Risk Assessment of *Listeria monocytogenes* in Ready-to-eat Meat from Plants to Consumption

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ABSTRACT

Listeriosis caused by *Listeria monocytogenes* (*L. monocytogenes*) has been of public concern since the 1980s. Among all the RTE food, deli meats are the major carrier for this pathogen. Eliminating or lowering the initial level of *L. monocytogenes* in RTE meat and poultry product in the plants is an important practice in reducing the risk of *L. monocytogenes* to the public due to the growth potential of *L. monocytogenes* in the RTE food product during storage. Research identifying the contamination at plants provided information for the Food Safety and Inspection Service (FSIS) to establish the Interim Final Rule, requiring the food processing plants that produce post-lethality exposed RTE meat and poultry product choose one of the three alternative plans to ensure good sanitation conditions during food processing or suppress the growth of *L. monocytogenes* during storage: post-processing treatment and use of growth inhibitor (Alternative1), post-processing alone (Alternative 2a) or use of growth inhibitor and sanitation program (Alternative 2b), and sanitation program alone (Alternative 3).

This research developed a comprehensive model that simulated the entire processes of RTE food production, taking into account potential transfer and growth of *L. monocytogenes* in RTE meat and poultry products. This plant-to-consumption model analyzed the effectiveness of the three alternative processes on reducing the *L. monocytogenes* in the RTE food products and also investigated the optimal sampling and sanitizing program. Results showed that formulation of food products with growth inhibitor has the greatest impact on reducing the risk of *L. monocytogenes*, followed by the post-processing treatment and sanitation intervention. Risk can also be reduced depending on alternatives. For example, 70% reduction if all are switched to alternative 2b and 91% reduction if all are switched to Alternative 1, compared with the current alternative selection by food establishments.

This study investigated several important factors in the sanitation program, analyzed the sensitivities of these factors, and proposed the reasonable improvement of the hold-and-test strategies by the plant-to-consumer mathematic model. Holding all the lots during the food contact surface (FCS) testing period instead of holding lots after finding the positive FCS would increase the detection rate of positive lots by three times. These results may help the food establishments under Alternative 3 choosing the proper sampling and sanitation program or switching to Alternative 1 or 2.

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Chapter 1 Introduction

With the outbreaks of listeriosis in the early 1980s, more and more attention has been paid to the investigation *of Listeria monocytogenes* in the food industry. In 2003, the Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS) developed a quantitative assessment of the relative risk of *L. monocytogenes* to public health among 23 categories of ready-to-eat (RTE) foods in the United States. Deli meats caused more deaths than any other food categories.

Subsequently, Endrikat et al. conducted another comparative risk assessment for *L. monocytogenes* in prepackaged versus retail-sliced deli meats with and without growth inhibitors (Endrikat et al. 2010), and found that 70% of the estimated annual deaths associated with *L. monocytogenes* occurred from retail-sliced deli meats without a growth inhibitor. This result suggested that it is possible to separate deli meats with less risk (prepackaged with growth inhibitor) from the high risk category in the report of FDA-FSIS 2003 (FDA/FSIS 2003).

As more and more data associated with *L. monocytogenes* appear in the literature, transport and fate of *L. monocytogenes* in the food chain becomes clearer. Using this data, it is possible to use a mathematic model to simulate the transport and growth of *L. monocytogenes* in the food supply system. This sort of model can be used in the risk assessment of *L. monocytogenes* in the RTE food and guide policy making of food safety in the RTE food industry. FSIS's interim final rule requires all the food processing facilities that produce post-lethality exposed RTE meat or poultry products to choose one of three alternatives based on a combination of post-processing treatment, growth inhibitor use, and sampling/sanitation to mitigate the risk of *L. monocytogenes* in the RTE food. The effectiveness of the three alternatives could be evaluated by a mathematical model consisting of the possible components, such as the cross-contamination in the plants and retail, the sampling process in the plants, the post-processing intervention, and growth of *L. monocytogenes* in the refrigerators.

1.1 Objective and scope of the this research

The objective and scope of the research is composed of three major sections: first is the risk ranking changes by the sub-categorization of the deli meats in the risk ranking model for *L. monocytogenes*; the second uses a risk assessment model to evaluate the effectiveness of post-processing intervention, growth inhibitor formulation and sanitation programs; the third uses the model to develop optimal test and hold strategies. The detailed objectives of this work are:

1) Conduct a relative risk ranking for RTE foods by subdividing deli meats based on slicing location and growth inhibitor use. The model is based on the previous risk assessment framework (FDA-FSIS 2003), and the results are compared with the previous risk ranking.

2) Develop a comprehensive risk assessment model of *L. monocytogenes* in RTE meat and poultry product from the plant to the time of consumption and resulting public health impact. The model incorporates food contact surface testing, product testing, sanitation, post-processing lethality, growth with and without inhibitors, retail cross-contamination, and differential consumer storage practices.

3) Determine the effectiveness of the three alternative interventions in reducing *L. monocytogenes* contamination in finished RTE product, and the subsequent risk to public health.

4) Analyze the most influential factors on the effectiveness in the interventions as well as the hold-and-test program in controlling the risk of *L. monocytogenes* in RTE meat and poultry product at the food facility plants under Alternative 3.

1.2 Organization of this dissertation

This dissertation consists of five chapters including the introduction, literature review and three manuscripts. The last three chapters are three complete manuscripts which serve as the major work of the author in the PhD program at Virginia Tech.

