Development of a Quantitative Microbial Risk Assessment Model for Foodborne *E. coli* O157:H7 Infection: The Risk of Consuming Lettuce

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The current study used a probabilistic Quantitative Microbial Risk Assessment (QMRA) framework to describe the change of *E. coli* O157:H7 concentration in lettuce through a foodborne pathway, to develop a predictive model for risk estimation for *E. coli* O157:H7 infection associated with lettuce. The model consisted of a series of pathogen-associated events including initial contamination, growth during cooling, cold storage and distribution, disinfection (chlorine, gaseous chlorine dioxide and gamma irradiation), and dose response after consumption. A modified Baranyi growth model was proposed which described the initial physiological state of *E. coli* O157:H7 as a function of the initial temperature. The modified Baranyi growth model was used to predict *E. coli* O157:H7 growth under realistic time-temperature profiles, accounting for the time dynamics of temperature fluctuation. The risk assessment model was constructed in an Excel spreadsheet and Monte Carlo uncertainty analysis was simulated using Crystal Ball. The results in the current study showed that temperature control was the key measure for minimizing the risk of *E. coli* O157:H7 infection associated with lettuce. Disinfecting contaminated lettuce using the hypothetical methods examined in the study had limited effectiveness in risk reduction. Temperature abuse occurring before or after the hypothetical disinfections significantly diminished the disinfection effect and contributed to increased risk. Of all simulated scenarios, the lowest risk was associated with adequate temperature control and irradiation (44 infections per 1000 consumptions [95%: 94 infection per 1,000 consumption; 5%: 5 infections per 1,000 consumption]). The model can be used to explore the public health impact of other potential strategies that can be adopted to minimize the risk of *E. coli* O157:H7, while taking into account the possible amplification of pathogen through the food chain.

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Chapter 1: Introduction

In recent years, *Escherichia coli* O157:H7 has emerged as a major cause of both outbreaks and sporadic cases of human diarrheal disease in North America and throughout the world (Griff & Boyce, 1998; Sparling, 1998; Woodward, Clark, Caldeira, Ahmed, & Rodgers, 2002). Mead *et al* have estimated that *E. coli* O157:H7 causes approximately 73480 illnesses annually in the U.S.; 85% (62456 cases) as the result of foodborne exposure (Mead, Slutsker, & Dietz, 2000).

Historically, foodborne *E. coli* O157:H7 infection was frequently associated with the consumption of foods of bovine origin. However, in the last few decades, a dramatic increase in the incidence of *E. coli* O157:H7 infection associated with fresh fruits and vegetables has occurred (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005; Sivapalasingam, Friedman, Cohen, & Tauxe, 2004). In the U.S., between 1982 and 2002, whereas 52% of the 350 *E. coli* O157:H7 outbreaks reported were caused by foodborne sources, ground beef and fresh produce were responsible for 75 and 38 outbreaks, respectively (Rangel et al., 2005).

Leafy green vegetables, such as lettuce, have been implicated in a number of large outbreaks of *E. coli* O157:H7, some of which had serious impact on public health (Hilborn, Mermin, & Mshar, 1999; Martin, Gustafson, Pelosi, Suarez, & Pierce, 1986; US FDA, 2006; US FDA, 2007). For example, two well-publicized outbreaks involving *E. coli* O157:H7-tainted lettuce served in Taco Bell and Taco John restaurants in the U.S. in 2006 sickened about 150 patrons in total, including 2 cases of hemolytic uremic syndrome (HUS) (US FDA, 2006; US FDA, 2007). These statistics have highlighted the concerns that leafy green vegetables may be an increasing source of *E. coli* O157:H7-associated illness, since leafy green consumption has increased dramatically during the last three decades. Leafy green vegetables have recently been ranked by the World Health Organization as the highest priority in terms of fresh produce

microbial safety from a global perspective (FAO/WHO, 2008) and second (with ground beef being the first) most significant cause of human foodborne illness caused by *E. coli* O157:H7 by Codex Alimentarius Committee (Codex Alimentarius Committee, 2002).

E. coli O157:H7 infection involving lettuce is receiving more and more attention. Leafy green vegetables are usually minimally processed without any kill steps; therefore, reduction of *E. coli* O157:H7 contamination to acceptable levels is essential to protecting public health. Given the serious impact of *E. coli* O157:H7 infection on health and the continued increase of lettuce consumption during recent years, prevention and control of *E. coli* O157:H7 on lettuce is a high priority for public health protection.

Epidemiological studies suggest that contamination of leafy green vegetables with *E. coli* O157:H7 is an event with very low probability (Arthur, Jones, Fabri, & Odumeruz, 2007; Johannessen, Loncarevic, & Kruse, 2002; Mukherjee, Speh, Dyck, & Diez-Gonzalez, 2004; Sagoo, Little, & Mitchell, 2001). Traditional food inspection approach by products sampling at various points of the food chain is apparently inadequate to address this problem. A more systematic risk management approach is to integrate quantitative risk assessment techniques into the development Hazard Analysis Critical Control Point (HACCP) plan. While HACCP plan identifies potential hazards and establishes measures for their control at the critical points in food production where it is essential to prevent or eliminate a food safety hazard (WHO, 1998a), risk assessment, on the other hand, quantifies the combined human health risk of multiple control-point deviations and therefore provides valuable information for risk management (Buchanan & Whiting, 1998).

Thus, the purpose of this project is to conduct a risk assessment for human health risk involving *E. coli* O157:H7-contaminated lettuce, in order to quantify the combined effects of current intervention strategies and to identify potential risk mitigation strategies. Findings from this project will hopefully be used by the C-EnterNet Health Canada group in the future.

Chapter 2: Background

2.1 *E. coli* O157:H7

E. coli O157:H7 belongs to a class of pathogenic *E. coli* known as enterohemorrhagic Escherichia *coli* (EHEC), with O and H designating its somatic antigen and flagella antigen, respectively (Buchanan & Doyle, 1997). Although most *E. coli* strains are harmless and found normally in the mammalian intestine, *E. coli* O157:H7 is a highly virulent strain that has toxin producing capabilities. It is sometimes referred to as verocytotoxin producing *E. coli* (VTEC) or shiga-like toxin producing *E. coli* (STEC).

Escherichia coli O157:H7 is the single most important EHEC serotype in relation to public health. It was first recognized as a human pathogen following two outbreaks of gastrointestinal illness in the U.S. associated with undercooked hamburger patties (Riley et al., 1983).Statistics suggested that, in Canada, the UK, Germany, Belgium, the Netherlands and Japan, *E. coli* O157:H7 has been associated with most outbreaks of EHEC infection and 70% to 80% of sporadic cases of classic HUS (Boyce, Swerdlow, & Griffin, 1995). The incidence of non-O157 EHEC is estimated to be only 20%-50% of that caused by *E. coli* O157:H7 (Mead *et al., 2000)*.

The importance of *E. coli* O157:H7 infection is derived from the severity of the disease. When ingested, *E. coli* O157:H7 exhibit effective acid resistance mechanisms that allow them to survive exposure to gastric acidity and finally to colonize in the large intestine, where they develop attaching and effacing (A/E) lesions in the host cells, induce inflammatory response and cause intestinal hemorrhaging by producing shiga toxins (Nataro & Kaper, 1998). Infectious dose of *E. coli* O157:H7 is found to be very small (10-1000 cells) (Harris et al., 2003).

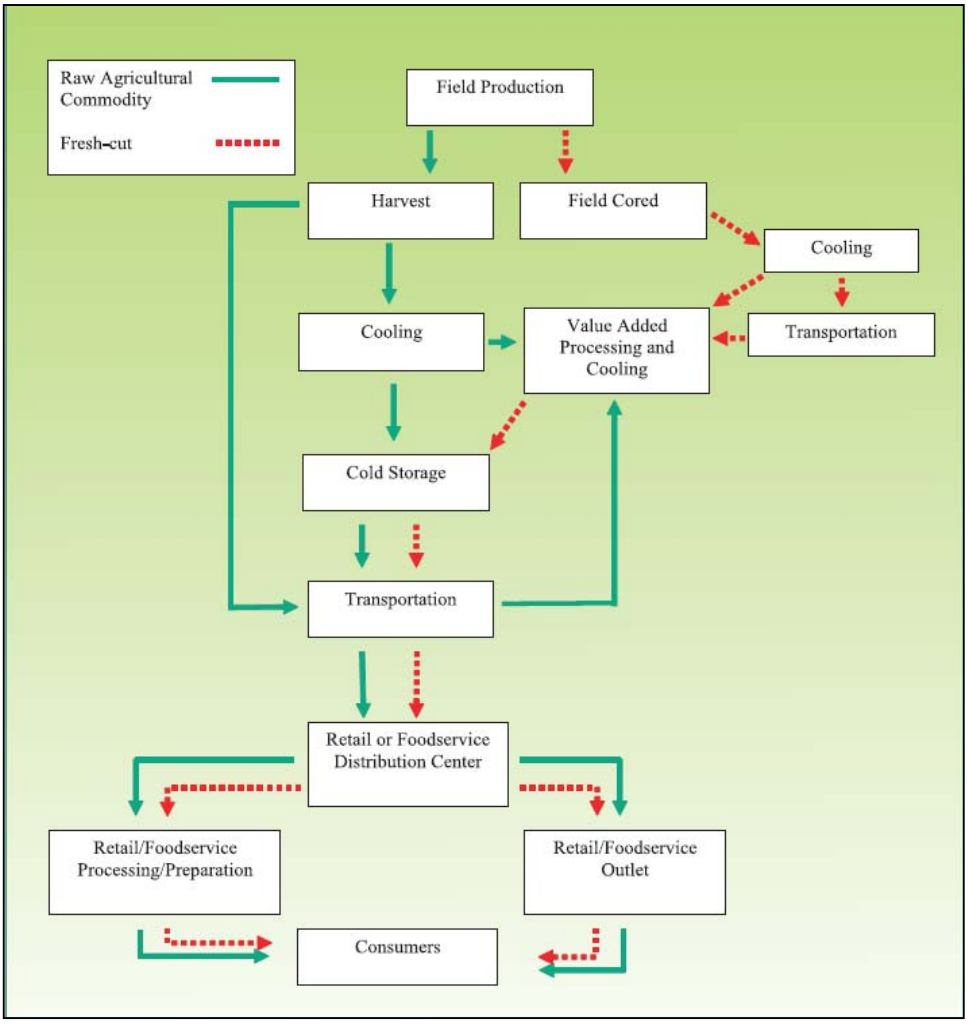
E. coli O157:H7 can affect people of all age; young children are most susceptible, followed by the elderly (Codex Alimentarius Committee, 2002).The incubation period of *E. coli*

O157:H7 disease ranges from two to five days (Harris et al., 2003).Clinical symptoms of *E. coli* O157:H7 infection begin with abdominal cramps and non-bloody diarrhea, which in more than 70% of the cases leading to bloody diarrhea (Bell, Goldoft, & Griffin, 1994). Furthermore, some of those infected may develop hemorrhagic colitis (grossly bloody diarrhea) and HUS, which is a systemic complication involving acute and chronic kidney failure, thrombotic thrombocytopenic purpura (TTP) and neurological sequelae (Nataro & Kaper, 1998). These complications may result in end-stage renal disease (ESRD), a serious chronic condition that can cause death (Nataro & Kaper, 1998).

2.2 Exposure Pathways

E. coli O157:H7 is widely distributed within the environment. It has been isolated from food and water for livestock, manure, soil, flies, domestic animals (e.g., cattle, sheep, pigs, horses, dogs) and wild animals (e.g. deer and birds) (Bach, McAllister, Veira, Gannon, & Holley, 2002). However, epidemiological studies demonstrate that dairy and beef cattle are primary reservoirs of *E. coli* O157:H7 (Bach et al., 2002). Cattle carry *E. coli* O157:H7 asymptomatically, shedding it intermittently and seasonally in their feces (Chapman, 2000).

In developed countries, most lettuce is now produced on an industrial scale (Figure 1). However, in practice, operations at each stage in the production-distribution-consumption chain are diverse and vary considerably.



(Adapted from (US FDA/CFSAN, 2006))

Figure 1. General Supply Chain Flow for Lettuce/Leafy Greens.

So far, outbreak investigations still do not provide sufficient information about how *E. coli* O157:H7 pathogens could have entered the lettuce supply chain and whether postharvest practices would have reduced or accentuated the contamination. In most cases, even extensive outbreak investigations failed to pinpoint the specific risk factors and the routes through which *E. coli* O157:H7 come into contact with lettuce (Ackers et al., 1998; California Food Emergency Response Team, 2007; Doyle & Erickson, 2008). A number of potential risk factors contributing to contamination during preharvest and postharvest phases have been proposed (Table 1).

Table 1. Possible Sources of *E.coli* O157:H7 on Lettuce.

Preharvest Sources	Outbreak Investigation	Experimental Studies
Feces	Ackers et al., 1998; Hilborn et al., 1999	
Irrigation water	Ackers et al., 1998	Wachtel, Whitehand, & Mandrell, 2002
Manure	Ackers et al., 1998	Solomon, Yaron, & Matthews, 2002
Wild and domestic animals;	Hilborn et al., 1999	
Lack of field sanitation	Hilborn et al., 1999	
Harvesting equipment		Gleeson & O'Beirne, 2005
Human handling (workers, consumers)	Harris et al., 2003	
Ice		Kim & Harrison, 2008
Wash and rinse water	Hilborn et al., 1999	
Improper storage (temperature, physical environment)		Wachtel & Charkowski, 2002
Cross-contamination with other foods	Harris et al., 2003	Wachtel & Charkowski, 2002; Wachtel, McEvoy, Luo, Williams-Campbell, & Solomon, 2003

The dynamics of the production environment at the farm level and the variety and diversity of postharvest practices present challenges to controlling possible outbreaks. Efforts must be made throughout the entire system to address this issue. Where the possibility of contamination cannot be excluded, developing effective intervention strategies to minimize or eliminate the risk is a priority for the produce industry. Attention has been paid to examining the survival and growth characteristics of *E. coli* O157:H7 on lettuce and the efficacy of disinfection treatments to eliminate the hazard. The following four sections review the research in these two areas.

2.3 *E. coli* O157:H7 Multiplication on Lettuce

Growth of *E. coli* O157:H7 may occur during cold storage, transportation, retail display or consumer storage, and is dependent on the interaction of a number of intrinsic and extrinsic factors. Operations during lettuce harvest and processing provide favorable conditions for the survival and proliferation of *E. coli* O157:H7 by creating cut surfaces where large amounts of nutrients are released (US FDA/CFSAN, 2001). It has been reported that packaging under modified atmosphere has no apparent effect on the survival and growth of *E. coli* O157:H7 (Abdul-Raouf, Beuchat, & Ammar, 1993).

Similar to other pathogens, temperature is an important factor determining the survival of *E. coli* O157:H7. High temperature during the storage, distribution or retail display of lettuce may result in the multiplication of *E. coli* O157:H7. Laboratory experiments revealed that given sufficient time, *E. coli* O157:H7 is capable of growing at 8°C (Rajkowski & Marmer, 1995). McEvoy *et al* (McEvoy, Luo, Conway, Zhou, & Feng, 2009) examined the survival and growth of *E. coli* O157:H7 under a simulated field temperature (30°C) and a refrigerated temperature (5°C) after *E. coli* O157:H7 were transferred onto lettuce during harvest. The result showed that *E. coli* O157:H7 populations increased by more than 2 log CFU/g in 8h at 30°C, whereas at 5°C, significant ($P<0.05$) growth or loss of viability of *E. coli* O157:H7 was not observed (McEvoy et al., 2009).

2.4 Disinfection

2.4.1 Chlorine

Water containing 50-200 mg/L of chlorine is the most common sanitizer in wash, spray, and flume waters used in the fresh fruit and vegetable industry (WHO, 1998b). Antimicrobial activity of chlorine treatment depends on the amount of free available chlorine (as hypochlorous

acid) in water that comes into contact with microbial cells. Since chlorine reacts with organic matter, its inhibitory activity is compromised when organic matter is present in water (Beuchat, 1999). In addition, human and environmental safety concerns have been raised about the production of chlorinated carcinogenic compounds as a result of the reaction between chlorine and organic matter (Ölmez & Kretzschmar, 2009). Moreover, Seo and Frank (Seo & Frank, 1999) found that *E. coli* O157:H7 are able not only to attach to the surface, trichomes, stomata and cut edges of lettuce, but also to penetrate into the internal tissue of lettuce via natural openings or cut surfaces as a result of environmental stresses (Takeuchi & Frank, 2000; Takeuchi & Frank, 2001). The penetration of *E. coli* O157:H7 into lettuce tissue may increase the resistance of *E. coli* O157:H7 to disinfectants.

A few studies investigated the effect of chlorine specifically on *E. coli* O157:H7 inoculated onto lettuce (Table 2). It is obvious that chlorine treatment at conventional concentrations (50-200 mg/L) has little effect in *E. coli* O157:H7 inactivation. Elevated risk of *E. coli* O157:H7 infection involving lettuce may be attributable to the failure of this disinfection treatment, which has important implications for public health. In an attempt to effectively reduce the microbial load on produce, alternative disinfection methods that are more efficacious than chlorine have been investigated; these will be discussed in the next few subsections.

Table 2. Studies about the Efficacy of Chlorine Treatments.

Investigation	Experimental details	Experimental results
Beuchat, Nail, Adler, & Clavero, 1998	Sprayed inoculated lettuce with water or 200ppm chlorine, then soaked for 10 min and rinsed with water	Spray treatments with 200ppm chlorine can further reduce *E.coli*O157:H7 populations by about 1 log CFU/g after soaking with water
Beuchat, 1999	Used bovine feces as a vehicle of inoculation, spayed inoculated lettuce with water or 200ppm chlorine, and stored for up to 15 days	Spray treatments with 200ppm chlorine and water were equally effective in eliminating *E.coli*O157:H7.
Li, Brackett, Chen, & Beuchat, 2001	Washed inoculated lettuce with 20ppm chlorine or water at 20 or 50°C and storage at 5°C or 15°C for 18 days.	Treatment with 20ppm chlorine at either 20 °C or 50 °C did not yield additional reductions in *E. coli* O157:H7 number, compared to water.

2.4.2 Chlorine Dioxide

Chlorine dioxide (ClO_2) is a powerful oxidizing agent that is about 2.5 times more oxidative than chlorine, but it is more stable and does not react with organic matter to produce carcinogenic compounds (WHO, 1998b). It can be used in gaseous or aqueous forms. The effectiveness of ClO_2 gas treatment is determined mainly by gas concentration, followed by the duration of treatment, relative humidity and temperature (Gómez-López, Rajkovic, Ragaert, Smigic, & Devlieghere).

Currently, the use of ClO_2 is not allowed to decontaminate fresh produce except in the U.S., where a maximum of 3ppm of ClO_2 is permitted for disinfection of whole fruits and vegetables (US FDA, 2001). Due to the development of technology that makes shipment of ClO_2 possible and its on-site generation safer, more and more research about the use of ClO_2 in fresh produce disinfection is being done.

Information regarding ClO_2 disinfection on foodborne pathogens and its influence on food quality has been reviewed (Gómez-López et al.). In general, ClO_2 effectively inactivates *E. coli* O157:H7 present on fresh fruits and vegetables, but complete elimination has not been observed. Application of gaseous ClO_2 has been more successful than that of aqueous ClO_2 due to the high penetrability of gaseous ClO_2. Table 3 outlines the results from studies about the effect of ClO_2 treatment on *E. coli* O157:H7 inoculated into lettuce, as well as the conditions under which the experiments were carried out.

Table 3. Efficacy of ClO_2 Treatment on *E.coli* O157:H7 Inoculated on Lettuce

Treatment	Concentration (mg/l)	Time (min)	Relative Humidity (%)	Log Reduction (log CFU)	Reference
Aqueous ClO_2	10	5	-	1.2	Singh, Singh, Bhunia, & Stroshine, 2002a
	20	15	-	1.7	Singh, Singh, Bhunia, & Stroshine, 2002b
Gaseous ClO_2	1	15	80	2.31	Singh, Singh, Bhunia, & Stroshine, 2002b
	4.1	20.5	36-84	1.57	Sy, Murray, Harrison, & Beuchat, 2005
	5	10	90-95	3.9	Mahmoud & Linton, 2008
	8.7	180	-	6.9	Lee, Costello, & Kang, 2004

According to these studies, gaseous ClO_2 is much more effective than aqueous ClO_2 at killing *E. coli* O157:H7 on lettuce and has the potential to be used as a sanitizer in industry. However, affecting the visual quality of lettuce is a concern when ClO_2 gas treatment is used. Research has generated conflicting results: although Lee *et al*. (Lee et al., 2004) reported no deteriorating visual quality in lettuce after treatment with ClO_2 gas at a concentration of 8.7 mg/l for 3h and 18-day storage at 4°C, immediate and adverse discolorations were observed by Mahmoud *et al*. (Mahmoud & Linton, 2008) and Sy *et al*. (Sy et al., 2005) after treatment with 0.5 mg/l and 1.4 mg/l ClO_2 for 10 min, respectively. More research into the use of ClO_2 is required.

2.4.3 Irradiation

Irradiation is a decontamination process that exposes the food to an appropriate level of ionizing radiation in the form of gamma rays, X-rays or electron beams to kill bacteria, viruses or insects that might be present. Use of irradiation in the industry has been legalized in the U.S. and Canada for fresh pork, poultry, spice, dry ingredients, potatoes and onions, etc. (CFIA, 2005; US FDA, 2008b). In August, 2008, as part of an overall antimicrobial strategy the U.S. FDA approved the irradiation of iceberg lettuce and spinach at a maximum absorbed dose of 4.0 kGy for disinfection (US FDA, 2008a).

Application of irradiation to lettuce was once considered unsuitable because of phytotoxic damage that might be encountered with high-dose irradiation (Thayer & Rajkowski, 1999). However, given that most surface disinfection treatments fail to assure lettuce safety, some attention has been paid to the value of irradiation as a disinfection method. Niemira (Niemira, 2007) compared the antimicrobial efficacy of chlorine washes and irradiation. Results showed that whereas treatments with 300ppm and 600ppm chlorine were only able to decrease the number of *E. coli* O157:H7 by less than 1 log, a 4-log reduction was achieved via irradiation at a

dose of 1.5 kGy (Niemira, 2007). Similarly, in another study, irradiation was found to kill as many as 5 log of *E. coli* O157:H7 in iceberg lettuce (Niemira, 2008). The relationships between the reduction of *E. coli* O157:H7 populations and irradiation dose have been found to be linear across the range of doses being tested in all but red leaf lettuce in which a tailing effect was observed (Niemira, Sommers, & Fan, 2002; Niemira, 2008).

