanitizers like chlorine cannot inactivate internalized bacteria (Niemira, 2008). Besides, there are increasingly demands for food industries to reduce their use of chemical additives to meet the consumers' needs for freshness of produce. The effects of washing and chlorine washing in detaching, killing, or transferring between contaminated and non-contaminated produce should be explored more and included in the risk model when relevant data and models are available.

Variability and uncertainty are two important concepts in QMRA. Variability represents inherent heterogeneity of a population, while uncertainty represents the lack of knowledge of parameter values. Separating variability and uncertainty in QMRA is an important issue (Vose, 2000; Nauta, 2000). However, correct separation of variability and uncertainty is difficult due to limited data availability and it requires more complicated modeling and simulation techniques (Nauta, 2000; Nauta, 2009). The variability, i.e., the stochastic process is the basis of a risk analysis model and the uncertainty about model parameters can be overlaid onto the model variability by using a two-dimensional modeling framework (Vose, 2000). However, most of the published risk assessments are one-dimensional. In this risk assessment a one-dimensional modeling framework was used in which variability and uncertainty are embedded in the risk model together. Probability distributions can be used to describe both variability and uncertainty.

A number of limitations and data gaps could be identified in this study. The ratio of VTEC to generic *E. coli* in soil was described in this study using the same distribution for VTEC ratio in irrigation water, as no available data is available for soil. Thus, this distribution may not represent the real situation in soil which may

affect the predicted results. As no data was found on soil water content at the time of harvest, it was assumed in this model that water content is 20%. In reality, this value may vary as a result of different harvesting practice or climate in different regions. Water content will affect the attachment of soil on harvesting tools. In the study of Yang et al. (2012), the attachment of soil on harvesting tools range from 0.05 to 31.26 g/blade. In addition, Yang et al. (2012) concluded that high water content in soil (representing a condition where lettuce is harvested soon after rain or irrigation) will increase the transfer of *E. coli* O157:H7 from soil to harvesting tools and subsequent transfer to lettuce. Thus more data are needed in order to quantify the variation of this parameter to better represent actual conditions at harvest. Cross contamination is a potential risk factor for lettuce contamination with pathogens during the processing. In this risk model, cross contamination was assumed as a result of transfer from contaminated lettuce to processing surfaces and subsequently transfer from processing surfaces to uncontaminated lettuce. In addition, prevalence of contamination is increased due to cross contamination. It was also assumed that pathogen cells are evenly distributed among contaminated lettuce after processing (the sum of the initially contaminated and the cross-contaminated lettuce) as little is known on how pathogen cells are distributed. Initial prevalence of contaminated lettuce at the beginning of processing and retail storage time for fresh-cut lettuce is also unknown.

The potential effect of transportation from farm to processing plant on concentration of *E. coli* O157:H7 in lettuce is not considered in this QMRA model due to lack of available data, assuming that lettuce is rapidly transported to processing

plant after harvesting and the change in *E. coli* O157:H7 concentration is negligible. Similarly, transportation of lettuce from processing plant to retail was not included in this QMRA model. Thus, the potential decline or growth during these transportations were not quantified which may affect the estimate of risk. In the growth model applied in this study, the lag phase was not included as the importance of lag time in modeling *E. coli* O157:H7 growth in lettuce is unknown.

Quantitative risk assessment can provide a way to model the food system in a systematic way, which can provide risk managers a comprehensive picture of key factors that impact the contamination levels of a certain pathogen along the supply chain of a certain food product. The QMRA model in this study was developed based on available data and can be used to provided estimates of the risks *E. coli* O157:H7 due to consumption of fresh-cut lettuce in the U.S. In addition, this QMRA also compared the relative risks for different potential interventions that could be applied at pre-harvest, processing, and post-processing of lettuce supply chain. Although limitations and assumptions lie within the model, the QMRA model provided a framework that is valuable to identify key factors and data gaps. The model is adaptable to provide better estimates as future research and available data could fill the gaps in the model and to evaluate different potential interventions that could be applied throughout the fresh-cut lettuce food chain when more data become available.

Chapter 6: Conclusions and Suggestions for Future Research

The QMRA model developed in this study could provide risk managers and policy-makers a systematic way to present the key factors along the farm-to-fork continuum of fresh-cut lettuce. Risk representing current practices for fresh-cut lettuce was estimated based on a thorough review of all available data. The predicted number of cases per years is comparable to other QMRA for lettuce. Beyond the current estimates, the QMRA model developed in this study provides a useful tool to assess how other risk factors can impact illness incidence, and to compare the relative efficacy of different pre-harvest, processing, and post-processing interventions. The results indicate that retail and home storage temperature was the most important factors affecting risk, suggesting risk management could focus on temperature control at retail and home storage level. The most effective intervention for *E. coli* O157:H7 management in fresh-cut lettuce is the combined treatment of ultrasound and organic acid during processing. Although a number of limitations and data gaps exist, the QMRA model described herein considered the whole farm-to-fork continuum of fresh-cut lettuce including infield production while explicitly modeled the cross contamination during processing, and for the first time incorporated a growth/death model that was able to quantify the decline of pathogen cells during transportation or storage.

Additional research is needed on distribution of pathogen cells on processed produce, determining the importance of lag time during storage and transportation stages, evaluation of impacts of strain virulence and host susceptibility on dose response relationship. Some critical data gaps were identified in this QMRA study

including, initial prevalence of contaminated lettuce at the beginning of processing, ratio of VTEC to generic *E. coli* in soil, soil water content at the time of harvest, retail storage time, and time and temperature during transportation from farm to processing plant and from processing plant to retail store. Transportation of lettuce from farm to processing plant and processing plant to retail should be included in the future when time-temperature data are available to quantify the potential increase or decline of pathogen cells on lettuce. Two-dimensional modeling to characterize uncertainty and variability in model parameters on the risk estimate should be performed in future risk assessments.

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