Chapter 2 is composed of a comprehensive literature review on the history of *L. monocytogenes*, associated outbreaks, the risk assessment of *L. monocytogenes* in food, the regulations, and the research conducted in recent years.

Chapter 3 evaluated the influence of deli meats sub-categorization on the risk ranking for *L. monocytogenes* in ready-to-eat food. By dividing deli meats into four subgroup- deli meats prepackaged or sliced at retail with and without growth inhibitor, the prepackaged deli meats with growth inhibitor could be switched from the very high risk region to moderate risk region.

Chapter 4 introduced the risk assessment plant-to-consumption model, incorporated processes in the food processing plants, the retail and consumers' home was introduced. This model analyzed the effectiveness of the three Alternatives on reducing *L. monocytogenes* in the RTE food products and also investigated the optimal sampling and sanitizing program. Results showed the formulation of food products with growth inhibitor has greater impact on reducing the risk of *L. monocytogenes* than the post-processing interventions and sanitation programs alone.

Chapter 5 evaluated the hold-and-test procedures required in food establishments that choose to follow Alternative 3. Several important factors in the hold-and-test program and the sensitivities of these factors were investigated and analyzed. Holding all the lots during the food contact surface (FCS) testing period instead of hold lots after finding the positive FCS sample would increase the detection rate of positive lots by three times while increasing the sampling size of the RTE food product could reduce the annual illnesses by up to 50%. These results may help the food establishments wisely choose the best alternatives or help the establishments under Alternative 3 to select the proper sampling and sanitation procedures.

Chapter 2 Literature Review

2.1. Background

Listeria monocytogenes (*L. monocytogenes*) is a facultative anaerobe, intracellular, and Gram-positive bacterium first described in 1926 in Cambridge, United Kingdom, as a cause of infection with monocytosis in laboratory rodents (Cliver 2006). In 1936, Burn in the United States affirmed listeriosis as a cause of both sepsis among newborn infants and meningitis in adults (Schaffner 2003). Up to the 1980's, human listeriosis remained a relatively obscure disease attracting limited attention, although large outbreaks of considerable morbidity and mortality but of unknown transmission occurred. Since a series of outbreaks in the 1980s, *L. monocytogenes* became recognized as causing an extremely serious, invasive, and often life-threatening food-borne disease with a high economic burden to both public health services and the food industry (Kusumaningrum et al. 2004).

When it is compared to other major foodborne diseases, listeriosis is a rare occurrence, but the fatality rate is very high (i.e., approximately 20% compared to 0.8% for other foodborne illnesses) (Buchanan et al. 1997). It is recognized as one of the most virulent foodborne pathogens with fatality rates (20% to 30%) (Yu et al. 2011; Aziza et al. 2006). Responsible for approximately 1,455 hospitalizations and 255 deaths in the United States annually (Scallan et al. 2011), listeriosis is the leading cause of death among foodborne illnesses. Listeriosis affects primarily pregnant women, newborns, and adults with weak immune systems (people with HIV infection, cancer, organ transplants, or advanced ages). Due to its frequent pathogenicity in causing meningitis in newborns, pregnant mothers are often advised not to eat soft cheeses, which may be contaminated with of *L. monocytogenes* and permit growth of them (Keskinen et al. 2008). Elderly people (greater than 65 years old) are the second most susceptible population group (Figure 2-1), and the proportion is increasing with about 21.9% - 45.6% of the whole illnesses cause by *L. monocytogenes* (Muñoz et al. 2012). In Julian et al.'s study performed from 1971 to 1999, 42% of the patients were older than 65 years and the mortality was as high as 61% (Julian et al. 2001).