Compared to other disinfection methods, irradiation is a promising method of lettuce disinfection for many reasons other than its high efficacy: irradiated lettuce is free of chemical residues; irradiation treatment can be conducted either before or after packaging; and irradiation only causes minimal environmental pollution. As well, it has been reported that irradiation doses of up to 0.5kGy had no meaningful impact on the texture of Boston, iceberg, green leaf and red leaf lettuce (Hagenmaier & Baker, 1997; Niemira *et al*., 2002), although the threshold doses at which noticeable softening occurs in lettuce have yet to be determined. However, market acceptance of the application of this technology on lettuce is still questionable. Furthermore, the high costs of irradiation-associated facilities and equipment make it unaffordable for small firms.

2.5 Dose Response

Dose-response assessment, or hazard characterization, describes the relationship between the level of microbial exposure and the likelihood of infection, making it possible to estimate the risk of human infection following exposure to pathogens via either foodborne or environmental pathways (Buchanan, Smith, & Long, 2000). Because of ethical considerations, these data are usually unavailable for highly infectious pathogens, such as E. coli O157:H7 (Buchanan et al., 2000). Based on the results from the empirical modeling of dose-response relationships, Strachan *et al.* (Strachan et al., 2005) fitted a Beta-Poisson model to the data obtained from eight foodborne and environmental *E. coli* O157:H7 outbreaks.

2.6 Study Rationale

Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs) and Hazard Analysis Critical Control Point (HACCP) − have been developed to advise growers and the industry about effective on-farm and in-plant sanitation programs (US FDA/CFSAN, 2006). Due to the complex production environment at the farm level, as well as the diversity and complexity that exist in the production chain and industry, prevention of contamination alone is clearly impossible to guarantee lettuce free of pathogens in most cases. Moreover, research has uncovered the ability of *E. coli* O157:H7 to survive on lettuce for extended periods of time. If *E.coli* O157:H7 is able to persist on lettuce for an extended period of time, it is possible that the infectious dose that remains may have grown. Thus, the implementation of effective intervention strategies to minimize or even eliminate the *E. coli* O157:H7 risk is an industry priority, especially when the produce industry is now increasingly dependent on importing lettuce from worldwide sources to fill domestic demand.

Research has investigated the efficacy of various disinfection treatments along with the behavior of *E. coli* O157:H7 on lettuce. However, quantitative studies regarding the combined effect of interventions at various postharvest steps in terms of health risk mitigation have not been done, which presents a major challenge to decision makers in industry and public health officers. Thus, the purpose of this project is to develop a modeling framework for evaluating the effectiveness of risk mitigation strategies in eliminating *E. coli* O157:H7 from lettuce, to provide information necessary for HACCP development and evaluation. Below are the research questions which will be addressed and discussed in the discussion section:

(a) What is the likelihood of *E. coli* O157:H7 infection as a result of consuming contaminated lettuce at various levels of contamination?

(b) Whether increased health risk is attributable to *E. coli* O157:H7 proliferation as a result of temperature abuse? If so, what portion of the risk can be reduced if temperature abuse

were successfully prevented?

(c) To what extent does the current disinfection method (treatment with chlorine, treatment with chlorine dioxide or irradiation) minimize the risks of consuming lettuce contaminated with *E. coli* O157:H7? What additional risk reduction can be expected from using alternative disinfection strategies? What is the major uncertainty in the successful application of these technologies?

(d) What are the effects of the stochastic model versus the threshold model for dose-response in predicting risk, given a specific estimate of the exposure level?

Chapter 3: Methodology

3.1 Model Depiction

Fitted to the exposure model was a hypothetical food system. Iceberg lettuce was assumed to be contaminated with *E. coli* O157:H7 during harvest by an unknown source. Before arriving at the restaurant, the lettuce traveled down the food system through various operation units, namely, cooling and cold storage, processing and distribution to the restaurant. The food pathway ended when the lettuce was disinfected with chlorine after delivery to the restaurant (Figure 2).

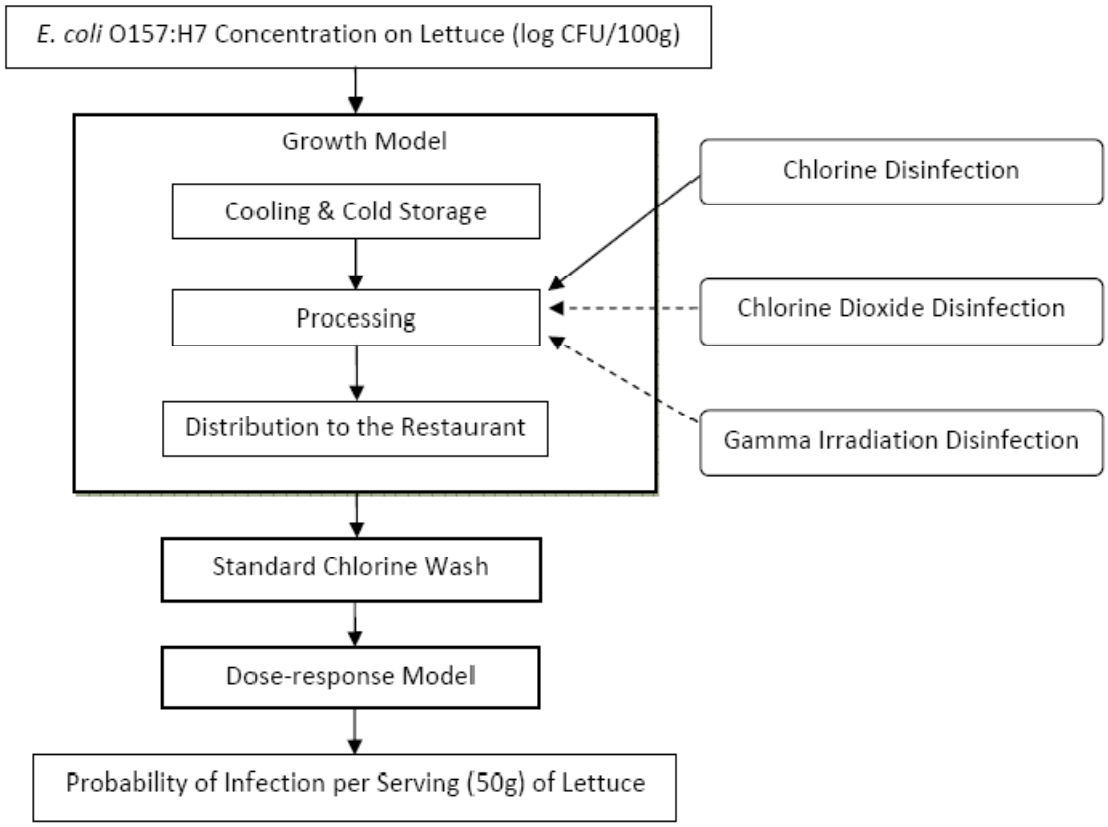


Figure 2. Flow Diagram of the Model for Quantitative Risk Assessment of *E. coli* O157:H7 on Lettuce.

The primary model input was the population of *E. coli* O157:H7 present on 100 grams of lettuce and the final output of the exposure model was defined as the probability distribution of the ingested dose in colony-forming units (CFU) in 50 grams (one serving) of lettuce, with which the risk of infection was predicted by the dose-response model.

The exposure model was constructed by linking the inputs and outputs of the consecutive modules in the cold chain, which referred to the intermediate stages in the supply chain from cooling and cold storage to distribution to the restaurant (Figure 2). In the cold chain, change of *E. coli* O157:H7 population size in 100 grams of lettuce was modeled under different temperature conditions which included low-risk temperature scenarios and high-risk temperature scenarios. Within each given temperature scenario, the effects of disinfection methods, i.e., chlorine wash (Cl), gaseous chlorine dioxide (ClO_2), and gamma irradiation (irradiation), were explored. Considering that chlorine was widely accessible and easy to use, chlorine wash was the baseline disinfection method and assumed to have been implemented after arriving at the restaurant. During processing, only one of the three disinfection methods mentioned above was assumed to be used. Therefore, four intervention strategies were constructed: baseline (1×Cl), chlorine wash (2×Cl), ClO_2 disinfection (ClO_2+Cl), as well as irradiation (irradiation+Cl). These designs allowed for the examination of risk reduction as a result of using alternative disinfection. The reason why disinfection was assumed to take place at the processing plant was because it was more feasible and cost-effective to apply disinfection at a centralized place.

Note that cross-contamination may be another factor that could contribute to increased risk. Modeling cross-contamination may be done in future studies but was not within the scope of the study. Since the effect of temperature abuse in the restaurant has been modeled elsewhere (Franz, Tromp, Rijgersberg, & van der Fels-Klerx, 2010), by assuming that temperature was well controlled at the restaurant, the current study focused on assessing the effect of temperature trajectories in the cold chain on the risk of infection.

Assumptions made for this model included the following:

a) *E. coli* O157:H7 were homogeneously distributed in lettuce;

b) Heads of lettuce were packaged individually;

c) No cross-contamination occurred;

d) Temperature was well controlled below 5°C in the restaurant;

e) One serving (50 grams) of lettuce was consumed at a time.

Distributions were identified to represent model variables where possible. Description of each variable and its associated uncertainty were summarized in Appendix A.

3.2 Initial Contamination Level

Initial contamination level referred to the concentration of *E. coli* O157:H7 present in the lettuce after harvest. A number of microbial surveys have been conducted at the farm, the distribution, or the retailing levels to quantify the prevalence of *E. coli* O157:H7 in lettuce or leafy produce in North America and Europe (Abadias, Usall, Anguera, Solsona, & Viñas, 2008; Arthur et al., 2007; Bohaychuk et al., 2009; Mukherjee et al., 2004; Mukherjee, Speh, Jones, Buesing, & Diez-Gonzalez, 2006; Sagoo, Little, Ward, Gillespie, & Mitchell, 2003). To date, no *E. coli* O157:H7 was found in any of these studies. A probability distribution for the contamination level cannot be established until better concentration data are available. As "proof of concept", three relatively low level of inoculum sizes (1, 2 or 3 logCFU/g) were used as the model input. Although the lack of good data limited the ability of this model to make geographically-related predictions at the population level, the model design set out to establish a framework that could be further developed at a future point in time.

3.3 Microbial Growth Model

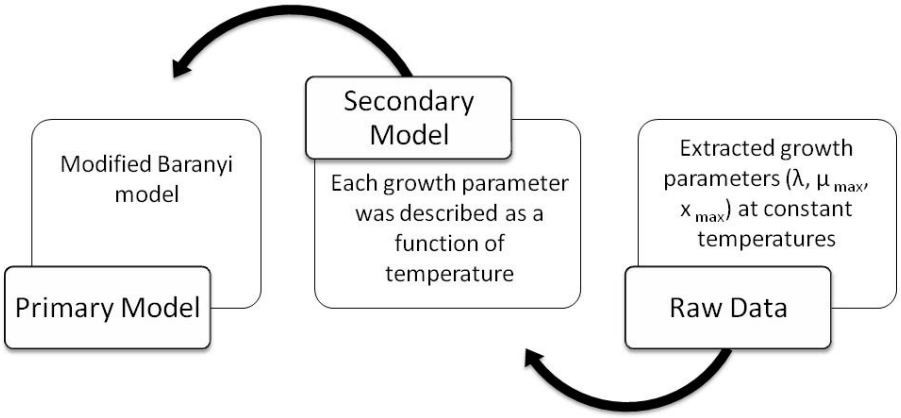


Figure 3. Development Process of the Growth Model for *E. coli* O157:H7 on Lettuce.

To construct the microbial growth model, raw log counts of *E. coli* O157:H7 were used to fit a sigmoidal growth curve at each constant temperature (Figure 3). Then, the parameters of these growth curves were further described as a function of temperature, which were known as secondary models. Finally, these secondary models were substituted into the modified Baranyi model to estimate *E. coli* O157:H7 growth under dynamic temperature.

3.3.1 Data Source

Predictive microbiology has been widely used to predict microbial growth in specific food environments, based on influencing intrinsic and extrinsic factors incorporated into the mathematical model (J. Baranyi & Roberts, 2004; Isabelle & André, 2006). The log counts of *E.coli* O157:H7 in lettuce were observed by Koseki *et al.* (2005) at a series of constant temperatures (5°C, 10°C, 15°C, 10°C and 25°C). The number of data point at each temperature varied from 10 to 44. These data are freely available in the Combase Database[1]. Using these data, Koseki *et al*. parameterized a growth model, the Baranyi model (J. Baranyi, Robinson, Kaloti, & Mackey, 1995), for *E. coli* O157:H7 on shredded lettuce. However, this model performed poorly

[1] Combase is an online database developed through international collaboration. It consists of useful microbial growth and survival data for the development of microbial models and is freely available to public.

when comparing the predicted counts with the viable counts of *E. coli* O157:H7 on lettuce under dynamic temperature conditions (Koseki & Isobe, 2005). In the study, the same data set was used to build a slightly different growth model.

3.3.2 Primary Growth Model Fitting

The primary growth model used in the current study was the same as the one used by by Koseki *et al.* (2005). It was the coupled sets of differential functions (Equation 1) developed by Baranyi and Roberts (J. Baranyi, Robinson, Kaloti, & Mackey, 1995).

Equation 1

$$\frac{dq}{dt} = \mu_{\max} q, q(0) = q_0$$
$$\frac{dx}{dt} = \mu_{\max} \frac{q}{q+1}(1 - \frac{x}{x_{\max}})x, x(0) = x_0$$

where μ_{max} was maximum specific growth rate in terms of lnCFU/h; x_{max} was the maximum population density in lnCFU/g; λ as the lag-phase duration in hours, referring to the length of time until maximum exponential growth occurs; q was a dimensionless quantity representing the initial physiological state of the cells.

Using the DMFit web edition[2], growth curves were fitted for a series of constants temperatures (Appendix B). The growth parameters (μ_{max}, x_{max} and λ) were extracted for all temperatures except 5 ˚C (Appendix C). In a constant environment, α, and q_0 were different ways to denote the physiological state of the cells (Equation 2, 3).

Equation 2

$$\alpha_0 = e^{-\lambda\mu}$$

[2] DMFit is an online model fitting tool for bacterial growth curves. Using inputted time and bacterial log counts, it provides graphic representation of a bacterial growth data set and generates growth parameters, such as maximum growth rate and lag.

Equation 3

$$q_0 = \frac{\alpha_0}{1-\alpha_0}$$

Fitting of the growth data and analysis of the results were done in lnCFU/g units but numbers reported in the current study are in $\log_{10}$CFU/g for easier interpretation.

3.3.3 Secondary Growth Model Fitting

Secondary model described how the model parameters (μ_{max}, x_{max} and q_0) vary with environmental conditions. The original Baranyi model assumed that the q_0 stayed relatively constant regardless of the temperature, when the pre-inoculation history of the cells was identical (J. Baranyi et al., 1995). However, based on empirical data, q_0 at 10 °C was about 100-fold different from that at 25 °C. Contradictory evidence to this assumption was also found in other studies, which suggested a temperature-dependency in q_0 (Mellefont, McMeekin, & Ross, 2003; Swinnen, Bernaerts, & Van Impe, 2006). Instead of adapting the original Baranyi model where q_0 was treated as a constant, in the study, q_0 was characterized by a quadratic function of initial temperature within the temperature range of 10°C to 25°C (Table 4). Therefore, the growth model used in the current study was coined as modified Baranyi model. The relationship between x_{max} and temperature was described by a linear equation, while μ_{max} was described using the simple square root model of Ratkowsky *et al.* (1982).

Table 4. The Relationship between Growth Model Parameter and Temperature for *E. coli* O57:H7.

Parameter	Secondary Model	Adjusted R^2
$X_{max}(T)$	X_{max} = 0.056T + 5.335	0.914
$\sqrt{\mu(T)}$	$\sqrt{\mu}$ = 0.032T - 0.147	0.998
$q_0(T)$	q_0 = -0.0039T^2 + 0.0993T + 0.0157	0.623

The three secondary functions were then substituted into the Baranyi model, allowing all the parameters to be temperature dependent. This system was solved numerically by the fourth order Runge-Kutta method (Chitode, 2010) which was a standard numerical approximation technique used to solve ordinary differential equation (Equation 4). The cell concentration was calculated iteratively as the cell concentration at the previous time interval plus the weighted-average derivative of the four estimates of the derivative. Each time interval was decided as 20 minutes to avoid oscillation (Equation 5).

Equation 4

$$\frac{dy}{dx} = f(x, y)\,,\ y(0) = y_0$$

Equation 5

$$y_{i+1} = y_i + \frac{1}{6}(k_1 + 2k_2 + 2k_3 + k_4)h$$
$$k_1 = f(x_i, y_i)$$
$$k_2 = f(x_i + \frac{1}{2}h, y_i + \frac{1}{2}k_1 h)$$
$$k_3 = f(x_i + \frac{1}{2}h, y_i + \frac{1}{2}k_2 h)$$
$$k_4 = f(x_i + h, y_i + k_3 h)$$

The process was programmed in Visual Basic from Microsoft Excel, by stating the initial contamination level, the length of the cold chain and step size (VBA codes can be found in Appendix D).

3.3.4 Growth Model Evaluation

In order to obtain data dynamic time- temperature data and *E. coli* O157:H7 cell counts for the use of model evaluation, two figures representing different scenarios from published study (Koseki & Isobe, 2005) were digitized, which allowed the graphical points to be accurately

converted back into numerical data. Starting temperatures in these two scenarios differ; 18°C in the first scenario (cool scenario) and 25°C in the second scenario (warm scenario).

The mean absolute relative error (MARE) is a standard goodness-of-fit statistics used for the evaluation of non-parametric curve. It is an indicator of the absolute deviation of the model estimates to the observed values. Therefore, a least positive value is desired.

Equation 6

$$MARE = \frac{1}{N}\sum\left|\frac{(O-P)}{O}\right| \times 100$$

In each of the evaluation scenarios, MARE was calculated for both the original Baranyi model and the modified Baranyi model (Table 5). The graphs with the predicted growth curves superimposed with the observed cell counts can be found in the Appendix E.

Table 5. Comparison of Model Performance in Two Dynamic Temperature Scenarios.

Temperature Profile	Growth Model	MARE (%)
Cool Scenario	Original Baranyi	2.83
	Modified Baranyi	4.14
Warm Scenario	Original Baranyi	36.33
	Modified Baranyi	1.40

Although the modified Baranyi model slightly overestimated growth in the cool scenario (MARE=4.14%), its predicted growth curve only deviated from the observation by 1.40% in the warm scenario, as opposed to 36.33% for the original Baranyi model.

3.3.5 Assumptions

For the maximum growth rate (μ_{max}) and the maximum population density (x_{max}), parameter uncertainty was represented by a normal distribution associated with the regression beta coefficient in the least-squares fitted secondary functions, under the assumption that the errors in the regression were normally distributed.

The uncertainty associated with the physiological state (q_0) of *E. coli* O157:H7 was captured with a uniform probabilistic distribution, the best estimate of which was estimated using the fitted function of initial temperature within the range of 10°C to 25°C, and the maximum and minimum values were arbitrarily defined as the q_0 times two and the q_0 divided by two, respectively. Univariate sensitivity analysis of the q_0 with respect to the model outcome was carried out by holding other variables constant at their point values derived from the secondary growth models.

3.4 Time-Temperature Profiles

3.4.1 Data Source

Time-varying temperature data were generously provided by Dr. Rediers (Rediers, Claes, Peeters, & Willems, 2009) from the Institute for Microbial Control of the Food Chain in Belgium, to analyze the effect of temperature on pathogen proliferation. Temperature was measured by data loggers attached to the leafy produce at one-minute intervals, following its route from harvest to the restaurant. In this study, four scenarios were chosen, including two low-risk scenarios and two high-risk scenarios with temperature abuse.

3.4.2 Curve Fitting

The purpose of curve fitting of the two dimensional time and temperature data was to functionalize data for confidentiality. Additionally, using function to describe the data was better than sampling point values directly from the data as it helped to smooth noise (i.e., measurement error) and avoid numerical artifacts resulting from over fitting the data.

The PROC REG procedure in the SAS package (SAS 9.1) was used to fit a cubic piecewise regression spline to each of the four data sets. The general principle of the curve fitting process was to characterize the change of temperature as much as possible in a parsimonious

form. For each curve, about 10 knots (the abscissa value of the joint points) were placed at the joint points where necessary. To start with, the section between each knot was fit by a third-degree polynomial including a linear term, a quadratic term and a cubic term. Then, any parameter that was not statistically significant in the regression function was removed from the model under the condition that removing this parameter did not compromise the visual fit of the curve. The SAS codes used for the curve fitting were included (Appendix F), as well as the estimated parameters and fitted curves (Appendix G). After the curve function was established, the instantaneous temperature was substituted by the function of time into the modified Baranyi model.

3.5 Disinfection Models

3.5.1 Chlorine Disinfection Model

The efficacy of chlorine in removing pathogens from the produce has been reviewed by Fonseca (Fonseca, 2006). Although the effectiveness of chlorine treatment varies by method of application, the treatment time and the concentration of effective ingredient, it was concluded that chlorine disinfection can only reduce pathogens populations by 1-2 log CFU/g (Fonseca, 2006). An attempt was made to derive a probabilistic model for the chlorine disinfection, but lack of disinfection model for industrial practices and the fact that relevant data were scattered by studies done under different conditions made it impossible.

For the sake of accounting for the disinfection effect of chlorine, it was assumed that chlorine wash can only removed *E. coli* O157:H7 from lettuce by 1 log CFU/g. This may be a conservative estimate in some case. No assumption was made with respect to the concentration of chlorine and the treatment time.

3.5.2 ClO_2 Disinfection Model

The length of the treatment time with gaseous ClO_2 at concentrations of 0.5-5.0 mg/L was found to be linearly related to the log reductions of *E. coli* O157:H7 population with high correlation coefficients (Mahmoud & Linton, 2008). Higher ClO_2 concentration and longer treatment times were associated with increased ClO_2 efficacy (Table 6). Yet, the benefit of using high concentration gas or extending treatment time was compromised by the browning problem of the leaves (Mahmoud & Linton, 2008). For the study, 1 mg/L and 4 minutes were chosen as the concentration and treatment time. In the literature review, it was found that internalization of pathogen may affect the efficacy of disinfection. To date, no study has quantified the effect internalization has on the efficacy of disinfection and data is unavailable to model the possibility of internalization.

Table 6. Inactivation Kinetics of *E. coli* O157:H7 on Lettuce by Gaseous ClO_2.

ClO_2 concentration (mg/l)	D_{10} *(min)
0.5	7.2±0.4
1.0	3.4±0.2
1.5	3.3±0.2
3.0	3.2±0.1
5.0	2.9±0.1

- D_{10} : Time required to achieve a 90% reduction in the number of microorganisms (Mahmoud & Linton, 2008)

The first-order kinetic model (Equation 7) was used to describe the inactivation kinetics of ClO_2 for *E. coli* O157:H7:

Equation 7

$$\log(N) = \log(N_0) - \frac{t}{D_{10}}$$

where t was the length of treatment time and D_{10} was the decimal reduction time, which refers to the time required to achieve a 90% reduction in the number of microorganisms. A normal distribution was constructed for D_{10} to represent the parameter uncertainty (Appendix A).

3.5.3 Irradiation Model

The data found in the peer-reviewed literature about irradiating lettuce suggested that the first-order kinetic model was the most appropriate model to describe this process. The function form of the first order kinetics was the same as the one used for ClO_2 (Equation 7) except that the D_{10} here was the dosimetry necessary to reduce the number of pathogen by 90%. A range of D_{10} values were reported for different types of lettuce and the locations where *E. coli* O157:H7 attached to lettuce (Table 7). To be consistent with the type of lettuce data the ClO_2 disinfection model was derived from, the study used iceberg lettuce data with 0.5kGy treatment dose.

Table 7. D_{10} Values of *E. coli* O157:H7 on/in Various Types of Lettuce.

Types of lettuce	D_{10} (kGy) (*E. coli* O157:H7 on lettuce)	D_{10} (kGy) (*E. coli* O157:H7 in lettuce)	R^2
Green leaf	0.119[A]	0.37[B]	0.95[B]
Red leaf	0.123[A]	0.35[B]	0.79[B]
Boston	0.140[A]	0.45[B]	0.88[B]
Iceberg	0.136[A]	0.30[B]	0.95[B]
Romaine	N/A	0.39[C]	N/A

A: Niemira *et al.*, 2002; B: Niemira, 2008; C: Niemira, 2007

Accounting for the uncertainty associated with the D_{10} depending of the proportion of pathogens internalized into the lettuce tissue, D_{10} was calculated as the weighted average of the

D_{10}value for the situation when *E. coli* O157:H7 attached on the surface of lettuce (D_{10}= 0.136) and the D_{10} value for the situation when the pathogens were inside the lettuce (D_{10}=0.30). The weight ranged from 0 to 1 was generated by randomly sampling from a continuous uniform distribution.

3.6 Dose Response Model

The predicted exposure dose served as the input for the dose-response model to predict the risk of infection associated with the consumption of contaminated lettuce. The dose response model used in the study was the beta-Poisson model developed by Strachan (2005). This model (Equation 8) took into account the variations that exist in pathogen-host interactions and were derived using collated foodborne and environmental outbreak data from global sources.

Equation 8

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

where P was the probability that an exposed individual will become infected, D represented the dose, and α and β were parameters that describe the distribution of host susceptibility. It was assumed that each organism acted independently; one cell was capable of causing a finite probability of disease. The outcome of the dose response was the probability of disease resulting from various ingested doses. The best estimates of model parameter α and β are shown in Table 8.

Table 8. Best Estimates of Dose-Response Model Parameters.

Parameter	Best Estimate
α	0.0571
β	2.2183

3.7 Uncertainty Analysis and Monte Carlo Simulation

Uncertainty analysis refers to identification and quantification of the variance introduced into the risk model's output variables as model predictions due to propagation of uncertainty. The variance in the model outputs represents a hybrid distribution which contains some combination of uncertainty and variability (Morgan & Henrion, 1990).

The Monte Carlo approach has been widely used in stochastic models to simulate the outcome distribution by sampling from the probability distribution defined for the input and intermediate parameters (Vose, 2008). After the model forms for exposure, the distributional information for each variable and relevant constant for the models were defined, the stochastic model was constructed in an Excel spreadsheet and simulated using Crystal Ball (version 11.1.1, Oracle) as an add-on program. Descriptions of all variables involved in the assessment can be found in (Appendix A). Since there was no theoretical reason to treat any variables as correlated, no correlation was specified. First-order uncertainty analyses were performed. Each scenario was simulated using Monte Carlo sampling method for 10,000 iterations. Each of the iterations produced one estimate of the probability of *E. coli* O157 infection resulted from consuming one serving (50g) of contaminated lettuce.

Chapter 4: Results

4.1 Time-Temperature Profiles

In this study, four time-temperature profiles were chosen to analyze the consumer's risk of *E. coli* O157:H7 infection contributed by possible pathogen proliferation occurred in the post-harvest stage of the lettuce supply chain (the cold chain), as well as to investigate the effect of selected disinfection methods for the use of risk reduction in certain circumstances. Each of these time-temperature profiles described temperature situations which are realistic. Scenario 1 was a typical low-risk scenario where temperature was generally well controlled at all stages. Compared to Scenario 1, the three other scenarios differed in their initial temperature, duration and overall temperature trajectory (Table 9).

Table 9. Major Differences in the Four Temperature Profiles.

Scenario*	Initial Temperature	Duration	Major Difference in Temperature Trajectory
Scenario 1	20°C	75h	Adequate temperature control
Scenario 2	25°C	98h	Prolonged exposure to warm temperature before cooling
Scenario 3	25°C	52h	Slow initial cooling process with some temperature fluctuations
Scenario 4	20°C	77h	Abusive temperature while distributing lettuce to restaurants

* Time-temperature data were generously provided by Dr. Rediers from the Institute for Microbial Control of the Food Chain in Belgium (Rediers et al., 2009).

4.2 Disinfection Scenarios

In all hypothetical scenarios, the lettuce was sanitized by chlorine (Cl), ClO_2 or irradiation at the processing plant before transportation. When distributed to the restaurant, chlorine was used again as the last safeguard to minimize contamination. The overview for each scenario with the constructed temperature curve and points of disinfection are displayed in

Figure 4.A-D.

In Scenario 1, the lettuce was cooled to below 5°C, 2 hours after harvest (Figure 4-A). As the lettuce was distributed down the cold chain, temperature was maintained at around 3°C with minimal fluctuation during processing. Then it was exposed to a warm environment for a short period of time before being distributed to the restaurant.

Scenario 2 described a situation commonly encountered in summer time (Figure 4-B), when the temperature was relatively high (25°C) to start with and the lettuce was exposed to approximately 18°C for 20 hours prior to placement in on-farm refrigerators. During transpiration, temperature was maintained at around 3°C.

Scenario 3 replicated Scenario 1 in terms of the overall temperature trajectory (Figure 4-C). However, it had an elongated on-farm cooling process which took 20 hours before the temperature dropped to below 5°C.

Scenario 4 was a situation when temperature control was very rigorous half of the time, but in the other half of the time, temperature control failure occurred due to accidental circumstances. The lettuce was unrefrigerated and kept at 20°C for about 24 hours (Figure 4-D).

In the remainder of this section, growth curves predicted under these four temperature scenarios are presented on a case-by-case basis. The antimicrobial effects of different hypothetical disinfection treatments applied in the processing plant in terms of infection risk reduction, the overall change of pathogen concentration presented with uncertainty bounds, and the estimated risk associated with one serving of lettuce are also shown.

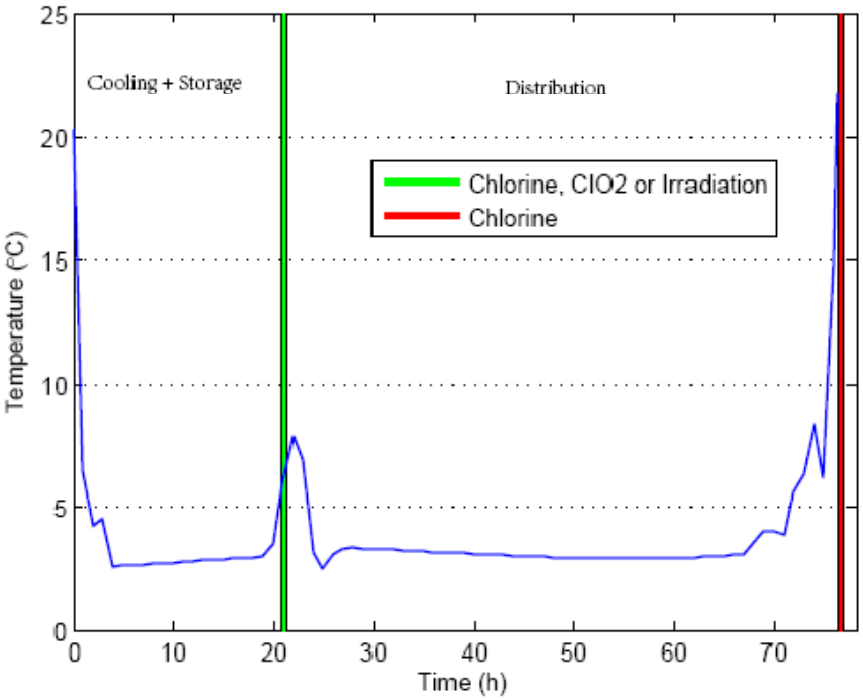


Figure 4-A. Temperature Profile of Lettuce throughout the Postharvest Supply Chain, Scenario 1.

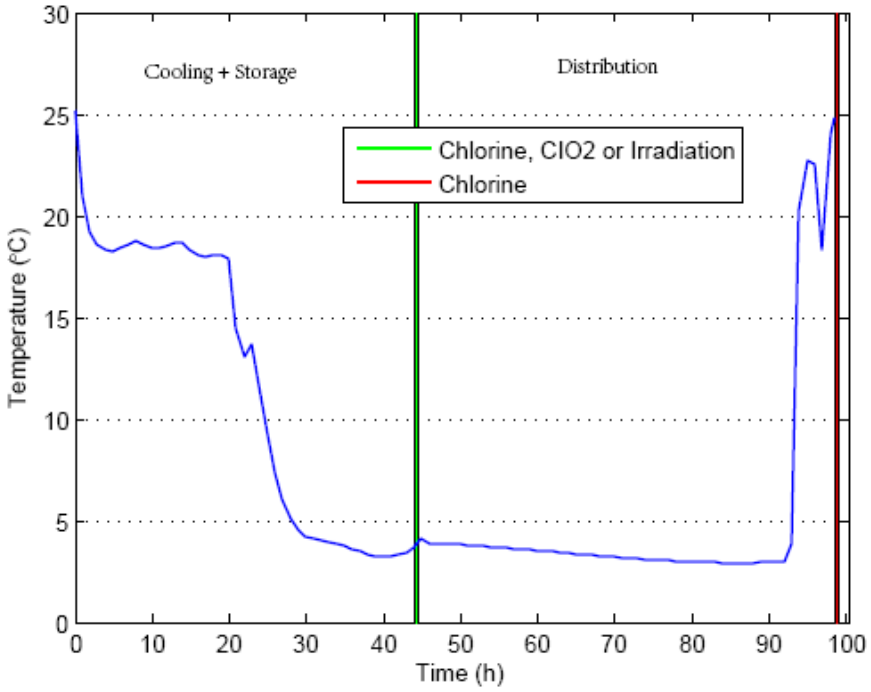


Figure 4-B. Temperature Profile of Lettuce throughout the Postharvest Supply Chain, Scenario 2.

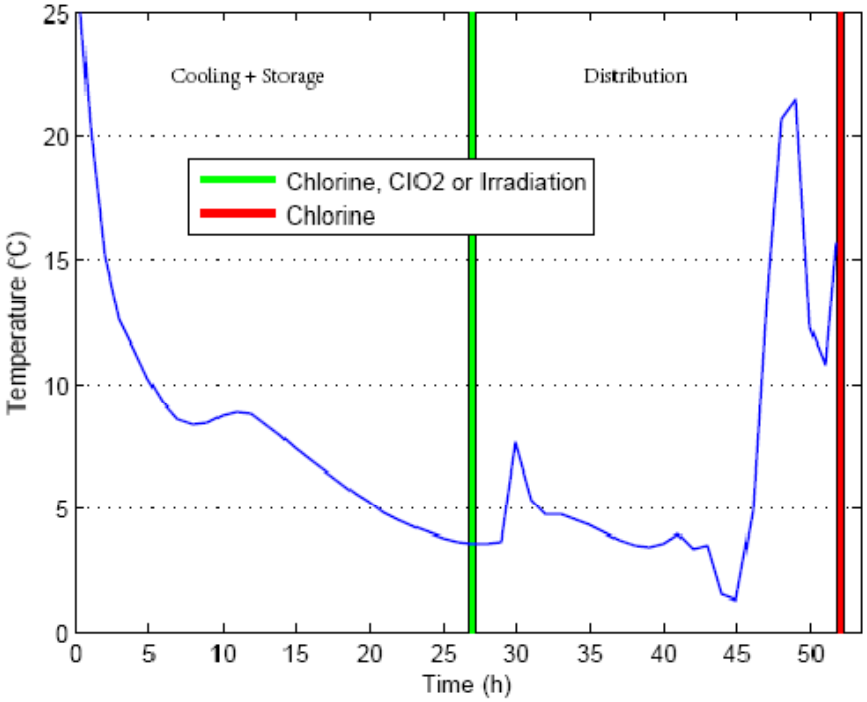


Figure 4-C. Temperature Profile of Lettuce throughout the Postharvest Supply Chain, Scenario 3.

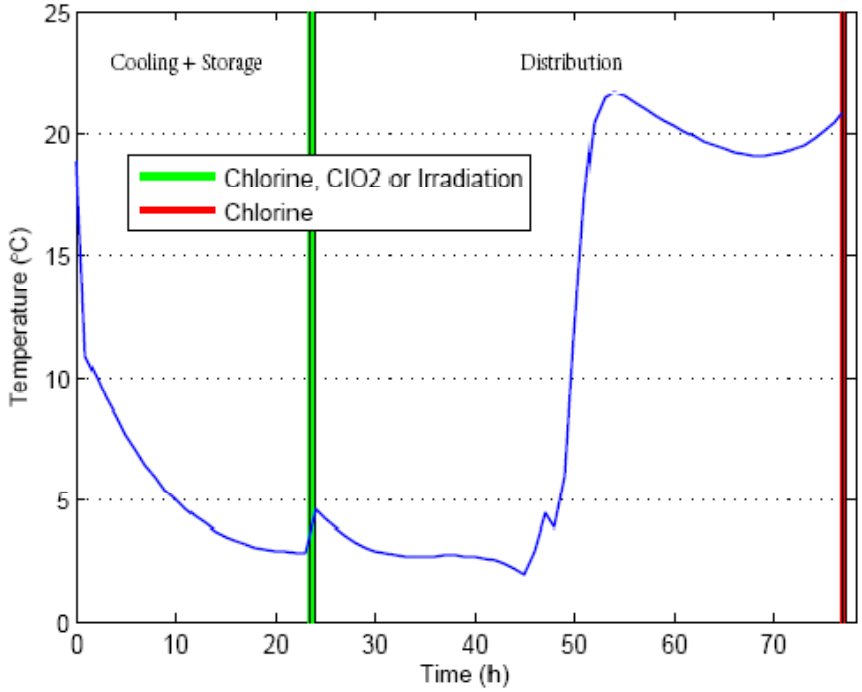


Figure 4-D. Temperature Profile of Lettuce throughout the Postharvest Supply Chain, Scenario 4.

4.3 Scenario 1

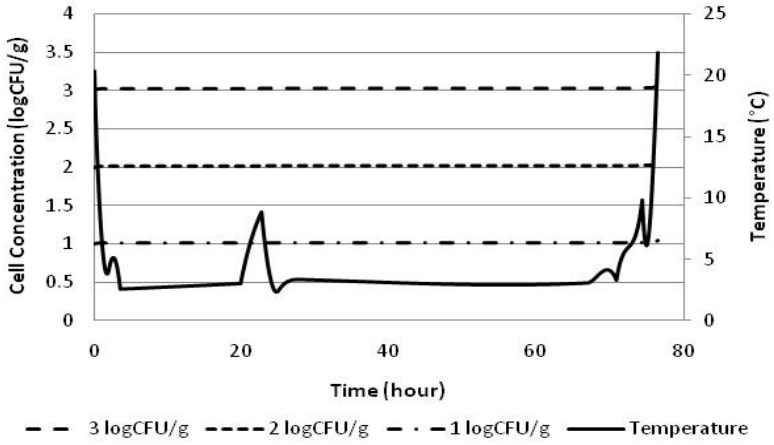


Figure 5. Predicted Growth Trajectory of *E. coli* O157:H7 in Lettuce, Scenario 1.

With stringent temperature control, the growth model in Scenario 1 predicted very minor increase in the *E. coli* O157:H7 population at all initial contamination levels (Figure 5). Relative growth was the ratio of net increase to the inoculum. There was little difference in the relative growth associated with different inoculum sizes (Table 10).

Table 10. Point Estimate of Final Microbial Concentrations, Scenario 1.

Inoculum (log CFU/g)	Inoculum (CFU/g)	Final Level (log CFU/g)	Final Level (CFU/g)	Net Growth (CFU/g)	Relative Growth (%)
1	10	1.05	11.11	1.11	11.12
2	100	2.07	117.76	17.76	17.76
3	1000	3.08	1205.04	205.04	20.50

Furthermore, evaluation of disinfection (Cl, ClO_2 and irradiation) efficacy was accomplished by converting the predicted post-processing *E. coli* O157:H7 concentration to the probability of infection using the dose response model. Thus, the disinfection strategies were compared in terms of the residual infection risks associated with one serving of lettuce (Table 10). Depending on the inoculum, irradiation could minimize the infection risk by as

much as 100 fold. The risk associated with chlorine or ClO_2 disinfection was within the same magnitude of the baseline risk.

Table 11. Post-ProcessingInfection Risk Associated with One 50g-serving of Lettuce, Scenario 1.

Inoculum (logCFU/g)	Baseline	Cl	ClO_2	Irradiation
1	0.269	0.168	0.149	0.003*
2	0.358	0.268	0.251	0.023*
3	0.437	0.358	0.343	0.096*

*indicated significant difference compared to the Baseline.

The Monte Carlo sampling method was used to propagate the variance associated with model parameters during simulation. Trend charts were produced for the medium contamination level (2 logCFU/g) using Crystal Ball to display how the predicted *E. coli* O157:H7 concentration and its variance changed over time in a series of confidence bands (Figure 6). Each confidence band was centered around the median of the estimate and represented the credibility intervals (i.e., certainty ranges) into which the actual values of the estimates fell. For instance, the blue band which represented the 90% credibility interval showed the range of values into which a microbial concentration had a 90% chance of falling.

These four trend charts represented four proposed disinfection scenarios. Model output as infection risk was extracted at six time points in the cold chain. Chlorine disinfection after the lettuce was distributed to the restaurant was the baseline scenario (the 6th point on Figure 6). At the processing plant (the 3rd point on Figure 6), there were four options of disinfection: baseline chlorine wash after delivery (1×Cl), chlorine wash (2×Cl), chlorine dioxide (ClO_2+Cl) and irradiation (irradiation+Cl).

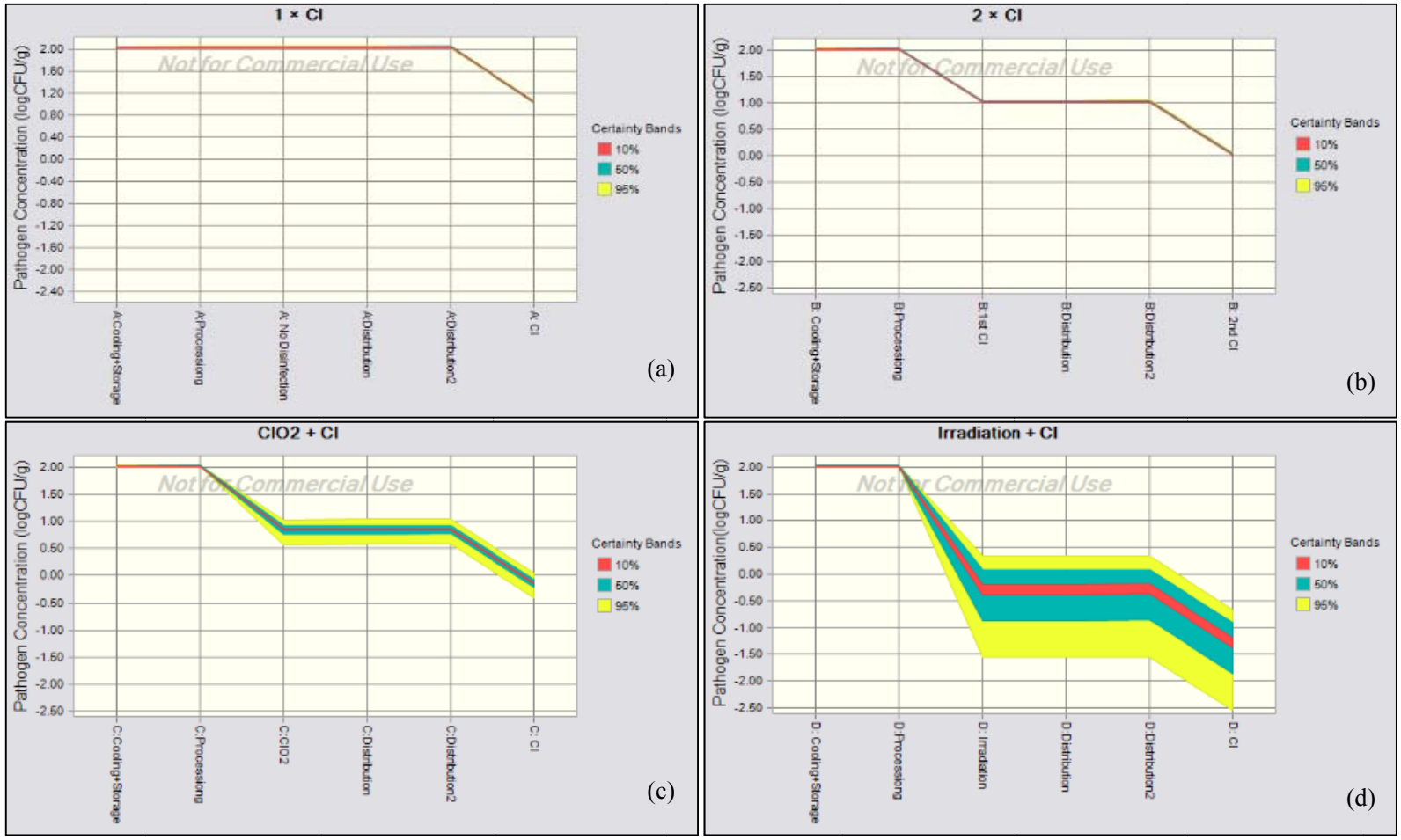


Figure 6. Change of Predicted *E. coli* O157:H7 Concentration and Associated Variance with Time, Scenario 1.