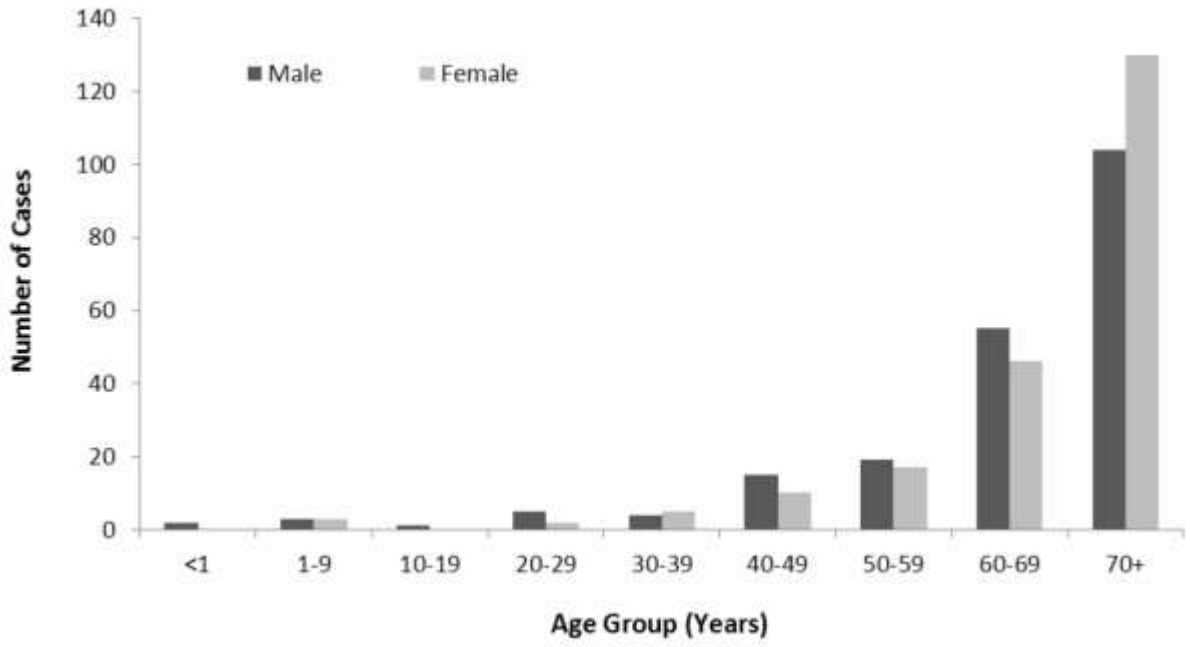

Figure 2-1. Patients with non-pregnancy-associated listeriosis, by age group and sex, *Listeria* Initiative, 2009 (N=421) (CDC 2009).

2.2 Historical Outbreaks

After *L. monocytogenes* was first described in 1926 resulting in six cases of sudden death in young rabbits and was named in 1940, it remained out of people's sight as foodborne pathogens for a long time. Around 1952, it was finally recognized as a significant cause of neonatal sepsis and meningitis in East Germany. Therefore, outbreaks of *L. monocytogenes* before that time were not identified.

Using outbreak data from 1998-2008, Batz et al. estimated that there are 44 sporadic cases of listeriosis for every identified outbreak case (Batz et al. 2011). Because of its virulence, this number is actually lower than many other food pathogens. The values for *Salmonella* and *Campylobacter* are 301 and 1702 respectively.

Many listeriosis outbreaks were confirmed after 1981, when *L. monocytogenes* was identified as a cause of foodborne illness. In 1981, more than 100 people in Canada were infected by *L. monocytogenes* with thirty-four of the infections occurring in pregnant women, resulting in nine stillbirths, and 23 infants infected. Among 77 non pregnant adults who developed typical disease,

there was nearly 30% mortality. The source of the outbreak was coleslaw produced by a local manufacturer (Sheen et al. 2010). Another outbreak of listeriosis in Halifax, Nova Scotia, involving 41 illnesses and 18 deaths, was linked to the consumption of coleslaw containing cabbage that had been fertilized with *L. monocytogenes*-contaminated raw sheep manure with epidemiological evidence (Dumen et al. 2009). From then on, a number of foodborne listeriosis outbreaks were reported and *L. monocytogenes* is widely recognized as one of the primary foodborne pathogens in the food industry (Cummins et al. 2008).

In 1985, in California, 142 people developed typical listeriosis with 93 cases for perinatal individuals, and 48 out of 49 cases for nonpregnant individuals were immune-compromised (Dufour 2011). The source of the bacteria was a brand of soft cheese became contaminated with unpasteurized milk during the manufacturing process.

In 2002, an outbreak of *L. monocytogenes* infections with 46 confirmed cases, seven deaths, and three stillbirths or miscarriages in eight states of US was linked to consuming sliced turkey deli meats. *L. monocytogenes* was found in one of the food products and multiple environmental samples from a poultry processing plant, with two isolates from floor drains indistinguishable from that of the outbreak clinical isolates (Sauders et al. 2009).

Table 2-1 lists the selected outbreaks in the USA since 1979. In the late 1990's through mid-2000's, many of the outbreaks were associated with FSIS regulated products: deli meats and frankfurters. Since then, most outbreaks have been associated with FDA regulated products: cheeses, fruits, and vegetables. Cartwright et al. have noted that the FSIS regulations and targeted sampling programs have reduced *L. monocytogenes* contamination of RTE meat and poultry, but similar reductions have not been made for FDA dairy products (Cartwright et al. 2013).

Table 2-1. Selected outbreaks of listeriosis in USA from 1979 to 2011 (Warriner et al. 2009; Cartwright et al. 2013; Silk 2013)

Year	Cases (Deaths)	Food Vehicle
1979	20 (5)	Raw vegetables
1983	49 (14)	Pasteurized milk
1985	142 (48)	Mexican cheese
1989	10	Shrimp
1994	48	Pasteurized chocolate milk
1998	108 (14)	Frankfurters
1998	4	Frankfurters
1999	6	Unknown
1999	4	Frankfurters
1999	5 (1)	Deli meats
1999	11	Pate
1999	2 (1)	Deli meats
2000	13	Mexican cheese
2000	30 (4)	Deli meats
2001	28	Deli meats
2002	54 (8)	Deli meats
2003	3	Unknown
2003	12 (1)	Mexican cheese
2005	32	Unknown
2005	7	Grilled chicken
2005	37 (1)	Deli meats
2005	36	Mexican cheese
2006	2 (1)	Unknown
2006	2	Taco salad
2006	3 (1)	Cheese
2007	5 (3)	Milk

Year	Cases (Deaths)	Food Vehicle
2008	5 (3)	Tuna salad
2008	20	Sprouts
2008	8	Mexican cheese
2009	12	Mexican cheese
2009	8	Mexican cheese
2010	8	Hog head cheese
2010	2	Sushi
2010	4	Unknown
2010	10	Pre-cut celery
2010	6	Mexican cheese
2011	2	Unknown
2011	2	Chive cheese
2011	147 (14)	Whole cantaloupe
2011	2	Mexican cheese
2011	15	Aged blue-vein cheese

In recent years, there has been an increasing trend in reported listeriosis in several European countries. Most of the cases are associated with the population of people with 65 years of age or above, uncorrelated with other factors such as geography, gender, ethnicity or infectious serotypes (Allerberger et al. 2010) and the same tread of listeriosis in aged people was found in most other cases. Although the Advisory Committee on the Microbiological Safety of Food (ACMSF) gave several hypotheses on the potential reasons for this increase (ACMSF 2009), no confirmed cause was found.