- The initial contamination level was 2 log CFU/g.
- Each band was centered around the median of the estimate and represented the credibility intervals (10%, 50% and 90%) the credibility intervals into which the actual values of the estimates fall. The upper and lower boundaries of the yellow band were the 95th and 5th percentiles of the estimate.
- 1×Cl Wash: baseline scenario, no disinfection during processing, chlorine applied after distribution to the restaurant; 2×Cl Wash: chlorine applied during processing; Gaseous ClO_2+Cl Wash: gaseous ClO_2 (1 mg/L, 4 minutes) applied during processing; Irradiation + Cl: gamma irradiation (0.5kGy) implemented during processing.

In general, the credibility intervals associated with the predicted *E. coli* O157:H7 concentrations were relatively tight, except in "irradiation + Cl". This was due to minimal predicted growth activity in the "irradiation + Cl" scenario. The width of the uncertainty spanning was a function of the number of variables. The tight uncertainty spanning associated with chlorine disinfection was a result of using point estimate for the disinfection function, rather than and uncertainty distribution (Figure 6. a, b), while the wide uncertainty spanning associated with irradiation resulted from the parameter uncertainty of D_{10} with respect to the various locations in lettuce where *E. coli* O157:H7 could attach to.

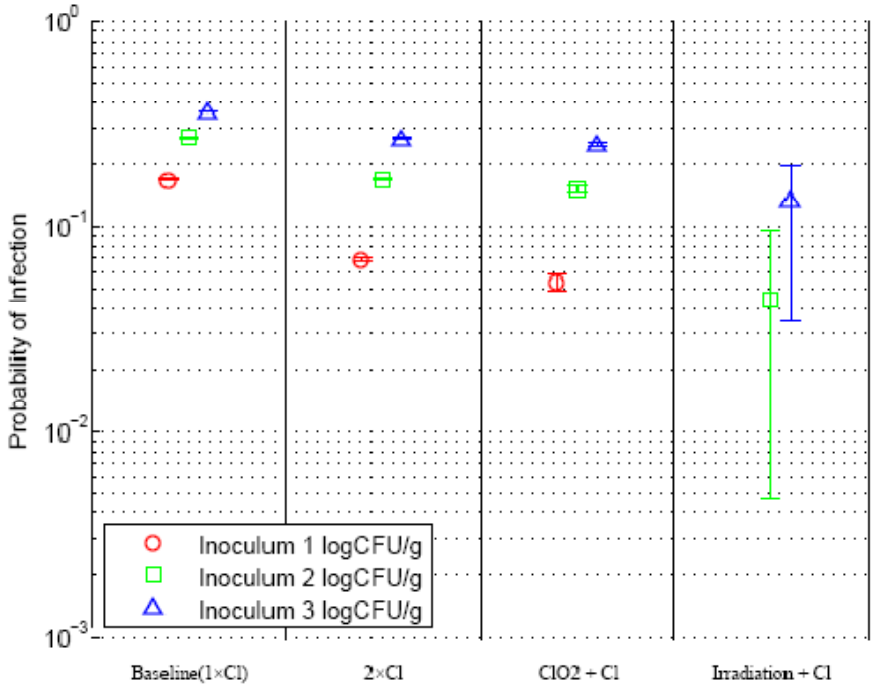


Figure 7. Predicted Median, 5th and 95th Percentiles of *E. coli* O157:H7 Infection Risk, Scenario 1.

Figure 7 demonstrated the comparative risks for all disinfection strategies, with the numerical results presented in Table 12. The 5th percentile, the median, and the 95th percentile of the estimated risk associated with one serving of lettuce were shown to reflect uncertainty. Compared with the baseline scenario, all strategies using ClO_2 and chlorine had only a limited antimicrobial effect. Applying irradiation can lower the risk by 10-100 fold, with the lowest risk

associated with the inoculum size of 2 log CFU/g (44 infections per 1000 consumptions [95%: 94 infection per 1,000 consumption; 5%: 5 infections per 1,000 consumption]). Note that in the "irradiation+Cl", the probability of infection associated with 1 log CFU/g inoculum was missing, because the residual pathogen load after irradiation at the processing plant exceeded the lower computational limit of the modified Baranyi growth model, which meant that the growth model was not able to predict post-processing pathogen loads.

Table 12. Probability of Infection Associated with the Consumption of One 50g-Serving of Lettuce, Scenario 1.

Inoculum		Baseline(1 × Cl)	2 × Cl	ClO_2 + Cl	Irradiation + Cl
1 logCFU/g	5th Percentile	0.167	0.067	0.049	N/A*
	Median	0.169	0.069	0.054	N/A*
	95th Percentile	0.171	0.070	0.059	N/A*
2 logCFU/g	5th Percentile	0.268	0.167	0.144	0.005
	Median	0.270	0.169	0.150	0.044
	95th Percentile	0.271	0.170	0.157	0.094
3 logCFU/g	5th Percentile	0.357	0.268	0.246	0.034
	Median	0.358	0.269	0.252	0.135
	95th Percentile	0.359	0.270	0.258	0.197

* *E. coli* O157:H7 load exceeded the lower computational limit of the growth model.

4.4 Scenario 2

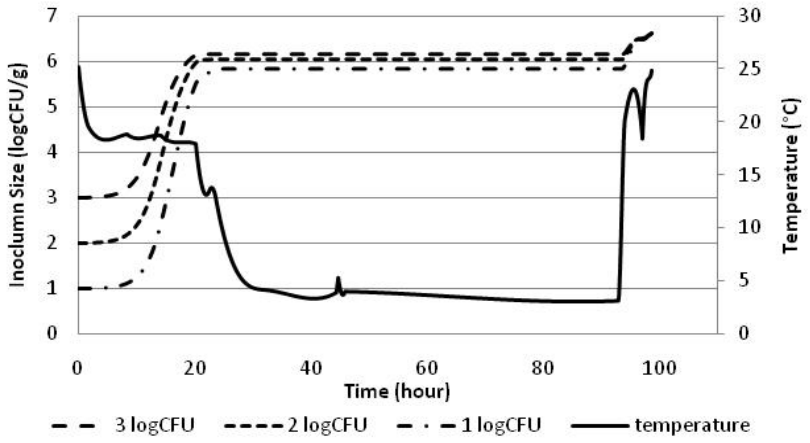


Figure 8. Predicted Growth Trajectory of *E. coli* O157:H7 in Lettuce, Scenario 2.

In Scenario 2, due to prolonged exposure to warm temperature during the cooling stage, the model predicted a significant increase in *E. coli* O157:H7 concentration (Figure 8). By the time the lettuce was cooled down, the *E. coli* O157:H7 concentration had reached the maximum population density, regardless of the initial contamination level. Accordingly, the relative growth was most significantly associated with the low inoculum, followed by the medium and the high inoculums (Table 13).

Table 13. Point Estimate of Final Microbial Concentrations, Scenario 2.

Inoculum (log CFU/g)	Inoculum (CFU/g)	Final Level (log CFU/g)	Final Level (CFU/g)	Net Growth (CFU/g)	Relative Growth (%)
1	10	6.63	4.22E+06	4.22E+06	4.22E+07
2	100	6.62	4.18E+06	4.18E+06	4.18E+06
3	1000	6.61	4.09E+06	4.09E+06	4.09E+05

The predicted residual infection risks associated with one serving of lettuce were equally high among all disinfection methods (Table 14). The health benefit of applying irradiation was diminished when lettuce was contaminated with a high concentration of *E. coli* O157:H7.

Table 14. Post-Processing Infection Risk Associated with One 50g-serving of Lettuce, Scenario 2.

Inoculum (logCFU/g)	Baseline	Cl	ClO_2	Irradiation
1	0.516	0.448	0.435	0.216
2	0.521	0.453	0.441	0.224
3	0.524	0.457	0.445	0.229

The uncertainty bounds in Figure 8 reflected the uncertainty with respect to both the behaviors of *E. coli* O157:H7 in lettuce and the efficacy of disinfectants.

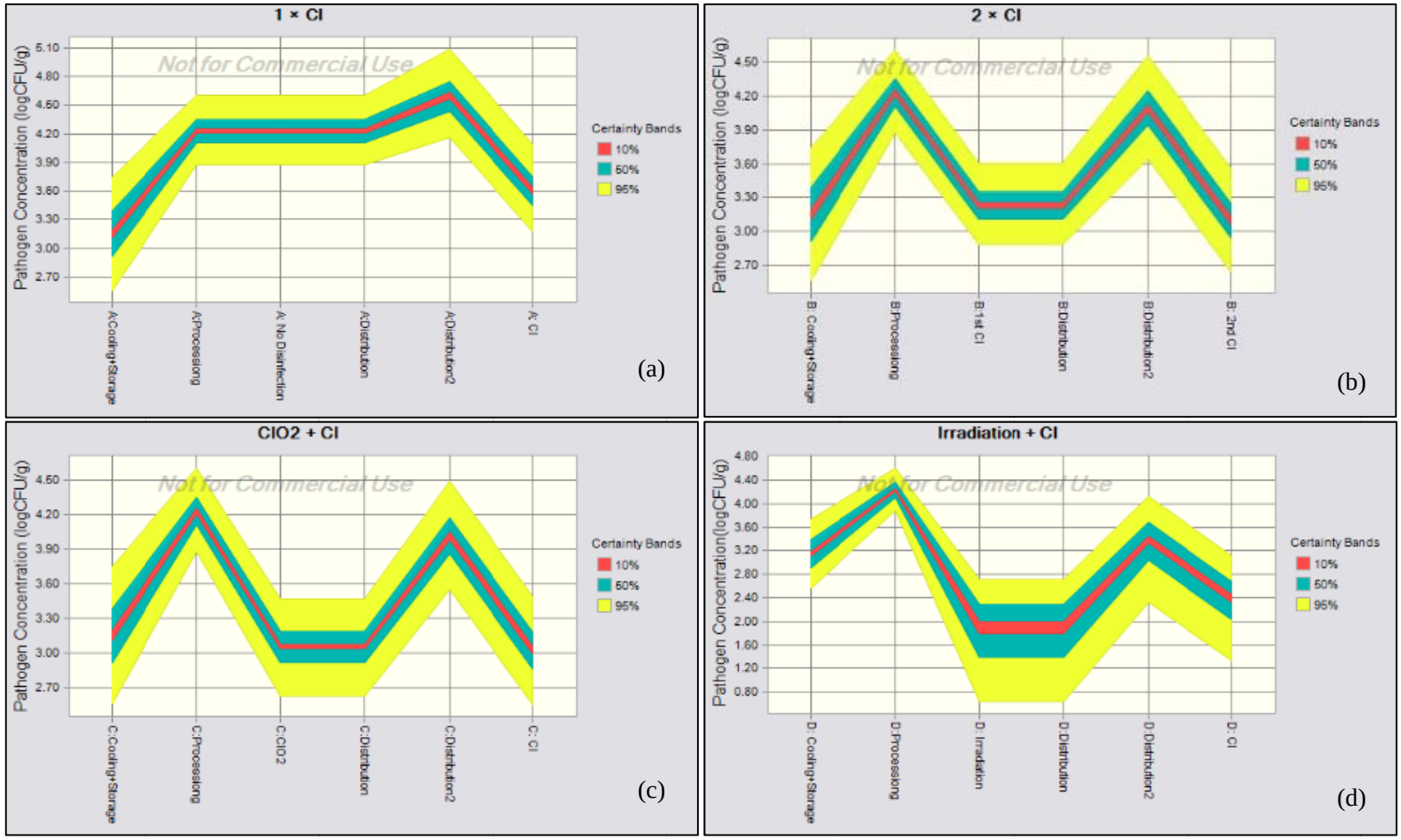


Figure 9. Change of Predicted *E. coli* O157:H7 Concentration and Associated Variance with Time, Scenario 2.

- The initial contamination level was 2 log CFU/g.
- Each band was centered around the median of the estimate and represented the credibility intervals (10%, 50% and 90%) the credibility intervals into which the actual values of the estimates fall. The upper and lower boundaries of the yellow band were the 95th and 5th percentiles of the estimate.
- 1×Cl Wash: baseline scenario, no disinfection during processing, chlorine applied after distribution to the restaurant; 2×Cl Wash: chlorine applied during processing; Gaseous ClO_2+Cl Wash: gaseous ClO_2 (1 mg/L, 4 minutes) applied during processing; Irradiation + Cl: gamma irradiation (0.5kGy) implemented during processing.

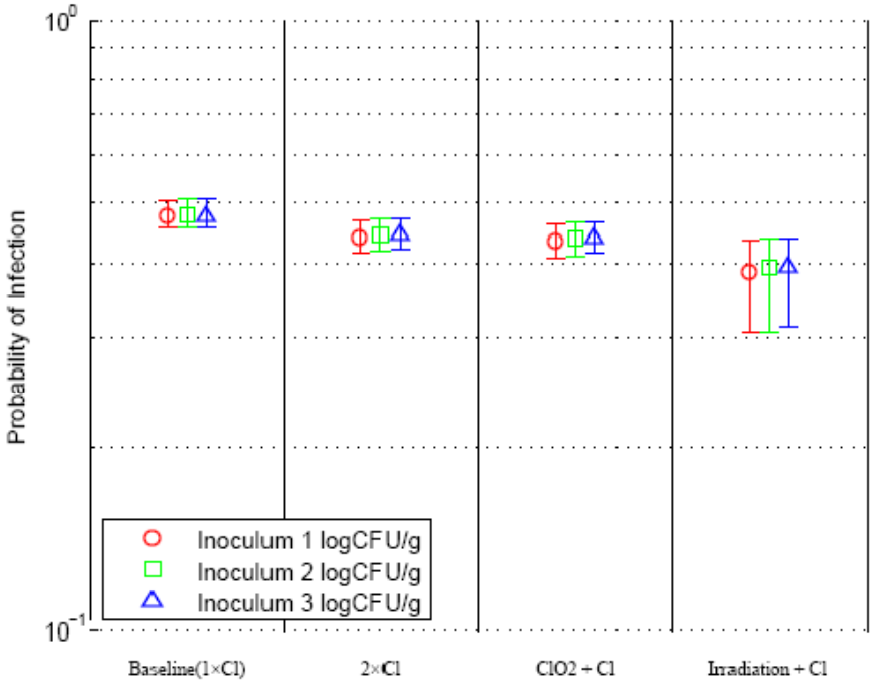


Figure 10. Predicted Median, 5th and 95th Percentiles of *E. coli* O157:H7 Infection Risk, Scenario 2.

In all disinfection strategies, the predicted risk of infection resulted from consuming one serving of contaminated lettuce was alarmingly high (Figure 10). The median predicted risk ranged from 40% to 48%. Compared to the baseline strategy, using one extra step of chlorine disinfection (2×Cl) or ClO_2 did not reduce the infection risk. Even with irradiation, one serving of contaminated lettuce was predicted to have a 39% chance of causing infection (95%: 31 infections per 100 consumptions; 5%: 44 infections per 100 consumptions) (Table 15). In this scenario, the risk of infection was independent of the initial contamination level.

Table 15. Probability of Infection Associated with the Consumption of One 50g-Serving of Lettuce, Scenario 2.

Inoculum		Baseline(1 × Cl)	2 ×Cl	ClO_2 + Cl	Irradiation + Cl
1 logCFU/g	5th Percentile	0.455	0.415	0.407	0.307
	Median	0.479	0.442	0.436	0.389
	95th Percentile	0.506	0.469	0.464	0.435
2 logCFU/g	5th Percentile	0.455	0.418	0.411	0.307
	Median	0.479	0.444	0.438	0.393
	95th Percentile	0.507	0.471	0.466	0.438
3 logCFU/g	5th Percentile	0.455	0.421	0.414	0.313
	Median	0.479	0.446	0.440	0.395
	95th Percentile	0.507	0.472	0.467	0.439

4.5 Scenario 3

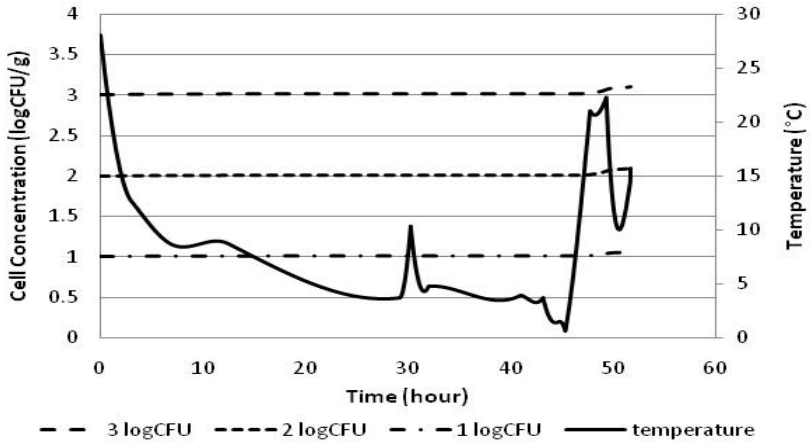


Figure 11. Predicted Growth Trajectory of *E. coli* O157:H7 in Lettuce, Scenario 3.

The predicted growth trajectories in Scenario 1 and 3 shared a few common characteristics. During the extended initial cooling process in Scenario 3, no growth was predicted, which was due to the relatively high initial temperature (25˚C), at which *E. coli* O157:H7 needed a longer time to adjust to the new environment (Figure 11).

Table 16. Point Estimate of Final Microbial Concentrations, Scenario 3.

Inoculum (log CFU/g)	Inoculum (CFU/g)	Final Level (log CFU/g)	Final Level (CFU/g)	Net Growth (CFU/g)	Relative Growth (%)
1	10	1.06	11.47	1.47	14.71
2	100	2.10	124.63	24.63	24.63
3	1000	3.11	1284.82	284.82	28.48

Table 17. Post-Processing Infection Risk Associated with One 50g-serving of Lettuce, Scenario 3.

Inoculum (logCFU/g)	Baseline	Cl	ClO_2	Irradiation
1	0.268	0.166	0.148	0.003
2	0.358	0.267	0.250	0.023
3	0.436	0.357	0.342	0.096

All trend charts displayed the similar pattern: the credibility intervals widened as predictions were made farther down the supply chain (Figure 12). Such phenomenon resulted from the propagation of uncertainty over time associated with one of the key variables, the physiological state (q). Forecast of this variable was dynamic throughout the simulation, because in the growth function, the instantaneous q was depended on the q from the previous interval and the maximum growth rate (μ_{max}).

The predicted health risks in all disinfection strategies as well as their associated uncertainty were similar to those produced for Scenario 1 (Figure 13). The lowest predicted risk was (79 infections per 1,000 consumptions [95%: 143 infection per 1,000 consumption; 5%: 6 infections per 1,000 consumption]) (Table 18).

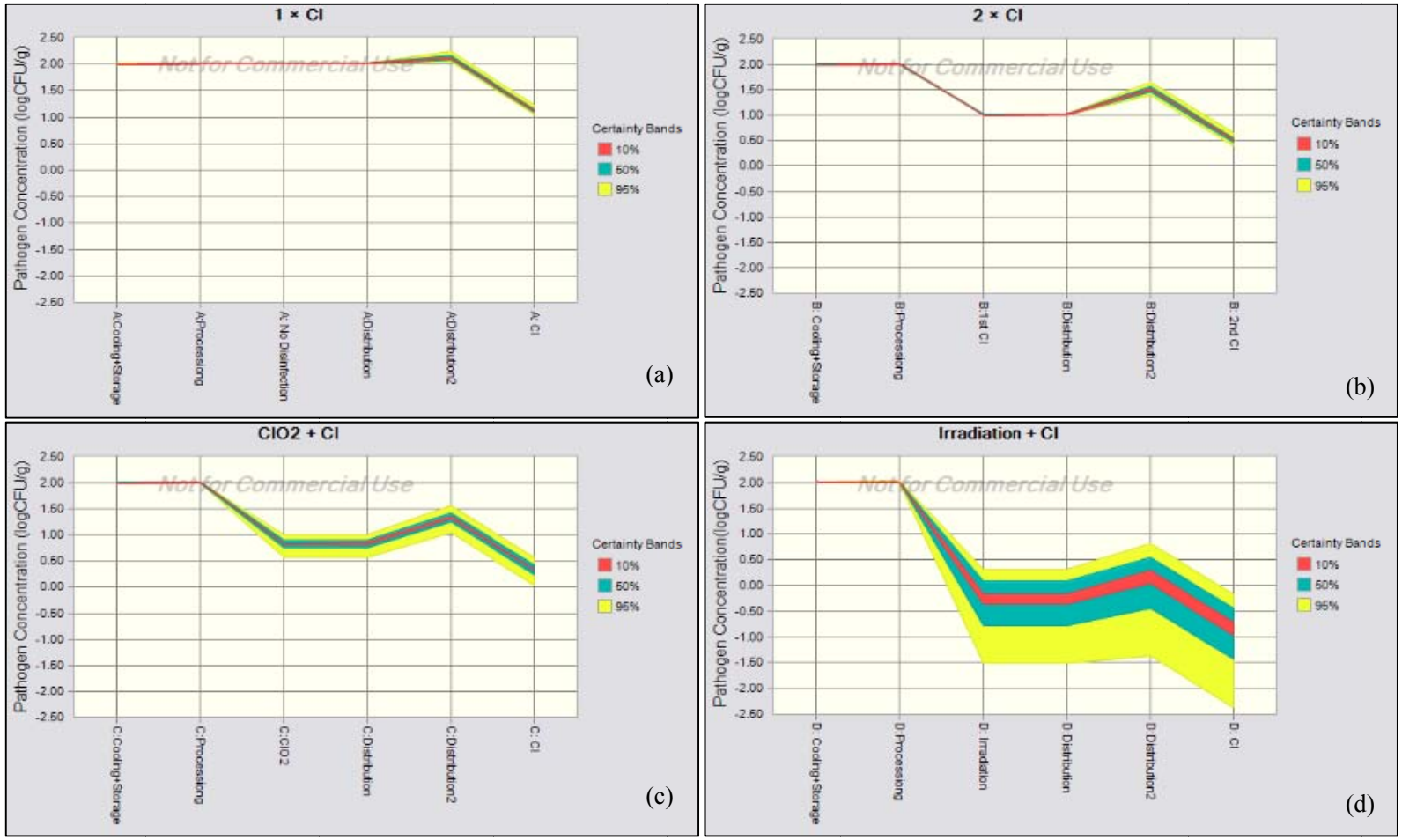


Figure 12. Change of Predicted *E. coli* O157:H7 Concentration and Associated Variance with Time, Scenario 3.

- The initial contamination level was 2 log CFU/g.
- Each band was centered around the median of the estimate and represented the credibility intervals (10%, 50% and 90%) the credibility intervals into which the actual values of the estimates fall. The upper and lower boundaries of the yellow band were the 95th and 5th percentiles of the estimate.
- 1×Cl Wash: baseline scenario, no disinfection during processing, chlorine applied after distribution to the restaurant; 2×Cl Wash: chlorine applied during processing; Gaseous ClO_2+Cl Wash: gaseous ClO_2 (1 mg/L, 4 minutes) applied during processing; Irradiation + Cl: gamma irradiation (0.5kGy) implemented during processing.

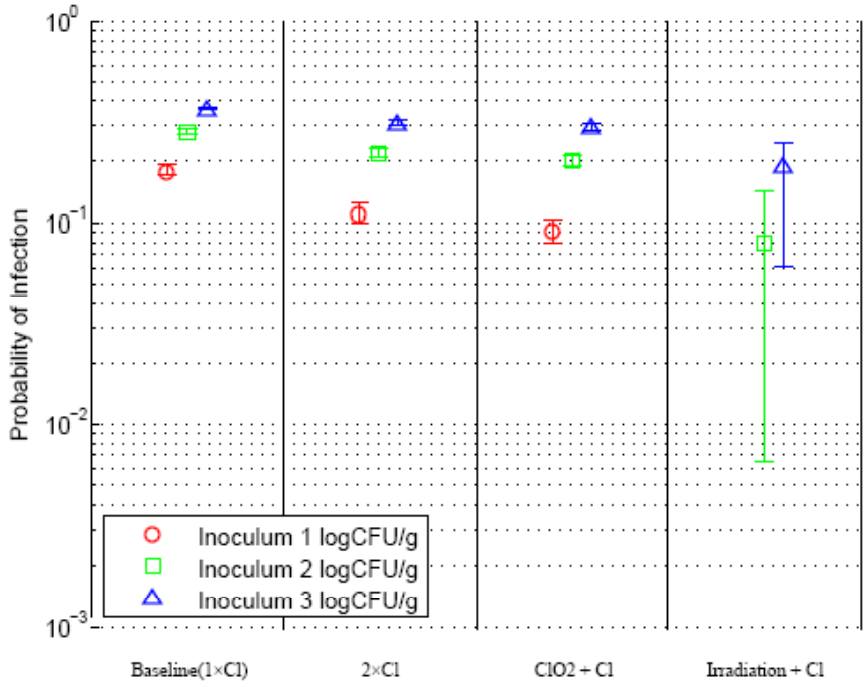


Figure 13. Predicted Median, 5th and 95th Percentiles of *E. coli* O157:H7 Infection Risk, Scenario 3.

Table 18. Probability of Infection Associated with the Consumption of One 50g-Serving of Lettuce, Scenario 3.

Inoculum		Baseline(1 × Cl)	2 ×Cl	ClO_2 + Cl	Irradiation + Cl
1 logCFU/g	5th Percentile	0.171	0.100	0.080	N/A*
	Median	0.178	0.110	0.090	N/A*
	95th Percentile	0.191	0.123	0.104	N/A*
2 logCFU/g	5th Percentile	0.271	0.207	0.188	0.006
	Median	0.277	0.218	0.200	0.079
	95th Percentile	0.287	0.231	0.214	0.143
3 logCFU/g	5th Percentile	0.359	0.300	0.284	0.061
	Median	0.363	0.308	0.294	0.188
	95th Percentile	0.370	0.319	0.305	0.247

* *E. coli* O157:H7 load exceeded the lower computational limit of the growth model.

4.6 Scenario 4

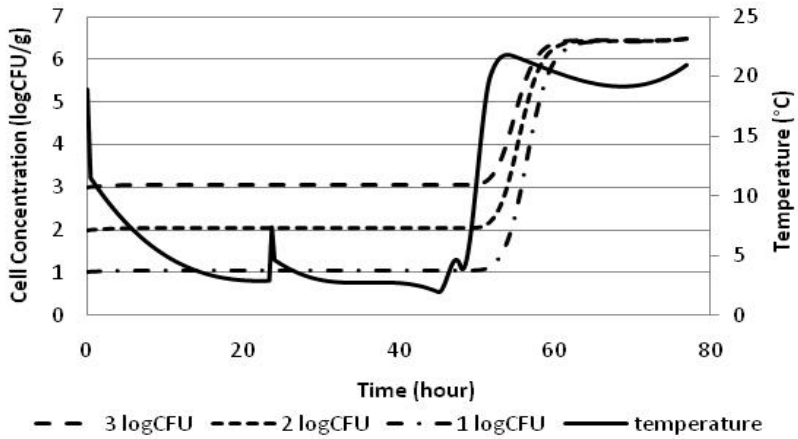


Figure 14. Predicted Growth Trajectory of *E. coli* O157:H7 in Lettuce, Scenario 4.

In Scenario 4, no growth was predicted in most parts of the cold chain where temperature was adequately controlled; however, substantial cell growth resulted due to the failure of temperature control which occurred at a later stage (Figure 14). Irradiation at the processing plant successfully reduced the residual infection risk by as much as 100 fold, when the inoculum size was 1 log CFU/g (Table 20). No significant risk reduction was predicted by chlorine or ClO_2. The magnitude of risk reduction achieved by irradiating lettuce was smaller as the initial contamination level became higher.

Table 19. Point Estimate of Final Microbial Concentrations, Scenario 4.

Inoculum (log FU/g)	Inoculum (CFU/g)	Final Level (log CFU/g)	Final Level (CFU/g)	Net Growth (CFU/g)	Relative Growth (%)
1	10	6.47	2.92E+06	2.92E+06	2.92E+07
2	100	6.47	2.93E+06	2.93E+06	2.93E+06
3	1000	6.47	2.94E+06	2.93E+06	2.93E+05

Table 20. Post-Processing Infection Risk Associated with One 50g-serving of Lettuce, Scenario 4.

Inoculum (logCFU/g)	Baseline	Cl	ClO_2	Irradiation
1	0.273	0.172	0.153	0.003
2	0.361	0.272	0.255	0.024
3	0.438	0.359	0.344	0.098

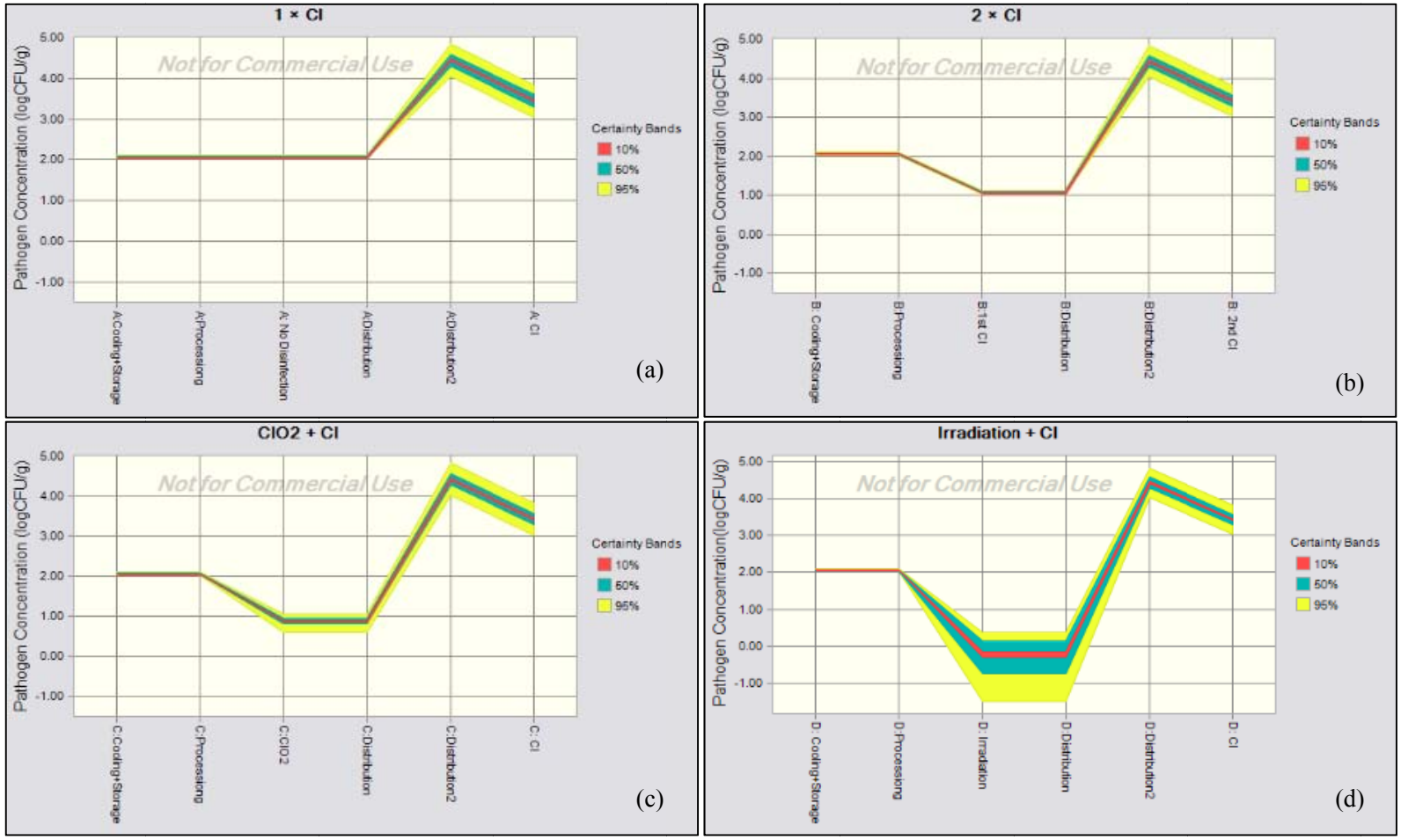


Figure 15. Change of Predicted *E. coli* O157:H7 Concentration and Associated Variance with Time, Scenario 4.

- The initial contamination level was 2 log CFU/g.
- Each band was centered around the median of the estimate and represented the credibility intervals (10%, 50% and 90%) the credibility intervals into which the actual values of the estimates fall. The upper and lower boundaries of the yellow band were the 95th and 5th percentiles of the estimate.
- 1×Cl Wash: baseline scenario, no disinfection during processing, chlorine applied after distribution to the restaurant; 2×Cl Wash: chlorine applied during processing; Gaseous ClO_2+Cl Wash: gaseous ClO_2 (1 mg/L, 4 minutes) applied during processing; Irradiation + Cl: gamma irradiation (0.5kGy) implemented during processing.

The infection risks estimated for the disinfection strategies in Scenario 4 were very high (Figure 16), the median estimates were 47 infections per 100 consumptions (95%: 49 infection per 100 consumptions; 5%: 44 infection per 100 consumptions) (Table 21).

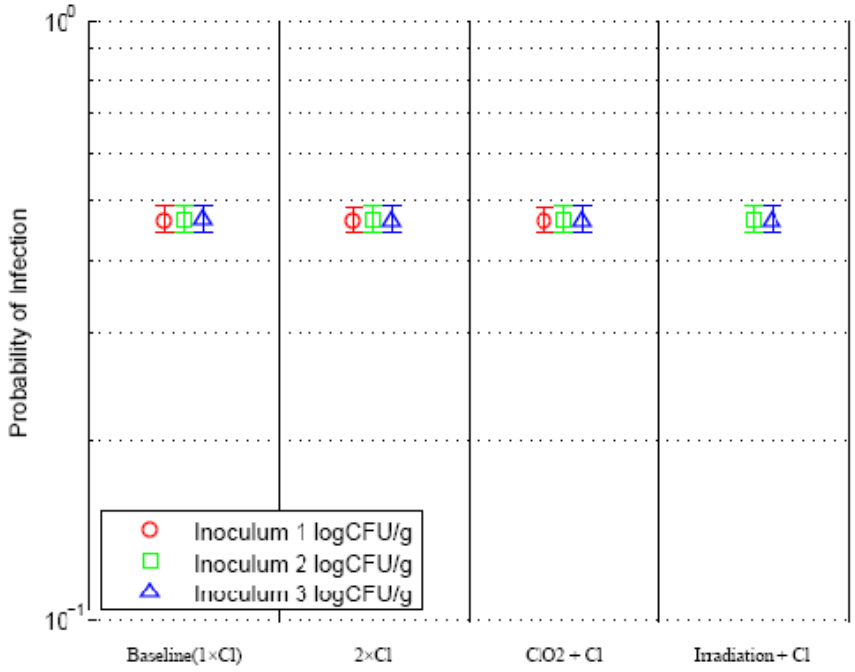


Figure 16. Predicted Median, 5th and 95th Percentiles of *E. coli* O157:H7 Infection Risk, Scenario 4.

Table 21. Probability of Infection Associated with the Consumption of One 50g-Serving of Lettuce, Scenario 4.

Inoculum		Baseline(1 × Cl)	2 ×Cl	ClO_2 + Cl	Irradiation + Cl
1 logCFU/g	5th Percentile	0.443	0.443	0.443	N/A*
	median	0.466	0.466	0.466	N/A*
	95th Percentile	0.490	0.489	0.489	N/A*
2 logCFU/g	5th Percentile	0.444	0.443	0.443	0.443
	median	0.467	0.466	0.466	0.466
	95th Percentile	0.491	0.490	0.490	0.490
3 logCFU/g	5th Percentile	0.445	0.443	0.443	0.443
	median	0.468	0.466	0.466	0.466
	95th Percentile	0.492	0.490	0.490	0.490

* *E. coli* O157:H7 load exceeded the lower computational limit of the growth model.

4.7 Summary of Findings

The results in the current study showed that temperature control was the key measure to minimize the risk of *E. coli* O157:H7 infection associated with consuming lettuce. Disinfecting contaminated lettuce using the hypothetical methods examined in the study had limited effects in reducing the risk of infection. In the case when temperature was low, the magnitude of risk reduction by disinfection was a function of the step of disinfection and the level of initial contamination.

Scenarios 1 and 3 belonged to the same type of scenario where temperature was well controlled. In accordance to low temperature, the model predicted negligible net increase of *E. coli* O157:H7 concentration across all three simulated initial contamination levels. When efficacies of all three disinfection methods (chlorine, ClO_2, and irradiation) were examined in terms of post-processing residual infection risk, no disinfection method except irradiation could reduce the level of risk by more than 10 fold. Yet, the predicted infection risks were generally high in all situations except when irradiation was implemented to disinfect lettuce with relatively low contamination level (1 logCFU/g). Low temperature alone was not sufficient to control *E. coli* O157:H7 at realistic log inoculums.

The time when temperature abuse occurred differed in Scenarios 2 and 4. With substantial pathogen growth, the predicted risks associated with one serving of lettuce were unacceptably high in both scenarios. All hypothetical disinfection methods could not reverse the adverse effect of abusive temperature; no matter if they were applied before or after the occurrence of temperature abuse. The final risk was independent of the initial contamination level, which implicated that failure of temperature control occurred in the cold chain could compromise any effort to minimize the initial contamination level at the farm level.

Table 22. Probability of Infection Associated with the Consumption of One 50g-Serving of Lettuce.

	Inoculums: 1 logCFU/g				Inoculums: 2 logCFU/g				Inoculums: 3 logCFU/g			
	Mean	**5th Percentile**	**Median**	**95th Percentile**	**Mean**	**5th Percentile**	**Median**	**95th Percentile**	**Mean**	**5th Percentile**	**Median**	**95th Percentile**
Scenario 1												
Baseline(1×Cl)	0.169	0.167	0.169	0.171	0.270	0.268	0.270	0.271	0.358	0.357	0.358	0.359
2×Cl	0.069	0.067	0.069	0.070	0.169	0.167	0.169	0.170	0.269	0.268	0.269	0.270
ClO_2+Cl	0.054	0.049	0.054	0.059	0.150	0.144	0.150	0.157	0.252	0.246	0.252	0.258
Irradiation+Cl	N/A*	N/A*	N/A*	N/A*	0.046	0.005	0.044	0.094	0.127	0.034	0.135	0.197
Scenario 2												
Baseline(1×Cl)	0.479	0.455	0.479	0.506	0.480	0.455	0.479	0.507	0.480	0.455	0.479	0.507
2×Cl	0.442	0.415	0.442	0.469	0.444	0.418	0.444	0.471	0.446	0.421	0.446	0.472
ClO_2+Cl	0.436	0.407	0.436	0.464	0.439	0.411	0.438	0.466	0.440	0.414	0.440	0.467
Irradiation+Cl	0.382	0.307	0.389	0.435	0.385	0.307	0.393	0.438	0.388	0.313	0.395	0.439
Scenario 3												
Baseline(1×Cl)	0.179	0.171	0.178	0.191	0.278	0.271	0.277	0.287	0.364	0.359	0.363	0.370
2×Cl	0.110	0.100	0.110	0.123	0.218	0.207	0.218	0.231	0.309	0.300	0.308	0.319
ClO_2+Cl	0.091	0.080	0.090	0.104	0.200	0.188	0.200	0.214	0.294	0.284	0.294	0.305
Irradiation+Cl	N/A*	N/A*	N/A*	N/A*	0.076	0.006	0.079	0.143	0.174	0.061	0.188	0.247
Scenario 4												
Baseline(1×Cl)	0.467	0.443	0.466	0.490	0.467	0.444	0.467	0.491	0.468	0.445	0.468	0.492
2×Cl	0.466	0.443	0.466	0.489	0.466	0.443	0.466	0.490	0.467	0.443	0.466	0.490
ClO_2+Cl	0.466	0.443	0.466	0.489	0.466	0.443	0.466	0.490	0.466	0.443	0.466	0.490
Irradiation+Cl	N/A*	N/A*	N/A*	N/A*	0.466	0.443	0.466	0.490	0.466	0.443	0.466	0.490

* *E. coli* O157:H7 load exceeded the lower computational limit of the growth model (see Discussion).

Chapter 5: Discussion

The current study adapted a probabilistic Quantitative Microbial Risk Assessment (QMRA) framework to describe the change of *E. coli* O157:H7 concentration in lettuce through the selected pathway, to develop a predictive model for risk estimation specific for *E. coli* O157:H7 infection associated with lettuce.

The model is not a direct reflection of reality, but rather an informative approximation of the complex system it is designed to represent. In industry, there is considerable variation in the pathway through which fresh produce is being distributed and the produce handlings that take place at operations units. The overall food chain in the study was constructed to resemble the general supply chain for lettuce adapted from the US Food and Drug Administration (FDA) (US FDA/CFSAN, 2006), which included a number of consecutive modules representing stages from cooling to distribution. The end point of the exposure model was set at the point of time when lettuce was delivered to the restaurant. It was assumed that no temperature violation occurred in the restaurant and that the lettuce was properly handled before consumption, because no data were available for this period. Thus, the predicted exposed dose was linked directly to the dose response model to estimate the risk of infection.

Empirical time-temperature profiles were chosen over a distribution of average temperature which could only broadly represent the temperature variability which existed in the industry as a whole. Realistic temperature profiles have been used by others to model microbial growth on the chilled ready-to-eat food in the school catering (Rosset, Cornu, Noël, Morelli, & Poumeyrol, 2004) and on leafy green vegetables consumed at salad bars (Franz et al., 2010). This study was unique in that it took into account the time dynamics of temperature to assess not only the impact of temperature control but also the efficacy of selected disinfection strategies on public health, using a scenario-specific approach.

A credible QMRA model should possess model input parameters of best-available quality,

reasonable assumptions, conceptual validity and operational soundness (Boone et al., 2009) (Appendix H). Throughout the iterative process of model development, the overall appropriateness of model was evaluated using a list of standardized criteria that addressed three aspects of model validity: model design, model formulation, and computation and computer implementation (Table 23). Each criterion was scored subjectively based on its level of fulfillment in the study. Comments arising from some of these criteria were further discussed.

Table 23. Criteria Used for Testing and Model Validation throughout the Development Process of Probabilistic Model.

Validation Criteria	Rating	Comments
Model Design		
Conceptual Validity	Medium	Efforts were made to increase the accuracy of model for its intended use. See discussion.
Biological realism	Medium	See discussion
Extrapolation	Medium	Growth model was extrapolated to low temperature with prediction consistent with the data from which the model was derived from
Generalizability	Unknown	No geographic or population data were employed
Model Formulation		
Appropriateness of model form	Medium	See discussion
Parsimony	High	the model was kept as simple as necessary
Data quality	Medium	See discussion
Sensitivity to significant changes in input parameters	High	Changes to key input parameters resulted in biologically appropriate changes to model outcomes
Robustness to extreme values	Medium	Model was robust when dealing with log inoculum across a wide range of values
Computation & Computer Implementation		
Computational/numerical correctness	High	No detectable mathematical misspecification
Internal consistency	High	Units of the same kinds of measure were consistent all through the model
Absence of numerical artifacts	Medium	Model was stable without numerical outcome oscillations at low dose
Computer Implementation	Medium	No errors identified in the syntax; still need to record the model in high-level language (e.g., Matlab)

5.1 Selection of Model Form

The iterative process of model assembly followed the guiding principle of parsimony and conceptual validity, with consideration of data availability and the importance of the process in relation to the predictive ability of the overall model (Wen, Kalff, & Peters, 1999). Due to the limited time and resources, the model was designed to focus mainly on the growth activity of *E. coli* O157:H7 in an idealized produce supply system. Neither cross-contamination processes nor any sorts of leaf partitioning or mixing practices were considered.