2.3 *L. monocytogenes* in natural environment

L. monocytogenes is commonly found in soil and water and on plant material, especially those undergoing decay. Decayed vegetation has been cited as the source of infection in numerous cases of listeriosis in farm animals, and may be the origin of contamination capable of spreading along the food chain (Mylius et al. 2007). Soil is often referred to as the source of *L.*

monocytogenes contamination particularly for silage (Zhao et al. 1998). The study of Weiss and Seeliger (Okutani et al. 2004) showed that *L. monocytogenes* was present in plant samples from 9.7% of cornfields, 13.3% of grainfields, 12.5 % of forests, and 23.1% of wildlife feeding areas examined in southern Germany. Surface soils had similar levels, but analysis of soil samples taken at a depth of 10 cm gave significantly fewer positive samples, indicating that vegetation is a principal component in *L. monocytogenes* contamination of the soil. *L. monocytogenes* has been found in the feces of a wide variety of healthy animal species, such as sheep, goats and cattle and so on.

The natural environment appears to be the initial reservoir for *L. monocytogenes* which can enter and pass along the food chain, but this contamination is usually of a low level and sporadic. It is significant that poultry products are more contaminated than beef, yet the environment in which beef cattle are raised presents a greater risk of contact with the organism than that of the intensively reared broiler chicken. However, in processing, poultry are exposed to greater risk of contamination from other carcasses and mechanical equipment than cattle (Schaffner et al. 2007). It is at the processing stage of food and fodder that amplification of numbers and persistent contamination occur, which in turn present a potentially more serious challenge to human and animal health (Figure 2-2).

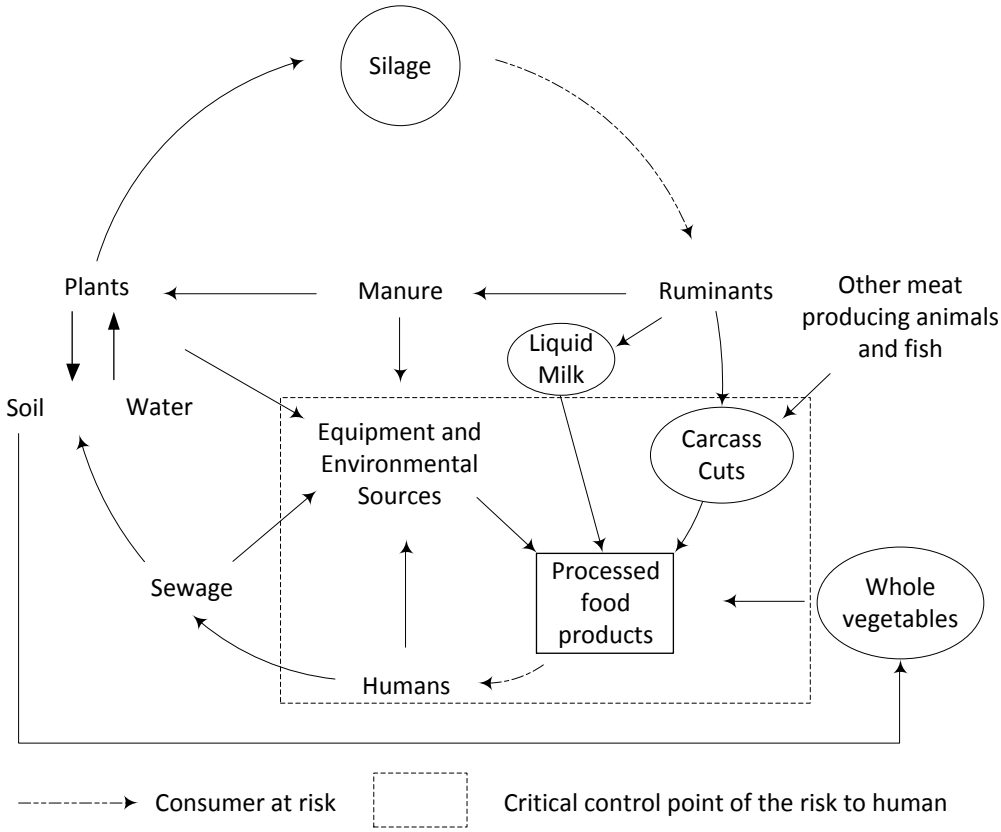

Figure 2-2. Spread of *L. monocytogenes* to the food chain from the natural environment. Revised from Ryser et al. (1999).

2.4 *L. monocytogenes* prevalence in food production facility and retail store

L. monocytogenes is also ubiquitous in the food production and retail environment, where could possibly account for a great portion of the *L. monocytogenes* outbreak.