5.1.1 Microbial Growth Model

The choice of the growth model can profoundly influence the model outcome, as the growth activity of *E. coli* O157:H7 was considered at all post-harvest stages in the study. A number of sigmoidal growth models, such as the modified Gompertz model and logistic model, were excellent at predicting pathogen growth at a constant temperature, but they did not have the capability of handling growth at fluctuating temperature in a consistent way (Van Impe, Nicolai, Martens, De Baerdemaeker, & Vandewalle, 1992). The Baranyi model, which consisted of a set of differential functions, had been used by many researchers to predict microbial growth under changing temperature (J. Baranyi et al., 1995; Bovill et al., 2000; Fujikawa, Kai, & Morozumi, 2004; Sutherland, Bayliss, Braxton, & Beaumont, 1997).

Some limitations of the Baranyi model regarding its basic assumption about the lag time were noted. The lag time was the delay in the growth of the microbial population due to a change in the environment. In the Baranyi model, the lag time and the initial physiological state were interchangeable indicators of the potential for pathogens to adjust to new environment. Baranyi and Roberts assumed that the initial physiological state of microorganisms remained constant, provided identical pre-inoculation history (J. Baranyi & Roberts, 1994). In a controlled experimental environment, the initial physiological state of pathogens may be relatively constant, but it is unlikely the case when the same microorganisms is present in a complex environment. Furthermore, this assumption has been refuted by contradictory

evidence generated from experimental studies (Alavi, Puri, Knabel, Mohtar, & Whiting, 1999; Koseki & Isobe, 2005; Mellefont et al., 2003; Swinnen et al., 2006). Alavi *et al*. revealed that the initial physiological state was dependent to a great extent on the incubation temperature (Alavi et al., 1999). Koseki and Isobe found that the value of the initial physiological state peaked at 15°C and decreased monotonically as temperature went up or down from 15°C (Koseki & Isobe, 2005).

To better characterize the growth response of *E. coli* O157:H7, the modified Baranyi model was developed in the study, with the physiological state described by a function of temperature instead of a static value. Non-parametric goodness-of-fit statistics (MARE) were used to examine the external validity of the model, with independent growth data obtained under dynamic temperature conditions as calibration standards (Koseki & Isobe, 2005). One can have some comfort with the model accuracy given that the model prediction provided a good fit to these calibration data. Further validation of the modified Baranyi growth model can be conducted with more independent growth data collected under a wider range of fluctuating temperature in the future.

5.1.2 Disinfection Models

Postharvest disinfection was a valuable way to reduce the microbial load on lettuce. Chlorine, ClO_2 and irradiation were chosen so that the study included the most common sanitizer (chlorine wash), a promising chemical sanitizer (ClO_2) and an effective but controversial physical decontamination method (gamma irradiation).

First-order kinetics was used for both ClO_2 and irradiation in the study. Although simple, these first-order kinetics were parsimonious and provided a statistically good fit to the observed relationship between the log reduction of *E. coli* O157:H7 and the treatment time for ClO_2 (R^2= 0.96) (Mahmoud & Linton, 2008) and irradiation (R^2=0.95) (Niemira, 2008). Yet, first-order kinetics may not always be the

right fit at all circumstances for ClO_2 disinfection or irradiation. It was valid for use under the conditions reported in these studies, and only to the extent that results obtained from these experimental studies reflected the true antimicrobial efficacies of these methods. The efficacy or lack of efficacy of chlorine has been widely debated, but no chlorine disinfection kinetics were found from all accessible information sources. For the purpose of risk assessment, it would be helpful to have a parametric model to characterize the effect of chlorine disinfection.

5.1.3 Dose Response Model

There were generally two types of dose-response models for pathogens: the threshold model and the non-threshold (or single hit) model. The threshold model postulates that there is a threshold level of pathogenic bacteria cells below which the bacteria do not cause infection; whereas the non-threshold model (e.g., exponential model or beta-Poisson model) provides a non-threshold sigmoidal function, assuming that one single cell is capable of causing a significant probability of disease. Unlike other foodboorne pathogens (e.g., *Listeria Monocytogenes*) *E. coli* O157:H7 is well-known for its extreme toxicity. There was no scientific evidence to support the existence of such a dose-response threshold for *E. coli* O157:H7 infection.

A number of non-threshold mathematical models had been used to describe the dose-response relationship for *E. coli* O157:H7. The beta-Poisson model is commonly accepted and had been used to model data for several foodborne and waterborne pathogens (Crockett, Haas, Fazil, Rose, & Gerba, 1996; C. N. Haas, Thayyar-Madabusi, Rose, & Gerba, 2000; C. Haas, Thayyar-Madabusi, Rose, & Gerba, 1999; Powell, Ebel, Schlosser, Walderhaug, & Kause, 2000). The beta-Poisson model was derived from the exponential model but it had an extra parameter that allows it to deal with highly skewed data with a long tail (Haas C.N., Rose J.B., Gerba C.P., 1999). It assumes that each organism can act independently with

an equal probability of causing infection. The beta-Poisson model was better than the exponential model as it accounted for the variability of the pathogen-host interaction probabilistically with a beta distribution, rather than unrealistically treating the host susceptibility as uniform in the general population. The current study adapted the beta-Possion model developed via meta-analysis of the outbreak attack rates and the ingested doses collected from a number of foodborne and environmental outbreaks (Strachan et al., 2005). This model was not subjected to some of the flaws inherent in using surrogate non-pathogen data or experimental animal data.

5.2 Biological Realism

One characteristic of *E. coli* O157:H7 that made it challenging to study was that *E. coli* O157:H7 cells could lose their growing abilities on agar but remain alive as a response to stress, which was called the viable but non-culturable (VBNC) state (Oliver, 2005). Decline in colony count of *E. coli* O157:H7 could be caused by cell death, extended lag time, or pathogens in a VBNC state, which had different implications on human health risk. Viable pathogens or pathogens that could not be cultured were still able to cause infection. Decline of *E. coli* O157:H7 population at 5°C or less had been investigated by many researchers with mixed observations. It was found that decline of *E. coli* O157:H7 population varied by the storage conditions and the length of storage time (Koseki & Isobe, 2005; McEvoy et al., 2009; Theofel & Harris, 2009). Whereas some observed about 1 log CFU/g decrease in *E. coli* O157:H7 population inoculated on lettuce, at 4°C and 5°C for a 14-day storage period (Abdul-Raouf et al., 1993; Chang & Fang, 2007), others showed no growth or loss of viability of *E. coli* O157:H7 at 5°C over 5 days(Koseki & Isobe, 2005). Development of a strict decline model taking into account all these three possibilities would benefit from *E. coli* O157:H7 population data counted with more sensitive detection methods, such as polymerase chain reaction. In this study, a fail-safe choice was made to model pathogen

growth without considering the occurrence of bacterial decay, with the assumption that at 5°C or lower, *E. coli* O157:H7 remained viable over time.

More often than not, empirical data were available only for typical conditions, but risk assessment concerned about event in the conditions that were more extreme than typical. This was also true when attempting to assess the potential growth activity of *E. coli* O157:H7 in lettuce at low temperature. The extrapolation method is a common strategy used in risk assessment to bridge the gap between the condition of interest and data of relevance (Covello & Merkhofer, 1993). The secondary growth model described the microbial growth parameter (λ, μ_{max}, x_{max}) as a function of an extrinsic factor, i.e., temperature, and served as the link between the sigmoidal Baranyi model (fitted with the DMFit web edition) and the differential Baranyi model. Functionalizing *E. coli* O157:H7 behavior at low temperature was very challenging because existing evidence was sparse and not collected in a time series. In the study, prediction of *E. coli* O157:H7 growth at 3-10 °C was accomplished by extrapolating secondary growth models derived at temperatures ranging from 10°C to 25°C. While problematic, extrapolation may be justified by the fact that the predicted change of *E. coli* O157:H7 population at 5°C was consistent with empirical observations.

The trajectory of growth curve depended on the growth and decay rate of the microbial populations. Microbial growth was influenced by the interactions of many extrinsic growth-determining factors. It was unknown how behavior of *E. coli* O157:H7 in lettuce, or other leafy green vegetables, was affected by extrinsic conditions other than temperature, such as water activity, pH value and the antibacterial properties of lettuce itself. It was possible that the lettuce composition (e.g., water activity and pH value) becomes slightly changed over time, and eventually became unfavorable for pathogens. Temperature was the only extrinsic factor considered by the current model. Caution should be taken when using this model to predict growth in situations where other extrinsic factors are not as stable.

The antimicrobial effect of disinfectants was complicated by the possibility of *E. coli* O157:H7 penetrating into the internal tissue of lettuce via natural openings or cut surfaces as a result of environmental stresses (Seo & Frank, 1999; Takeuchi & Frank, 2000; Takeuchi & Frank, 2001). Penetration of *E. coli* O157:H7 into lettuce tissue may increase the resistance of *E. coli* O157:H7 to disinfectants. For instance, in order to reduce the total *E. coli* O157:H7 load by 90%, 0.3 kGy irradiation was required when *E. coli* O157:H7 located within the lettuce tissue; when the same pathogen was present on the leaf surface, only 0.136 kGy was required (Niemira et al., 2002; Niemira, 2008). Similar information was unavailable for chlorine and ClO_2. Surface disinfection methods (e.g., Cl_2 and ClO_2) were reported largely ineffective on *E. coli* O157:H7 present within lettuce, due to the limited access of these chemical disinfectants to the internalized pathogens (WHO, 1998b).

5.3 Data Quality

The quality of model input parameters was one important indicator of model reliability. To assure transparency, the overall strength of parameters in the model was evaluated with four data quality criteria (proxy, empirical basis, methodological rigor and validation) proposed by Boone *et al.* (Boone et al., 2009).

5.3.1 Growth Data

Prespecified arbitrary initial contamination levels were used as the primary model inputs, because empirical numbers were absent in all reviewed published literature and accessible grey literature that were desired to quantify the prevalence and concentration of *E. coli* O157:H7 in the fresh produce (Abadias et al., 2008; Arthur et al., 2007; Bohaychuk et al., 2009; Mukherjee et al., 2004; Mukherjee et al., 2006; Sagoo et al., 2003) .

The growth model was parameterized with *E. coli* O157:H7 growth data in shredded lettuce as published by Koseki and Isobe (Koseki & Isobe, 2005). The authors used conventional plating method to observe changes of *E. coli* O157:H7 population with realistic inoculum sizes (4.71-4.86 log CFU/g) in a series of temperature from 5°C to 25°C (Appendix B). However, the relative small number of temperature points examined in this experimental study limited the potential for using the data to extract parameter information pertaining *E. coli* O157:H7 growth at low temperature. Nonetheless, this was so far the only study designed to examine growth of *E. coli* O157:H7 in lettuce in a time series with rigorous experimental method.

Substantial amount of research had been conducted, in an attempt to characterize the growth behavior of *E. coli* O157:H7. The empirical findings were, however, as diverse as the environmental conditions under which those studies were carried out. Whereas Francis and O'Beirne reported up to 2 log CFU/g increase of *E. coli* O157:H7 population in shredded lettuce at 8 °C within 5 days (Francis & O'Beirne, 2001), Delauquis *et al*. found no change in microbial population at 10°C over 14 days (Delaquis, Stewart, Cazaux, & Toivonen, 2002). The data used for the study produced an increase of 1 log CFU/g at 10°C over 3 days, which was in line with an increase of a 1.5 log CFU/g at 12°C over 3 days reported by Addul-Raouf (Abdul-Raouf et al., 1993). Until we gain an in-depth understanding of the microbiology of *E. coli* O157:H7, it is not clear whether this model might produce over/under estimates of the actual growth.

5.3.2 Disinfection Data

The efficacy of chemical disinfection is influenced by concentration, temperature, and time of exposure. Existing data were insufficient for developing an empirical probabilistic distribution to capture the variability in the disinfection conditions (i.e., treatment time and concentration) occurrring in the fresh produce industry. For each disinfection method in the current study, realistic point values of treatment

time and disinfectant concentration were chosen, with the uncertainty of the D_{10} values represented by a normal distribution.

Using a point value of treatment time or disinfectant concentration instead of a probabilistic distribution did not allow examining the parametric variance of disinfection practices or identifying the most effective disinfection strategy. Yet, given that the study was designed to assess the contribution of alternative disinfection strategy to risk reduction, this choice served the modeling purpose well as a proof-of-concept.

The internalization of pathogen can influence the disinfection effect and ultimately the risk of infection. The model incorporated recent empirical data about the efficacy of irradiation for inactivating *E. coli* O157:H7 attached both to the surface of the lettuce and within the lettuce. However, all disinfection models were set up in the spreadsheet in such a way that D_{10} values accounted for the effect of internalized pathogens can be included when required data become available in the future.

5.3.3 Dose Response Data

Adapting the beta-Poisson dose response model developed by Strachan *et al*. (2005) may be a conservative choice. The Strachan's model assumed much higher virulence of *E. coli* O157:H7, compared to the model generated from rabbits, but was comparable with the Shigella surrogate model (Crockett et al., 1996; C. N. Haas et al., 2000). However, the choice may be justified by the fact that *E. coli* O157:H7 was a particularly toxic strain; as few as 10 *E. coli* O157:H7 cells were reported to be sufficient to cause measurable infection (Harris et al., 2003).

The dose response model took into account the variability of the natural susceptibility in the general population, but was not able to capture highly susceptible groups (e.g., children and the elderly)

due to the absence of specific data. For simplicity, only the best estimates of the model parameters were used in the study.

5.4 Local Sensitivity

Global sensitivity analysis refers to the analysis of the effect on model predictions of simultaneous variation of all model input and intermediate variables. Ideally, a global sensitivity analysis should have been performed to help identify the parameters important to the model output. In the current study, given that the risk of infection was predicted on a scenario-specific basis, global sensitivity analysis was not applicable. Nominal range sensitivity analysis is a less computationally intense local sensitivity analysis method that tested the sensitivity of intermediate model outcome to the change in value of specific variables. This method was employed to analyze the sensitivity of one growth parameter at a time while holding other parameters constant.

Temperature was an important input variable, but the model output was relatively insensitive to abrupt changes in temperature at a short period of time, because the Baranyi differential function dealt with growth in a continuous way with the first derivative always being continuous. This was considered biologically reasonable as it took into account the cells' previous inoculation history.

The physiological state of microorganism (q_0) and the initial contamination level were two key variables identified by their relative importance to model output. As an adaptation component in the growth model, q_0 increased monotonically with time at the maximum growth rate. Therefore, the initial value of q_0 played an important role in determining how fast the inoculum adjusted to the new environment and subsequently reached the exponential growth phase (Baranyi et al., 1995). The hypothetical uniform distribution associated with q_0 consisted of a range of biologically plausible values. Changes in the value of q_0 did not affect the shape of the predicted curve, but when q_0 was large, the

growth curve was elevated proportionally. On the other hand, the relationship between the initial contamination level and the final *E. coli* O157:H7 concentration depended on the temperature trajectory. As shown in

Figure 10 and Figure 14, growth curves converged as a result of abusive temperatures, which suggested a lack of dependence of predicted growth on the initial contamination level. When the net growth was minimal, the final concentration was dependent of the initial contamination level.

5.5 Model Robustness

Robustness of the model was fulfilled if its response was numerically reasonable while parameters varied over their defined range of values. The minimum floor of the growth function was set at 5°C under which it was assumed that *E. coli* O157:H7 population remained stable without growth or decline. The stability of model was examined with visual inspection regarding potential oscillations from the 4^{th} order Runge-Kutta numerical approximation used in the growth function. In general, the model was stable and did not exhibit oscillatory behavior.

The modified Baranyi model can be classified as a deterministic predictive growth model particularly suitable for describing large population size of microorganisms. When the log inoculum was smaller than zero, which indicated the average of the probabilistic exposure dose was smaller than one organism, the model failed to compute growth. This shortcoming was evidenced in the study in the scenarios when log *E. coli* O157:H7 concentration was reduced to less than zero after disinfection. However, if what was indicated by the dose response model was true, minimal but non-zero dose of *E. coli* O157:H7 in the exposure prediction would still be a concern from a public health point of view.

5.6 Computer Implementation

Implementation of mathematical formulas required coding in appropriate computer language. Assessment of computer implementation was generally accomplished by reviewing computer code line by line to ensure that the syntax and mathematical structure were accurate and free of errors. Intermediate and final outputs were checked against published results from which these models were derived. As an example, parameterized with empirical data and coded in Microsoft Excel VBA, the Baranyi growth model successfully replicated the published results generated by the author with the same data set (Koseki & Isobe, 2005).

Although efforts were made to ensure the computer realization was as accurate as possible, the possibility of software imprecision remained. Therefore, it is recommended to recode the model in higher-level software, e.g., Matlab, to check the results of new software implementation against the current results.

Chapter 6: Future Work and Conclusions

Although the available data and approximations inherent in the model design limited the ability of the model to describe actual exposure and mechanisms of pathogen infection, development of a risk assessment was useful to identify critical gaps in currently published data. With relevant data, the risk model developed in this study could be improved in the future in the following areas:

- Quantification of the initial concentration and prevalence of *E. coli* O157:H7 on lettuce;
- Accounting for the occurrence of cross contamination;
- Increased understanding of the physiological state of *E. coli* O157:H7 and how the pathogen response differs by environmental situations ;
- Gaining further insight to the conditions under which microbial penetration occurred and the survival of *E. coli* O157:H7 following leaf internalization;
- Employing a more sophisticated stochastic growth model that accounts for the probability of exposing to small but non-zero amount of *E. coli* O157:H7;
- Incorporating *E. coli* O157:H7 dose response relationship regarding susceptible population groups, i.e., children and the elderly.

In conclusion, the quantitative microbial risk assessment model developed here can help to gain a quantitative insight into the risk of *E.coli* O157:H7 infection resulted from temperature control and disinfection occurring in the postharvest supply chain of lettuce. Analysis of various scenarios demonstrated the crucial effect of temperature control at the postharvest level. Disinfecting contaminated lettuce using the hypothetical methods examined in the study had limited effectiveness in reducing the risk of infection, especially in the case of temperature abuse. With empirical data collected by the C-EnterNe (Health Canada) in the future, this model can be further developed to make microbial risk prediction more relevant to Canada.

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Appendix A

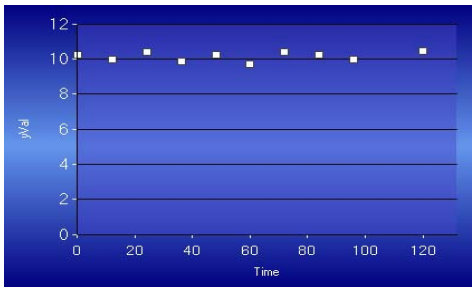
Overview of Food Pathway Parameters

Parameter	Description	Distribution	Unit	Reference
q_0, s_1	Initial physiological state, scenario1	Uniform (0.037, 0.146)	Unitless	
q_0, s_2	Initial physiological state, scenario2	Uniform (0.0009, 0.0036)	Unitless	
q_0, s_3	Initial physiological state, scenario3	Uniform (0.0009, 0.0036)	Unitless	
q_0, s_4	Initial physiological state, scenario4	Uniform (0.045, 0.178)	Unitless	
B_u	Maximum growth rate	Normal (0.049, 1.39E-06)	lnCFU/h	Koseki & Isobe, 2005
B_x	Maximum population density	Normal (0.129, 5.06E-04)	lnCFU/g	Koseki & Isobe, 2005
P	Percentage of *E. coli* O157:H7 residing within the lettuce	Uniform (0,1)	Unitless	
D_{10}, ClO_2	Decimal reduction time	Normal (3.4, 0.04)	Minute	Mahmoud & Linton, 2008
D_{10}, Cl_2	Decimal reduction time	-	Minute	
D_{10}, irradiation	Dosimetry of gamma irradiation	0.136, 0.3	kGy	Niemira et al., 2002; Niemira, 2008
t, ClO_2	ClO_2 treatment time	4	Minute	
t, Cl_2	Cl_2 treatment time	-	Minute	
α	Beta-Poisson model parameter	0.0571	Unitless	Strachan et al., 2005
β	Beta-Poisson model parameter	2.2183	Unitless	Strachan et al., 2005
T	Temperature in the cold chain	-	˚C	Rediers et al., 2009
t	Time in the cold chain	-	minute	Rediers et al., 2009

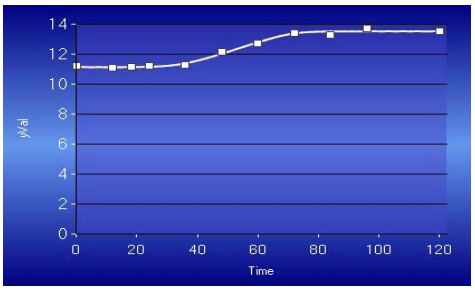
Appendix B

Graphical Representation of the Baranyi Growth Model Fitted to Microbiological Growth Data

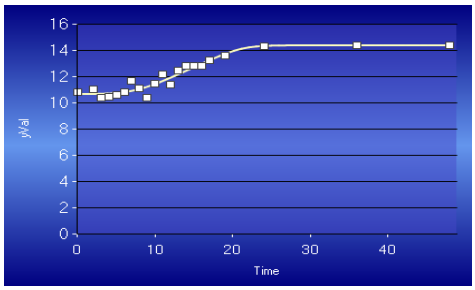
5˚C



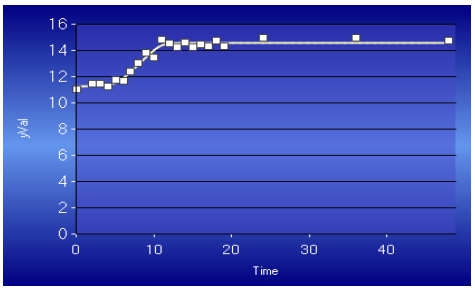
10˚C



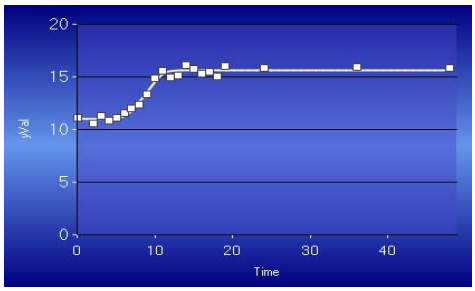
15˚C



20˚C



25˚C



- All sigmoidal curves were fitted with DMFit web edition using empirical data provided by Koseki and Isobe (Koseki & Isobe, 2005).
- No growth was observed at 5˚C; therefore it was not possible to fit with the Baranyi model.