Rorvik et al. investigated forty smoked salmon processing plants situated in middle and southern part of Norway for the occurrence of *L. monocytogenes* and other *Listeria spp*. in the smoked salmon and the drains. More than 25% of the salmon samples and drain samples were contaminated with *L. monocytogenes* and more than 40% with contamination of other *Listeria spp*. Multivariate analyses of data on the factors that including hygiene, management, production facilities of the plants and bacteriological results showed that job rotation was the strongest expressed risk factor for isolation of *L. monocytogenes* from the smoked salmon and well-maintained facilities and use of vats for salting of the fillets, showed a preventive effect (Rorvik et al. 1997).

According to a survey of *L. monocytogenes* in ready-to-eat foods in the US from retail markets at Maryland and northern California FoodNet sites, which contains meat, salad, cheeses and seafood, the overall prevalence was reported as 1.82%, ranging from 0.17% to 4.7%. Significant differences ($p < 0.05$) between the sampling sites were found, with higher prevalence for three categories in northern California and for two categories in Maryland. In-store-packaged samples had a significantly higher prevalence than manufacturer-packaged samples of luncheon meats, deli salads, and seafood salads. Most of the samples (16 of 21) with higher counts were manufacturer-prepackaged in the food processing facilities (Gombas et al. 2003). These results suggested that the increased prevalence of *L. monocytogenes* in food products comes from the practices at retail, and the initial contamination before packaging was more remarkable because the growth potential of *L. monocytogenes* during storage aggrandized the risk of *L. monocytogenes* at the point of consumption.

In France, total proportion of ready-to-eat foods contaminated with *L. monocytogenes* from 1995 to 1996 was 6.7% (Goulet et al. 2001). The incidence of *L. monocytogenes* in imported seafood products in Canada was 0.88% (1996-1997) and 0.3% (1997- 1998) (Farber 2000; Okutani et al. 2004). The presence of *L. monocytogenes* on 99 fresh and frozen chicken carcasses sourced from various retailers in Gauteng, South Africa was investigated and 19.2% of the carcasses were found to be contaminated. No significant difference in the proportion of carcasses with *L. monocytogenes* from different sources was found (van Nierop et al. 2005). In a recent investigation in Canada, a total of 800 meat and poultry products consisting of beef, chicken pork and turkey from the local retail marketplace were analyzed and *L. monocytogenes* was found in all of the products with occurrence of pathogens similar to the typical products retail products in many other international locales (Bohaychuk et al. 2006).

All of the studies mentioned previous suggest that *L. monocytogenes* exist widely in the environment of food production facilities. *L. monocytogenes* can also exist and persist in retail environments by characterizing *L. monocytogenes* isolates from 125 foods, 40 environmental samples and 342 clinical cases collected in New York State from 1997 to 2002 (Sauders et al. 2004). *L. monocytogenes* was found to be able to persist in retail environments for more than 1 year and a number of the subtypes at retail are common among human listeriosis cases (Sauders et al. 2009).

2.5 *L. monocytogenes* regulations

In response to the outbreaks of listeriosis that occurred during the 1980s and 1990s, U.S. federal regulatory agencies and the food industry embarked on a number of initiatives designed to control this pathogen. Many of these efforts are continuing today. In response to the recognition that *L. monocytogenes* can contaminate meat and dairy products, the Food and Drug Administration (FDA) developed the Dairy Safety Initiatives Program in April of 1986 (Schaffner 2004) and the USDA also developed the monitoring/verification program for *L. monocytogenes* in meat products in September of 1987 (Yu et al. 2011). Measures to exert control over *L. monocytogenes* contamination in the processing plant and its impact upon subsequent finished product contamination were effectively developed.

Considerable progress has been made in minimizing recalls through implementation of good manufacturing practices (GMPs), standard sanitation operating procedures (SSOPs) and Hazard Analysis and Critical Control Points (HACCP) programs. As a result of these efforts, Tappero et al. reported a 49% decrease in the rate of invasive listeriosis and a 48% decrease in numbers of deaths related to listeriosis between 1989 and 1993 (Tappero et al. 1995). The overall annual incidence rate of listeriosis in the United States during this period declined from 7.9 to 4.2 cases per million persons, and remains stable today at 5.0 cases per million persons. Continuing efforts include those of the FDA's Food Compliance Program for Domestic and Imported Cheese and Cheese Products, which issued guidelines in November of 1998 (Ross et al. 2000). These guidelines were developed in response to the recognition that cheese and cheese products can contain pathogenic bacteria such as *L. monocytogenes,* and cause human illness. The guidelines call for the FDA to conduct inspections of domestic cheese firms, to examine samples of imported and domestic cheese for microbiologic contamination, and to take appropriate regulatory action when violations are encountered (Ross et al. 2000). After the large listeriosis outbreak in 1998, a serial of public meetings were prompted by by large-scale product recalls due to contamination with *L. monocytogenes* or actual outbreaks of listeriosis. In these meetings, *Listeria* species were used as indicator organism for *L. monocytogenes* and scientific methods were discussed to detect and control *L. monocytogenes*.

From 1990 till now, the testing prevalence (the ratio of positive samples to the total samples) of *L. monocytogenes* in RTE foods at plants decreased continuously from 4.5% to 0.5%. FoodNet data on the incidence of foodborne illnesses for the United States in 2001 indicated that the incidence of infection from *L. monocytogenes* decreased between 1996 and 2001 from 0.5 to 0.3 cases per 100,000 people per year. However, the level then reached a plateau, from 2001 to 2010 (Figure 2-3).

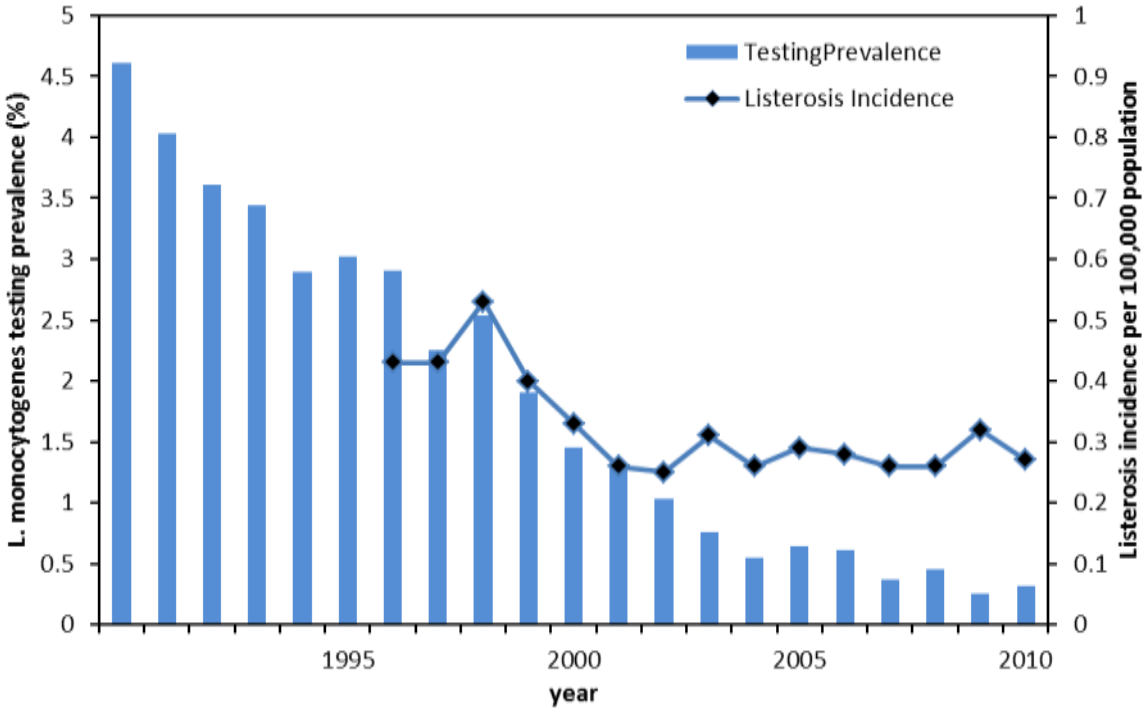

Figure 2-3. Prevalence of *L. monocytogenes* at plants versus listeriosis incidence.

Prevalence: http://www.fsis.usda.gov/PDF/Figure1_Micro_Testing_RTE_1990-2011.pdf;
Incidence: http://www.cdc.gov/foodnet/PDFs/Table2b.pdf

Regulatory legislation was implemented to minimize the prevalence of *L. monocytogenes* in the US. But implementing regulations to control *L. monocytogenes* was problematic due to the fact that the pathogen is highly ubiquitous in the environment, and highly virulent. Therefore, a balance had to be made with respect to implementing realistic limits for industry while ensuring adequate protection for consumers. To strike this balance the majority of trading nations undertook a risk-based approach when developing legislation. In contrast, the US Food Safety and Inspection Service made a significant move in classifying *L. monocytogenes* as an

adulterant. In practical terms this meant that the detection of *L. monocytogenes* on a food or food contact surface would trigger a product recall. The specific final rule of the FSIS/USDA was Federal Register Interim Final Rule 9 CFR Part 430, which is utilized to fulfill the requirement specified as below:

> *L. monocytogenes* can contaminate RTE products that are exposed to the environment after a lethality treatment (destroy/kill). *L. monocytogenes* is a hazard that an establishment must control through its HACCP plan, or prevent in the environment through a Standard Sanitation Operating Procedures (SSOP) or other prerequisite program if it produces RTE product that is exposed post-lethality. RTE product is adulterated if it contains *L. monocytogenes* or if it contacts surfaces contaminated with *L. monocytogenes*.

To comply with the regulations, food establishments must follow one of three alternative processes (Figure 2-4). For high risk foods such as RTE meat, Alternative 1 is preferred which combines a post-lethal decontamination step along with the addition of a growth inhibitor into the formulation. In Alternative 2 the establishments are offered the option of applying a post-lethal decontamination step or formulation of product to include a growth inhibitor along with a *Listeria species* sanitation program. Finally, Alternative 3 relies solely on having an effective sanitation program along with end-product testing. This is generally recommended for low risk foods (for example, salad vegetables), however, if used for RTE meats a hold-and-test policy must be enforced.