Appendix C

Estimated Maximum Growth Rate, Lag Time and Maximum Population Density

Temperature	Growth Parameter	Standard Error
Maximum Growth Rate (μ_{max}, log CFU/h)		
10°C	0.431	0.010
15°C	0.256	0.043
20°C	0.119	0.100
25°C	0.029	0.157
Lag time (λ, h)		
10°C	34.526	3.749
15°C	7.519	1.484
20°C	5.163	0.713
25°C	6.374	0.503
Maximum Population Density (x_{max}, logCFU/g)		
10°C	6.790	0.086
15°C	6.336	0.227
20°C	6.256	0.094
25°C	5.882	0.113

- All parameters were estimated with DMFit web edition using empirical data provided by Koseki and Isobe (Koseki & Isobe, 2005).

Appendix D

Microsoft Excel VBA Code Used For the Modified Baranyi Model

**********START OF CODE**********

```
Option Explicit

Dim QArray(0 To 500000) As Double
Dim QALength As Integer
Dim QStep As Double
Dim QLastTwoStep As Double
Dim CurrentIndex As Integer

Function GrowthModel_RK4(InitialValue As Double, InitialTime As Double, EndTime As Double, Q0 As
Double, Bu As Double, Bx As Double) As Double

   Dim y As Double
   Dim h As Double
   Dim k1 As Double, k2 As Double, k3 As Double, k4 As Double
   Dim roundEndTime As Double
   Dim i As Integer
   Dim increase As Double
   Dim T As Double

   On Error GoTo HandleError

   h = 20
   roundEndTime = (InitialTime + ((EndTime - InitialTime) \ h) * h) / 60
   QALength = ((EndTime - InitialTime) \ h) * 2 + 1

   InitialTime = InitialTime / 60#
   EndTime = EndTime / 60#
   h = h / 60#

  QArray(0) = Q0
   QStep = h / 2#

   T = InitialTime

   For i = 1 To QALength Step 1
      QArray(i) = Q_RK4_OneRound(QArray(i - 1), T, T + QStep, Bu)
      T = T + QStep
   Next i

   If roundEndTime < EndTime Then
      T = roundEndTime
      QLastTwoStep = (EndTime - roundEndTime) / 2#

      QArray(QALength + 1) = Q_RK4_OneRound(QArray(QALength), T, T + QLastTwoStep, Bu)
```

```
        T = T + QLastTwoStep
        QArray(QALength + 2) = Q_RK4_OneRound(QArray(QALength + 1), T, T + QLastTwoStep, Bu)

        QALength = QALength + 2
    End If


    y = InitialValue

    CurrentIndex = 0
    For T = InitialTime To roundEndTime - h Step h
        k1 = f_t_y2(T, y, QArray(CurrentIndex), Bu, Bx)
        CurrentIndex = CurrentIndex + 1
        k2 = f_t_y2(T + 0.5 * h, y + 0.5 * k1 * h, QArray(CurrentIndex), Bu, Bx)
        k3 = f_t_y2(T + 0.5 * h, y + 0.5 * k2 * h, QArray(CurrentIndex), Bu, Bx)
        CurrentIndex = CurrentIndex + 1
        k4 = f_t_y2(T + h, y + k3 * h, QArray(CurrentIndex), Bu, Bx)
        increase = 1 / 6 * (k1 + 2 * k2 + 2 * k3 + k4) * h
        If increase > 0 Then y = y + increase
    Next T

    If roundEndTime < EndTime Then
        h = EndTime - roundEndTime
        T = roundEndTime
        k1 = f_t_y2(T, y, QArray(CurrentIndex), Bu, Bx)
        CurrentIndex = CurrentIndex + 1
        k2 = f_t_y2(T + 0.5 * h, y + 0.5 * k1 * h, QArray(CurrentIndex), Bu, Bx)
        k3 = f_t_y2(T + 0.5 * h, y + 0.5 * k2 * h, QArray(CurrentIndex), Bu, Bx)
        CurrentIndex = CurrentIndex + 1
        k4 = f_t_y2(T + h, y + k3 * h, QArray(CurrentIndex), Bu, Bx)
        increase = 1 / 6 * (k1 + 2 * k2 + 2 * k3 + k4) * h
        If increase > 0 Then y = y + increase
    End If

    GrowthModel_RK4 = y

HandleError:
    If Err <> 0 Then MsgBox "T=" & T & ", Err=" & Err & ", " & Error(Err)

End Function


Function f_t_y2(Time As Double, y As Double, q As Double, Bu As Double, Bx As Double) As Double

    Dim M As Double
    Dim u As Double
    Dim bigT As Double

    On Error GoTo HandleError

    bigT = getT(Time)
```

```
    M = Bx * bigT + 12.269

    If bigT < 5 Then
            u = 0     'if Temperature<5C, u equals 0
    ElseIf bigT >= 5 Then
            u = (Bu * bigT - 0.2271) ^ 2
    End If

    f_t_y2 = u * q / (1 + q) * (1 - y / M) * y
    Exit Function

HandleError:
     If Err <> 0 Then MsgBox "time=" & Time & ", Err=" & Err & ", " & Error(Err)
End Function

Function Q_RK4_OneRound(PreValue As Double, PreTime As Double, NextTime As Double, Bu As Double)
As Double

    Dim y As Double
    Dim h As Double     ' h is actually the step
    Dim Time As Double
    Dim k1 As Double, k2 As Double, k3 As Double, k4 As Double  ' Four variants used in RK4

    On Error GoTo HandleError

    If PreValue > 100000000# Then
       Q_RK4_OneRound = PreValue
       Exit Function
    End If

    Time = PreTime
    h = NextTime - PreTime
    y = PreValue

    k1 = f_t_q(Time, y, Bu)
    k2 = f_t_q(Time + 0.5 * h, y + 0.5 * k1 * h, Bu)
    k3 = f_t_q(Time + 0.5 * h, y + 0.5 * k2 * h, Bu)
    k4 = f_t_q(Time + h, y + k3 * h, Bu)
    y = y + 1 / 6 * (k1 + 2 * k2 + 2 * k3 + k4) * h

    Q_RK4_OneRound = y
HandleError:
     If Err <> 0 Then MsgBox "time=" & Time & ", Err=" & Err & ", " & Error(Err)
End Function


Function f_t_q(Time As Double, q As Double, Bu As Double) As Double
    Dim u As Double
    Dim bigT As Double

    On Error GoTo HandleError
```

```
    bigT = getT(Time)

    If bigT < 5 Then
            u = 0     'if Temperature<5C, u equals 0
    ElseIf bigT >= 5 Then
            u = (Bu * bigT - 0.2271) ^ 2
    End If

    f_t_q = u * q
    Exit Function

HandleError:
     If Err <> 0 Then MsgBox "time=" & Time & ", Err=" & Err & ", " & Error(Err)

End Function
```

‘Time Temperature function, Scenario 1

```
Function getT(Time As Double) As Double

  Dim y As Double
  Dim x As Double

  On Error GoTo HandleError

  x = Time * 60   'change T from hour to minute

Const b01 = 18.8754558952
Const b02 = -0.2947861272
Const b03 = 0.0000099022
Const b04 = -0.0000000018
Const b05 = 0.2780259523
Const b06 = 0.2232240877
Const b07 = -0.3603787484
Const b08 = 0.0000062962
Const b09 = 0.1288649837
Const b10 = -0.0000000039
Const b11 = 0.0004950322
Const b12 = -0.0000022422
Const b13 = 0.0017125941
Const b14 = -0.0000003793
Const b15 = 0.0005779601
Const b16 = 0.0000029303
Const b17 = -0.0000003003

  y = b01 + b02 * x + b03 * x ^ 2 + b04 * x ^ 3
  If x > 25 Then y = y + b05 * (x - 25)
  If x > 1400 Then y = y + b06 * (x - 1400)
```

```
 If x > 1420 Then y = y + b07 * (x - 1420) + b08 * (x - 1420) ^ 2
 If x > 1440 Then y = y + b09 * (x - 1440)
 If x > 1780 Then y = y + b10 * (x - 1780) ^ 3
 If x > 2700 Then y = y + b11 * (x - 2700) ^ 2 + b12 * (x - 2700) ^ 3
 If x > 2870 Then y = y + b13 * (x - 2870) ^ 2 + b14 * (x - 2870) ^ 3
 If x > 3100 Then y = y + b15 * (x - 3100) ^ 2 + b16 * (x - 3100) ^ 3
 If x > 3300 Then y = y + b17 * (x - 3300) ^ 3

  getT = y


 Exit Function

HandleError:
    If Err <> 0 Then MsgBox "time=" & x & ", Err=" & Err & ", " & Error(Err)

End Function
```

‘Time Temperature function, Scenario 2

```
Function getT(Time As Double) As Double

 Dim y As Double
 Dim x As Double

 On Error GoTo HandleError

 x = Time * 60   'change T from hour to minute

   Const b01 = 25.2211751830596
Const b02 = -0.09138956872187
Const b03 = 0.00035876400986
Const b04 = -0.000000065556343
Const b05 = -0.000277281113982
Const b06 = -0.009557803363525
Const b07 = 0.000060589380211
Const b08 = -0.008262757131685
Const b09 = 0.000068658483021
Const b10 = -0.08639484303839
Const b11 = 0.000451143117825
Const b12 = -0.000923203162937
Const b13 = 0.000638711916958
Const b14 = 0.000000075557664
Const b15 = 0.051540637192484
Const b16 = 0.000680520198474
Const b17 = -0.00000000989912
Const b18 = -0.15223875323306
Const b19 = -0.000696057604959
```

```
Const b20 = -0.030147165734108
Const b21 = 0.009795076304211
Const b22 = -0.000089638342888
Const b23 = 0.00887502423335
Const b24 = 0.000088494432305
Const b25 = 0.35680405374695
Const b26 = -0.002838685614399
Const b27 = 0.000019531037764


  y = b01 + b02 * x + b03 * x ^ 2 + b04 * x ^ 3
  If x > 110 Then y = y + b05 * (x - 110) ^ 2
  If x > 500 Then y = y + b06 * (x - 500) + b07 * (x - 500) ^ 2
  If x > 850 Then y = y + b08 * (x - 850) + b09 * (x - 850) ^ 2
  If x > 1210 Then y = y + b10 * (x - 1210) + b11 * (x - 1210) ^ 2
  If x > 1350 Then y = y + b12 * (x - 1350) ^ 2
  If x > 1420 Then y = y + b13 * (x - 1420) ^ 2
  If x > 1950 Then y = y + b14 * (x - 1950) ^ 3
  If x > 2660 Then y = y + b15 * (x - 2660) + b16 * (x - 2660) ^ 2 + b17 * (x - 2660) ^ 3
  If x > 2680 Then y = y + b18 * (x - 2680)
  If x > 2750 Then y = y + b19 * (x - 2750) ^ 2 + b20 * (x - 2750)
  If x > 5570 Then y = y + b21 * (x - 5570) ^ 2 + b22 * (x - 5570) ^ 3
  If x > 5640 Then y = y + b23 * (x - 5640) ^ 2 + b24 * (x - 5640) ^ 3
  If x > 5820 Then y = y + b25 * (x - 5820) + b26 * (x - 5820) ^ 2 + b27 * (x - 5820) ^ 3
   getT = y


 Exit Function

HandleError:
    If Err <> 0 Then MsgBox "time=" & x & ", Err=" & Err & ", " & Error(Err)

End Function
```

‘Time Temperature function, Scenario 3

```
Function getT(Time As Double) As Double

  Dim y As Double
  Dim x As Double

  On Error GoTo HandleError

  x = Time * 60

Const b01 = 28.0891598668899
Const b02 = -0.147413710867
Const b03 = 0.000321840103599
```

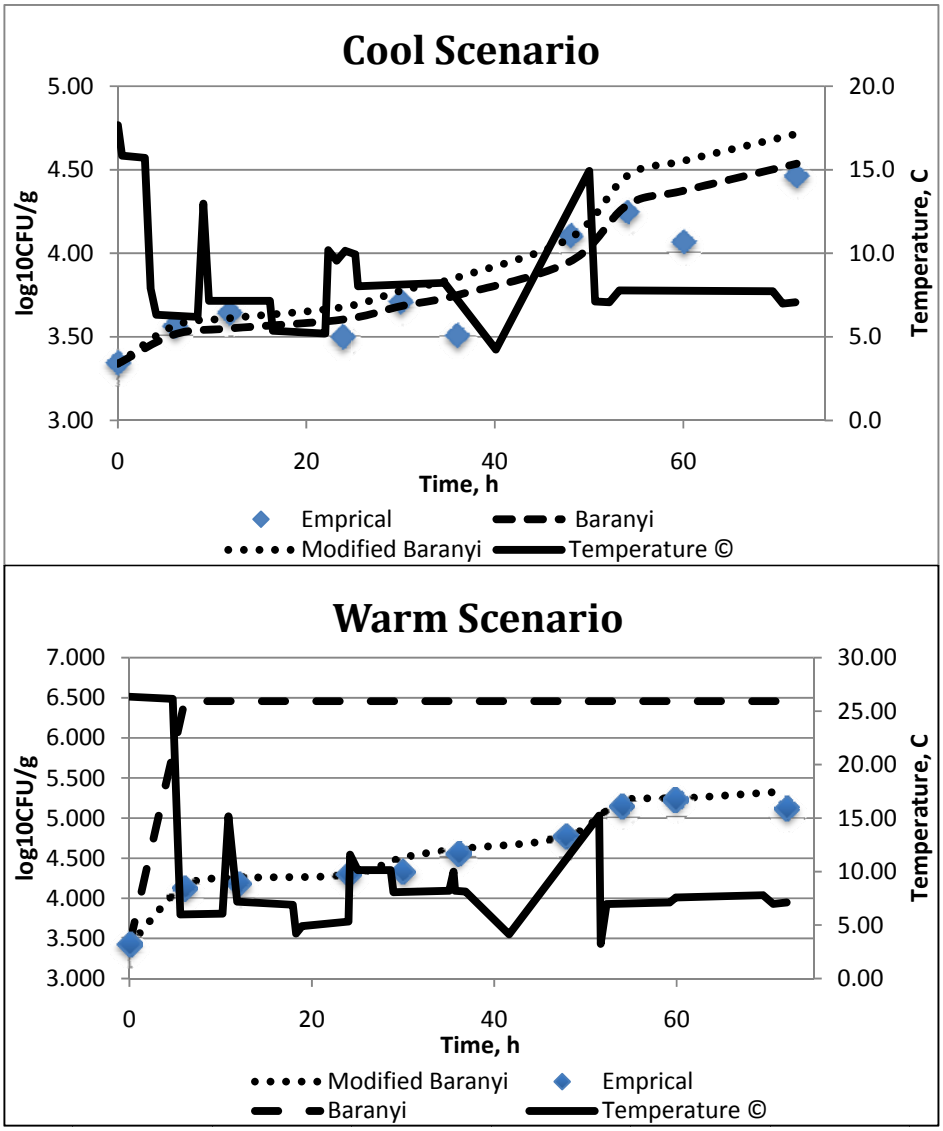
```
Const b04 = 0.000000123345982
Const b05 = -0.000407058601291
Const b06 = -0.000000268010873
Const b07 = 0.000072278516405
Const b08 = 0.00000014850604
Const b09 = 0.001670667015698
Const b10 = -0.000002052301744
Const b11 = -0.35985813304664
Const b12 = -0.034783434784276
Const b13 = -0.000665861067844
Const b14 = 0.000002092472699
Const b15 = -0.09568240882596
Const b16 = 0.000259404032428
Const b17 = -0.000002807911835
Const b18 = -0.012567885427135
Const b19 = -0.000205440859568
Const b20 = 0.000001355451592
Const b21 = -0.000990886813785
Const b22 = 0.14305844864343
Const b23 = 0.001468556124605
Const b24 = -0.19426428667278
Const b25 = 0.00062761961296
Const b26 = -0.000006585563724
Const b27 = 0.003193962216409
Const b28 = -0.41548772904001
Const b29 = 0.52481856531246

  y = b01 + b02 * x + b03 * x ^ 2 + b04 * x ^ 3
  If x > 180 Then y = y + b05 * (x - 180) ^ 2
  If x > 420 Then y = y + b06 * (x - 420) ^ 3
  If x > 750 Then y = y + b07 * (x - 750) ^ 2 + b08 * (x - 750) ^ 3
  If x > 1750 Then y = y + b09 * (x - 1750) ^ 2 + b10 * (x - 1750) ^ 3
  If x > 1815 Then y = y + b11 * (x - 1815)
  If x > 1920 Then y = y + b12 * (x - 1920) + b13 * (x - 1920) ^ 2 + b14 * (x - 1920) ^ 3
  If x > 2590 Then y = y + b15 * (x - 2590) + b16 * (x - 2590) ^ 2 + b17 * (x - 2590) ^ 3
  If x > 2460 Then y = y + b18 * (x - 2460) + b19 * (x - 2460) ^ 2 + b20 * (x - 2460) ^ 3
  If x > 2680 Then y = y + b21 * (x - 2680) ^ 2
  If x > 2720 Then y = y + b22 * (x - 2720) + b23 * (x - 2720) ^ 2
  If x > 2865 Then y = y + b24 * (x - 2865) + b25 * (x - 2865) ^ 2
  If x > 2960 Then y = y + b26 * (x - 2960) ^ 3 + b27 * (x - 2960) ^ 2 + b28 * (x - 2960)
  If x > 3100 Then y = y + b29 * (x - 3100)

   getT = y


 Exit Function

HandleError:
    If Err <> 0 Then MsgBox "time=" & x & ", Err=" & Err & ", " & Error(Err)

End Function
```

‘Time Temperature function, Scenario 4

```
Function getT(Time As Double) As Double

  Dim y As Double
  Dim x As Double

  On Error GoTo HandleError

  x = Time * 60

Const b01 = 18.8754558952
Const b02 = -0.2947861272
Const b03 = 0.0000099022
Const b04 = -0.0000000018
Const b05 = 0.2780259523
Const b06 = 0.2232240877
Const b07 = -0.3603787484
Const b08 = 0.0000062962
Const b09 = 0.1288649837
Const b10 = -0.0000000039
Const b11 = 0.0004950322
Const b12 = -0.0000022422
Const b13 = 0.0017125941
Const b14 = -0.0000003793
Const b15 = 0.0005779601
Const b16 = 0.0000029303
Const b17 = -0.0000003003

  y = b01 + b02 * x + b03 * x ^ 2 + b04 * x ^ 3
  If x > 25 Then y = y + b05 * (x - 25)
  If x > 1400 Then y = y + b06 * (x - 1400)
  If x > 1420 Then y = y + b07 * (x - 1420) + b08 * (x - 1420) ^ 2
  If x > 1440 Then y = y + b09 * (x - 1440)
  If x > 1780 Then y = y + b10 * (x - 1780) ^ 3
  If x > 2700 Then y = y + b11 * (x - 2700) ^ 2 + b12 * (x - 2700) ^ 3
  If x > 2870 Then y = y + b13 * (x - 2870) ^ 2 + b14 * (x - 2870) ^ 3
  If x > 3100 Then y = y + b15 * (x - 3100) ^ 2 + b16 * (x - 3100) ^ 3
  If x > 3300 Then y = y + b17 * (x - 3300) ^ 3
   getT = y

 Exit Function

HandleError:
    If Err <> 0 Then MsgBox "time=" & x & ", Err=" & Err & ", " & Error(Err)

End Function

**********END OF CODE**********
```

Appendix E

Model Evaluation with Empirical Dynamic Temperature Data



- Empirical data obtained by digitizing graphical data reported in the literature (Koseki & Isobe, 2005);
- Baranyi: Original Baranyi model developed by Baranyi and Roberts (Baranyi & Roberts, 1994);
- Modified Baranyi: Baranyi model with the initial physiological state (q_0) defined as a function of initial temperature.
- Calculated mean absolute relative errors (MARE) were shown in Methodology.

Appendix F

SAS Code for Curve Smoothing

********** Scenario 1 **********;

```
data one;
set work.exp051(keep=X B1);
X1=X;
X2=X**2;
X3=(X>10)*(X-10);
X4=(X>120)*((X-120)**2);
X5=(X>210)*(X-210);
X6=(X>210)*((X-210)**2);
X7=(X>1190)*(X-1190);
X8=(X>1190)*((X-1190)**2);
X9=(X>1190)*((X-1190)**3);
X10=(X>1360)*(X-1360);
X11=(X>1360)*((X-1360)**2);
X12=(X>1500)*((X-1500)**2);
X13=(X>1680)*((X-1680)**3);
X14=(X>4010)*((X-4010)**2);
X15=(X>4010)*((X-4010)**3);
X16=(X>4250)*(X-4250);
X17=(X>4250)*((X-4250)**2);
X18=(X>4250)*((X-4250)**3);
X19=(X>4460)*(X-4460);
X20=(X>4460)*((X-4460)**2);
X21=(X>4460)*((X-4460)**3);
run;

ods trace on / listing;
ods output ParameterEstimates=Par;
proc reg data=one;;
model B1=X1-X21;
output out=exp051 predicted=pB1;
run;
title 'REG analysis of expansion variables';
title2 'Full Model: x1-x21';
quit;
ods trace off;

proc print data=Par;
format Estimate 30.15;
run;
```

```
legend label=none value=('B1' 'predicted B1') position=(bottom left inside)
mode=share down =2;
proc gplot data=exp051;
plot (B1 pB1)*x/overlay legend=legend;
run;
```

```
*************** Scenario 2 ************;
data two;
set work.exp054(keep=X Y);
X1=X;
X2=X**2;
X3=X**3;
X4=(X>110 )*((X-110)**2);
X5=(X>500)*(X-500);
X6=(X>500 )*((X-500)**2);
X7=(X>850)*(X-850);
X8=(X>850)*((X-850)**2);
X9=(X>1210)*(X-1210);
X10=(X>1210)*((X-1210)**2);
X11=(X>1350 )*((X-1350)**2);
X12=(X>1420)*((X-1420)**2);
X13=(X>1950 )*((X-1950)**3);
X14=(X>2660)*(X-2660);
X15=(X>2660)*((X-2660)**2);
X16=(X>2660)*((X-2660)**3);
X17=(X>2680)*(X-2680);
X18=(X>2750)*((X-2750)**2);
X19=(X>2750)*((X-2750)**1);
X20=(X>5570 )*((X-5570)**2);
X21=(X>5570)*((X-5570)**3);
X22=(X>5640 )*((X-5640)**2);
X23=(X>5640)*((X-5640)**3);
X24=(X>5820)*(X-5820);
X25=(X>5820 )*((X-5820)**2);
X26=(X>5820 )*((X-5820)**3);
run;

ods trace on / listing;
ods output ParameterEstimates=Par;
proc reg data=two;
model Y=X1-X26;
output out=exp054 predicted=pY;
```

```
run;
title 'REG analysis of expansion variables';
title2 'Full Model: x1-x26';
quit;
ods trace off;

proc print data=Par;
format Estimate 30.15;
run;
legend label=none value=('Y' 'predicted Y') position=(bottom left inside)
mode=share down =2;
proc gplot data=exp054;
plot (Y pY)*x/overlay legend=legend;
run;



************ Scenario 3 *************;

data three;
set work.exp061(keep=X Y);
X1=X;
X2=X**2;
X3=X**3;
X4=(X>180)*((X-180)**2);
X5=(X>420)*((X-420)**3);
X6=(X>750)*((X-750)**2);
X7=(X>750)*((X-750)**3);
X8=(X>1750 )*((X-1750)**2);
X9=(X>1750)*((X-1750)**3);
X10=(X>1815)*(X-1815);
X11=(X>1920)*(X-1920);
X12=(X>1920)*((X-1920)**2);
X13=(X>1920)*((X-1920)**3);
X14=(X>2590 )*(X-2590);
X15=(X>2590)*((X-2590)**2);
X16=(X>2590)*((X-2590)**3);
X17=(X>2460)*(X-2460);
X18=(X>2460)*((X-2460)**2);
X19=(X>2460)*((X-2460)**3);
X20=(X>2680 )*((X-2680)**2 );
X21=(X>2720 )*(X-2720 );
X22=(X>2720 )*((X-2720)**2);
X23=(X>2865 )*((X-2865)*1);
X24=(X>2865)*((X-2865)**2);
X25=(X>2960 )*((X-2960)**3);
X26=(X>2960 )*((X-2960)**2);
```

```
X27=(X>2960 )*((X-2960)**1);
X28=(X>3100 )*(X-3100);
run;

ods trace on / listing;
ods output ParameterEstimates=Par;
proc reg data=three;;
model Y=X1-X28;
output out=exp061 predicted=pY;
run;
title 'REG analysis of expansion variables';
title2 'Full Model: x1-x28';
quit;
ods trace off;

proc print data=Par;
format Estimate 30.15;
run;

legend label=none value=('y' 'predicted y') position=(bottom left inside)
mode=share down =2;
proc gplot data=exp061;
plot (Y pY)*x/overlay legend=legend;
run;



************ Scenario 4 *************;

data four;
set work.exp063(keep=X Y);
X1=X;
X2=X**2;
X3=X**3;
X4=(X> 25)*(X-25);
X5=(X>1400)*(X-1400);
X6=(X>1420)*(X-1420);
X7=(X>1420)*((X-1420)**2);
X8=(X>1440)*(X-1440);
X9=(X>1780)*((X-1780)**3);
X10=(X>2700)*((X-2700)**2);
X11=(X>2700)*((X-2700)**3);
X12=(X>2870)*((X-2870)**2);
X13=(X>2870)*((X-2870)**3);
X14=(X>3100)*((X-3100)**2);
X15=(X>3100)*((X-3100)**3);
```

```
X16=(X>3300 )*((X-3300)**3);
run;

ods trace on / listing;
ods output ParameterEstimates=Par;
proc reg data=four;;
model Y=X1-X16;
output out=exp063 predicted=pY;
run;
title 'REG analysis of expansion variables';
title2 'Full Model: x1-x16';
quit;
ods trace off;

proc print data=Par;
format Estimate 30.15;
run;
legend label=none value=('y' 'predicted y') position=(bottom left inside)
mode=share down =2;
proc gplot data=exp063;
plot (Y pY)*x/overlay legend=legend;
run;
```

Appendix G

Piecewise Cubic Regression Fitted to Time-Temperature Profile

Scenario 1:

Variable	DF	Estimate	StdErr	tValue	Probt
Intercept	1	20.332477320177500	0.10893	186.65	<.0001
X1	1	-0.375306460433790	0.01274	-29.46	<.0001
X2	1	0.001502300491138	0.00001098	136.85	<.0001
X3	1	0.066927695415670	0.01367	4.89	<.0001
X4	1	-0.002290984105323	0.00002117	-108.23	<.0001
X5	1	0.090217016161230	0.00125	72.06	<.0001
X6	1	0.000788713845252	0.00001132	69.68	<.0001
X7	1	0.050730114095757	0.00075605	67.10	<.0001
X8	1	-0.000127063768643	0.00000440	-28.87	<.0001
X9	1	0.000000160167311	4.048354E-9	39.56	<.0001
X10	1	-0.125186522302920	0.00151	-82.75	<.0001
X11	1	0.000435237736999	0.00000374	116.31	<.0001
X12	1	-0.000543767024861	0.00000655	-83.02	<.0001
X13	1	-0.000000160084423	4.055784E-9	-39.47	<.0001
X14	1	0.000111718193996	0.00000367	30.43	<.0001
X15	1	-0.000000459864614	1.571306E-8	-29.27	<.0001
X16	1	0.087270335482540	0.00251	34.79	<.0001
X17	1	-0.000317718888709	0.00001855	-17.13	<.0001
X18	1	0.000002308822291	7.152008E-8	32.28	<.0001
X19	1	-0.306412503850460	0.00499	-61.38	<.0001
X20	1	0.003134900975001	0.00006772	46.29	<.0001
X21	1	-0.000011802356281	4.153751E-7	-28.41	<.0001

Scenario 2

Variable	DF	Estimate	StdErr	tValue	Probt
Intercept	1	25.221175183059600	0.04683	538.53	<.0001
X1	1	-0.091389568721870	0.00117	-78.09	<.0001
X2	1	0.000358764009860	0.00000608	58.98	<.0001
X3	1	-0.000000065556343	5.87512E-10	-111.58	<.0001
X4	1	-0.000277281113982	0.00000655	-42.32	<.0001
X5	1	-0.009557803363525	0.00050295	-19.00	<.0001
X6	1	0.000060589380211	0.00000105	57.67	<.0001
X7	1	-0.008262757131685	0.00055649	-14.85	<.0001
X8	1	0.000068658483021	0.00000114	60.21	<.0001
X9	1	-0.086394843038390	0.00102	-84.43	<.0001
X10	1	0.000451143117825	0.00000447	100.99	<.0001
X11	1	-0.000923203162937	0.00000907	-101.79	<.0001
X12	1	0.000638711916958	0.00000550	116.22	<.0001
X13	1	0.000000075557664	9.27371E-10	81.48	<.0001
X14	1	0.051540637192484	0.00276	18.66	<.0001
X15	1	0.000680520198474	0.00004037	16.86	<.0001
X16	1	-0.000000009899120	3.83234E-10	-25.83	<.0001
X17	1	-0.152238753233060	0.00675	-22.55	<.0001
X18	1	-0.000696057604959	0.00004034	-17.26	<.0001
X19	1	-0.030147165734108	0.00242	-12.44	<.0001
X20	1	0.009795076304211	0.00003545	276.28	<.0001
X21	1	-0.000089638342888	4.265453E-7	-210.15	<.0001
X22	1	0.008875024233350	0.00007458	118.99	<.0001
X23	1	0.000088494432305	3.70718E-7	238.71	<.0001
X24	1	0.356804053746950	0.00676	52.82	<.0001
X25	1	-0.002838685614399	0.00013240	-21.44	<.0001
X26	1	0.000019531037764	0.00000103	18.98	<.0001

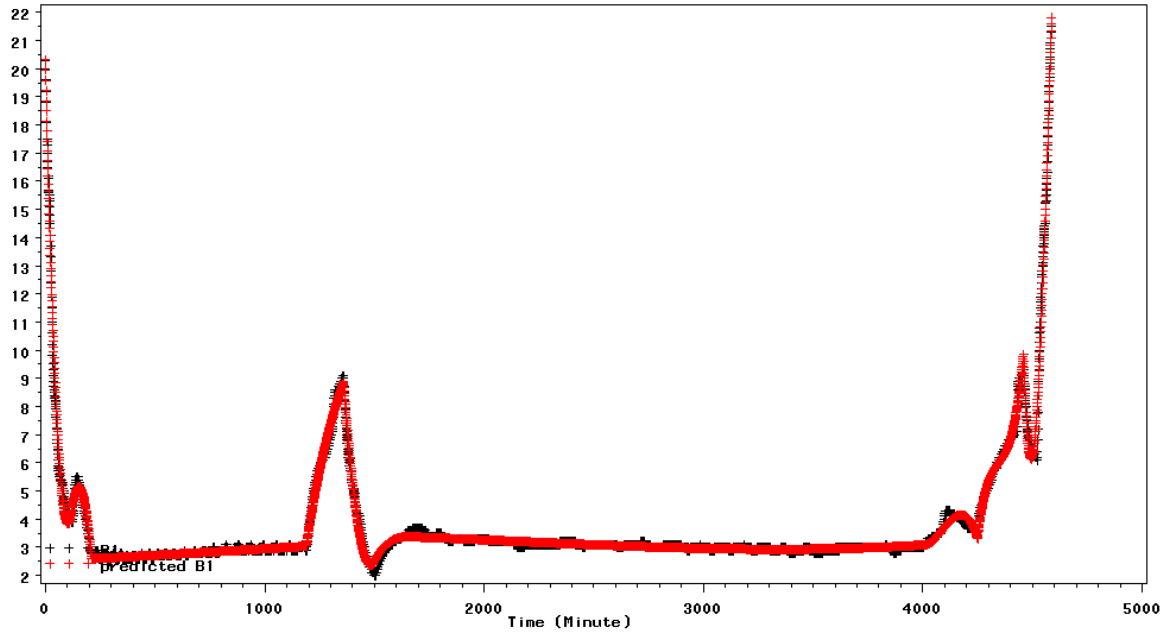
Scenario 3

Variable	DF	Estimate	StdErr	tValue	Probt
Intercept	1	24.591348981412800	0.06416	383.30	<.0001
X1	1	-0.074501386639630	0.00045713	-162.98	<.0001
X2	1	0.000083694596245	6.768617E-7	123.65	<.0001
X5	1	-0.000000189295316	2.425882E-9	-78.03	<.0001
X6	1	0.000108859990239	0.00000247	44.12	<.0001
X7	1	0.000000189566566	2.121565E-9	89.35	<.0001
X8	1	0.001742809810341	0.00003860	45.15	<.0001
X9	1	-0.000002213453644	1.294414E-7	-17.10	<.0001
X10	1	-0.367233435576320	0.00822	-44.68	<.0001
X11	1	-0.034395526421568	0.00567	-6.07	<.0001
X12	1	-0.000649227461010	0.00005447	-11.92	<.0001
X13	1	0.000002257244507	1.319667E-7	17.10	<.0001
X14	1	-0.095677601078760	0.01334	-7.17	<.0001
X15	1	0.000259501882604	0.00016128	1.61	0.1077
X16	1	-0.000002807233758	9.873663E-7	-2.84	0.0045
X17	1	-0.012584313230888	0.00826	-1.52	0.1279
X18	1	-0.000205319746871	0.00014944	-1.37	0.1696
X19	1	0.000001354733853	7.894645E-7	1.72	0.0863
X20	1	-0.000990876398192	0.00030143	-3.29	0.0010
X21	1	0.143058303316500	0.01811	7.90	<.0001
X22	1	0.001468546837308	0.00017657	8.32	<.0001
X23	1	-0.194264415382940	0.00979	-19.85	<.0001
X24	1	0.000627616246598	0.00018359	3.42	0.0006
X25	1	-0.000006585575745	9.802394E-7	-6.72	<.0001
X26	1	0.003193960714469	0.00016100	19.84	<.0001
X27	1	-0.415487840075530	0.01407	-29.54	<.0001
X28	1	0.524818631863140	0.16609	3.16	0.0016

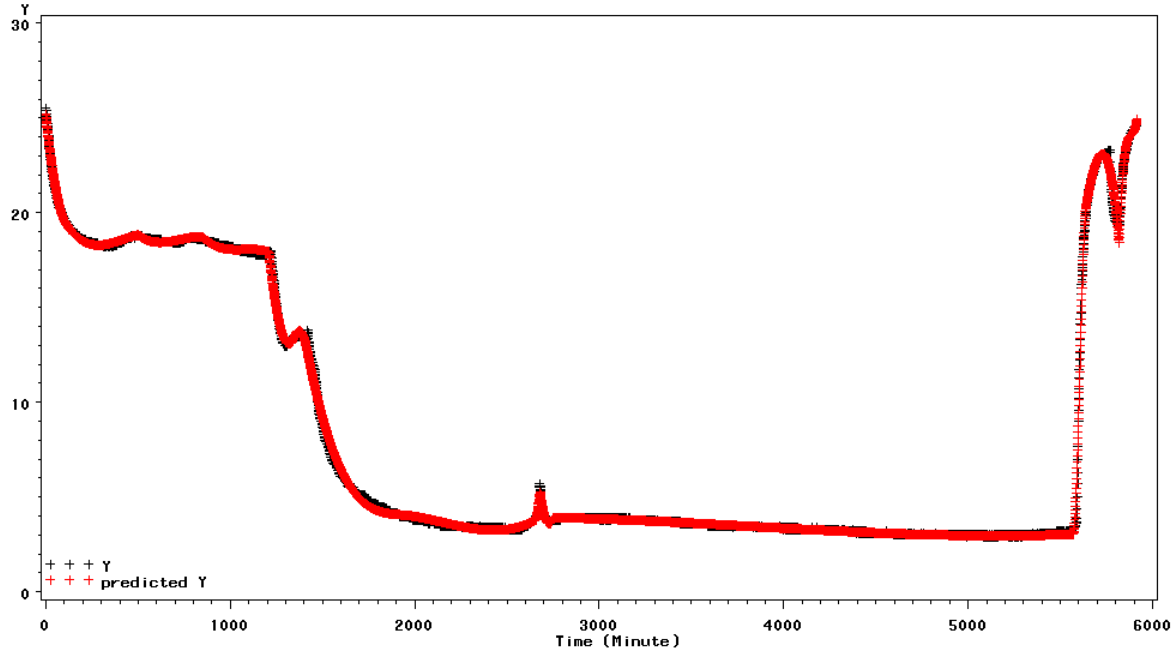
Scenario 4

Variable	DF	Estimate	StdErr	tValue	Probt
Intercept	1	18.875455895202500	0.08446	223.49	<.0001
X1	1	-0.294786127222160	0.00367	-80.34	<.0001
X2	1	0.000009902240553	2.863243E-7	34.58	<.0001
X3	1	-0.000000001763646	1.3221E-10	-13.34	<.0001
X4	1	0.278025952251190	0.00373	74.45	<.0001
X5	1	0.223224087728040	0.00384	58.16	<.0001
X6	1	-0.360378748375840	0.00731	-49.33	<.0001
X7	1	0.000006296222003	4.465615E-7	14.10	<.0001
X8	1	0.128864983719280	0.00382	33.76	<.0001
X9	1	-0.000000003943900	2.26576E-10	-17.41	<.0001
X10	1	0.000495032172002	0.00000771	64.22	<.0001
X11	1	-0.000002242170208	3.554957E-8	-63.07	<.0001
X12	1	0.001712594057819	0.00001810	94.61	<.0001
X13	1	-0.000000379333377	2.673068E-8	-14.19	<.0001
X14	1	0.000577960106756	0.00000922	62.66	<.0001
X15	1	0.000002930282926	1.842084E-8	159.07	<.0001
X16	1	-0.000000300287060	4.824885E-9	-62.24	<.0001

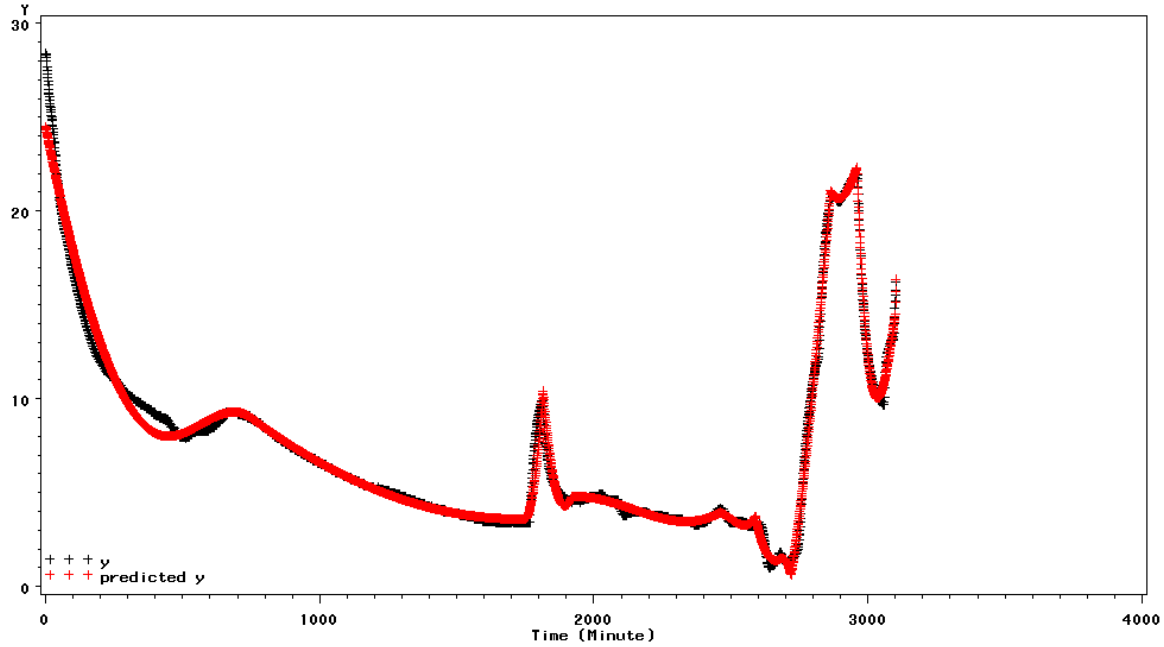
Piecewise Cubic Regression Fitted to Time—Temperature Profile, Scenario 1



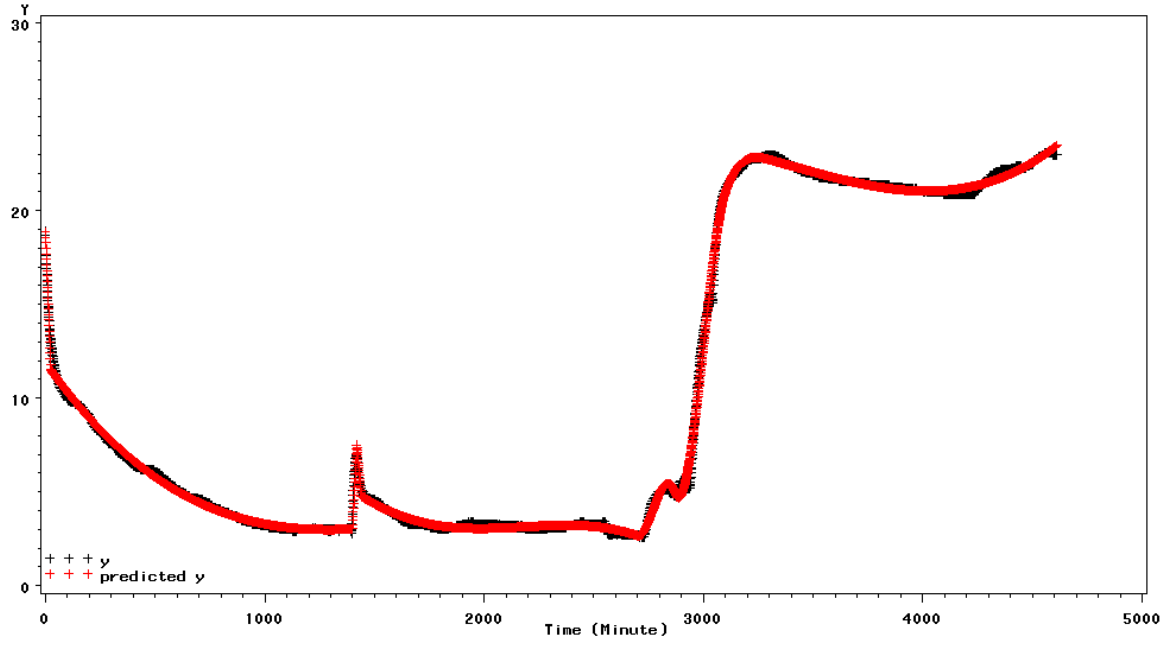
Piecewise Cubic Regression Fitted to Time—Temperature Profile, Scenario 2



Piecewise Cubic Regression Fitted to Time—Temperature Profile, Scenario 3



Piecewise Cubic Regression Fitted to Time—Temperature Profile, Scenario 4



Appendix H

NUSAP Matrix Used to Score the Parameter Strength

Pedigree Criteria

Score	Proxy	Empirical basis	Methodological rigor	Validation
4	Exact measure of the desired quantity (e.g., measurements from the same geographically representative area as that being investigated	Large sample direct measurements, recent data, controlled experiments	Best available (method) practice in well-established discipline (accredited method for sampling/ diagnostic test)	Compared with independent measurements of the same variable over long domain, rigorous correction of errors
3	Good fit or measure (e.g., measurements used from another geographical area but representative	Small sample, direct measurements, less recent data, uncontrolled experiments, low nonresponse rate	Reliable method common within established discipline, best available practice in immature discipline (sampling/diagnostic test)	Compared with independent measurements of closely related variable over shorter period
2	Well correlated but not measuring the same thing (e.g. large geographical differences, less representative)	Very small sample modeled/derived data/indirect measurements, structured expert opinion	Acceptable method but limited consensus on reliability	Compared with measurements not independent, proxy variable, limited domain
1	Weak correlation (e.g., very large geographical differences, low representativity)	One expert opinion, rule-of-thumb estimate	Preliminary methods with unknown reliability	Weak, very indirect validation
0	Not clearly correlated	Crude speculation	No discernible rigor	No validation
A				
B				

- Rows A and B were used to register missingness in two categories. A = no score due to insufficient information, B = no score due to insufficient expertise.

Adapted from Boone *et al*. (Boone et al., 2009)