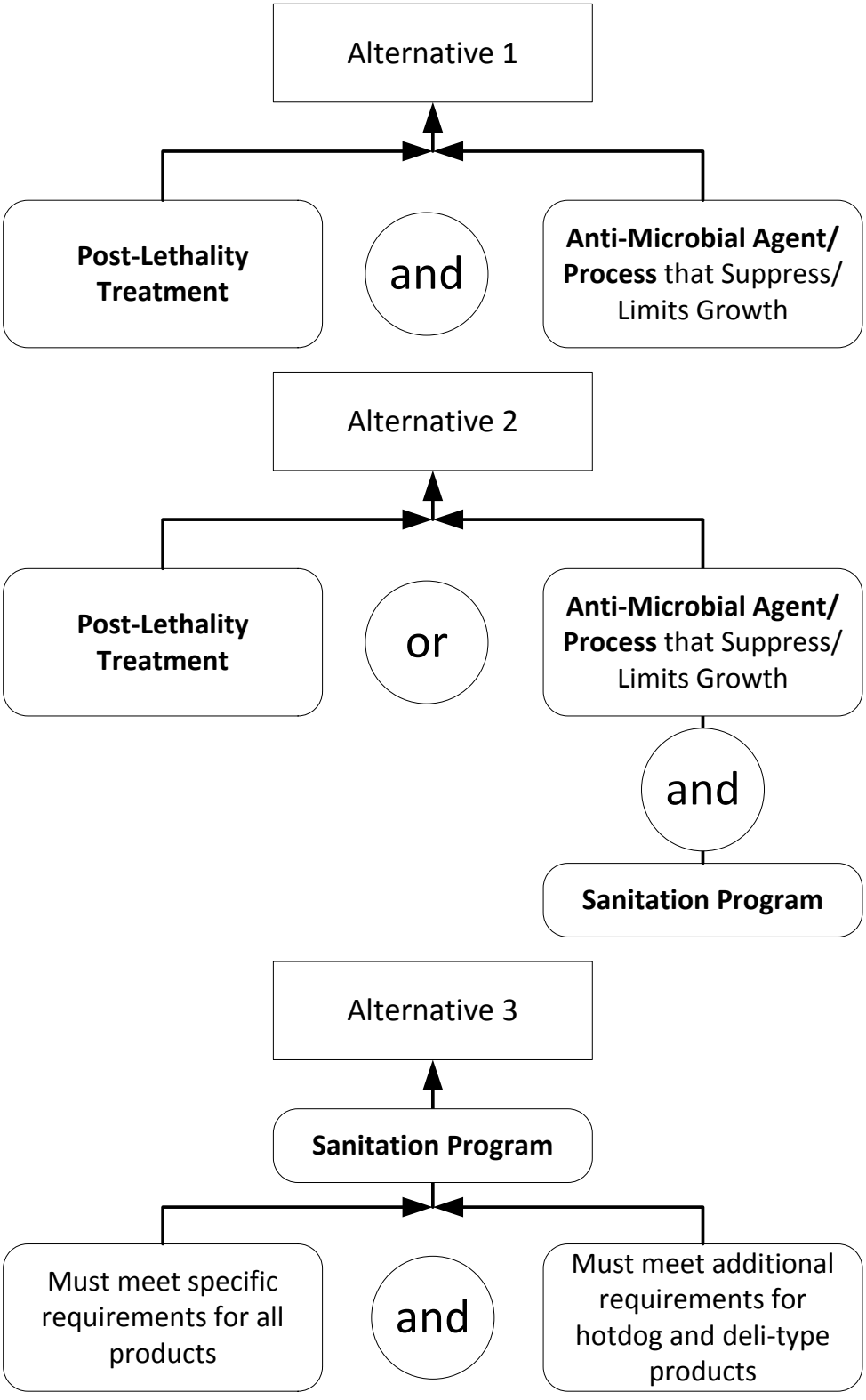

Figure 2-4. Alternatives to control *L. monocytogenes* in ready-to-eat food processing operations as recommended by the USDA.

2.6 Risk assessment of *L. monocytogenes*

It is important to identify which foods pose the greatest risk and identify which processes are most related to the safety of ready-to-eat foods. Over the past decade, the United States Department of Agriculture/Food Safety and Inspection Service (FSIS) and the Department of Human Health Services/Food and Drug Administration (FDA) have conducted several microbiological risk assessments to guide federal policies in an attempt to control and reduce listeriosis in the U.S. In 2003, the FDA and FSIS developed a quantitative microbial risk assessment to determine the relative risk of listeriosis among 23 categories of ready-to-eat foods to the total U.S. population and three age-based subpopulations (neonatal, intermediate-age, and elderly). This risk assessment showed that deli meats caused the greatest risk of listeriosis in the US, accounting for approximately 89% of all listeriosis cases per year (FDA/FSIS 2003).

In the following risk assessment, FSIS evaluated which processing practices were most effective in mitigating food safety risks associated with deli meats (FDA/FSIS 2003). This risk assessment revealed that using both growth inhibitors and post-lethality interventions was more effective than using either of these interventions alone or simply testing and sanitizing food contact surfaces. These findings formed the basis of FSIS's interim final rule for *L. monocytogenes* in ready-to-eat meat and poultry products in federal establishments (USDA 2003). In January 2004, the Food Safety and Inspection Service (FSIS) assembled a team to assess and measure the effectiveness of the new regulation (Interim Final Rule 9 CFR Part 430) to control *L. monocytogenes* in ready-to-eat (RTE) meat and poultry products. Later, FSIS completed another peer reviewed risk assessment that indicated that approximately 83% of the listeriosis cases attributed to deli meats were associated with deli meats sliced at retail (FSIS 2009; Endrikat et al. 2010).

2.7 *L. monocytogenes* in plants

2.7.1 Sampling

In order to verify the effectiveness of sanitation programs in establishments, sampling on food contact surface and other relevant environmental surfaces is required for Alternative 2 and Alternative 3, and recommended for Alternative 1. Under 9 CFR 430, an establishment with deli

and hotdog products in Alternative 3 must provide enough evidence for testing of food contact surface (FCS).

The establishment must provide FCS testing on all the identified sites that could contaminate product with a sample plan at a frequency no less than the recommended minimum sampling frequency. The minimum sampling frequency varies with the size of the plants, with 4, 2, and 1 sample per month per line for large volume plant, small volume plant and very small volume plant, respectively. FSIS recommends higher frequency of FCS testing in order to accumulate supportable data faster to ensure that the establishment's sanitation program is effective and appropriate to keep *L. monocytogenes* out of the production environment. The extra data would also further support that a plant is not producing an adulterated product and may help the plant to decide to reduce its FCS testing frequency at some point in the future. Some researchers have reported that the sampling program under this minimum sampling frequency is ineffective and suggested sampling program with more frequency. Nevertheless, more samplings imply more expense on *Listeria* testing and the benefit-cost ratio should be investigated to determine the optimum sampling program.

Besides the FCS testing in the establishment using Alternative 3, FSIS has continued a regulatory microbiological testing program on RTE meat and poultry products since 1983. With more understanding of the risk of *Listeria* in various food production establishments and under different food categories, FSIS amended the regulation on sampling program from 2004 to 2009. Before 2004, the establishments were randomly selected for regulatory samples from different sub-populations or from the total population of establishments producing RTE products.

FSIS initiated a new project identified as RTE001 in 2005 based on the continued project ALLRTE and RTERISK1. In this program, the sampling scheduled each month is requested from a list of establishments with the highest risk ranking for *L. monocytogenes*, which is based on several factors including the RTE Alternatives used by the establishments, the volume of production for post-lethality exposed products and the sample results from previous testing for *L. monocytogenes.* In 2006, FSIS implemented RLm (Routine *Listeria monocytogenes* Risk-based Sampling Program), phase 2 of *L. monocytogenes* risk-based sampling program aiming at detecting *L. monocytogenes* contamination from three types of samples: post-lethality

environmentally exposed RTE meat and poultry products, RTE food contact surfaces and noncontact environmental sources in conjunction with a comprehensive food safety assessment. With an incident where an unusual proportion of a product using growth inhibitor was found to be *L. monocytogenes* positive, FSIS modified RTE001, increasing the percent of samples scheduled in establishments reporting production of products with an antimicrobial or growth inhibitor but without post-lethality processing (Alternative 2b). Later, the RTE001 was incorporated into FSIS Directive 10,240.4, February 3, 2009. Directive 10, 240.4 provided a hierarchy of products to sample that divided in the deli products into those that are sliced in the inspected establishment and those shipped intact to be sliced at grocery store deli counters and other retail outlets.

2.7.2 Hold-and-Test program

In the FSIS's interim final rule in 2003 on the control of *L. monocytogenes* in ready-to-eat (RTE) meat and poultry products, most processors of RTE products are required to conduct microbiological testing of product contact surfaces. The rule states that establishments using antimicrobial agents or processes under Alternative 2 and establishments producing non-hotdog or non-deli products under Alternative 3 must identify the conditions under which they will implement hold-and-test procedures. "Hold-and-test" is a procedure that identifies the conditions under which the establishment will hold product pending test results following an *L. monocytogenes* or an indicator organism positive FCS test result. The rule describes the hold-and-test procedures to be followed by establishments producing hotdog and deli products under Alternative 3. Under Alternative 3, an establishment producing a hotdog or deli product that obtains a positive for *L. monocytogenes* or an indicator organism such as *Listeria spp.* in follow up testing on food contact surfaces must hold lots of product that may have become contaminated by the food contact surface and must sample and test these lots before release into commerce. In addition, establishments producing RTE products must identify conditions under which the establishment will implement hold-and-test procedures following a positive test for *Listeria spp.* or *L. monocytogenes* on a food contact surface.

FSIS provided the hold-and-test scenario flowchart which the establishments can directly use or develop their own hold-and-test scenario. This flowchart illustrates what an establishment could

do in case of a FCS testing positive for *Listeria spp.* or *Listeria-like* organisms, and when a follow-up FCS test is positive. The repeated positive FCS testing would imply an inadequacy of the sanitation system indicating that the establishments should investigate and reassess the sanitation program and the equipment layout to determine the cause of the contamination. When one FCS is positive, the establishment will take corrective action such as intensified cleaning and sanitizing, and test the FCS again. If another positive FCS occurs during the follow-up testing, the establishments must hold the applicable product lot and destroy or rework with a process destructive of *L. monocytogenes* if the products are positive for *L. monocytogenes*. The FCS should be tested until the establishment corrects the problem as indicated by the test result. If second FCS is found to be positive, the product on that day that the second FCS are available would be tested for *L. spp.* or *L. monocytogenes*; while the products during the testing period should be hold. Then, if the lots were positive for *L. monocytogenes*, destroy the tested product or rework products, and test the held products.

Figure 2-5 demonstrates the general process of hold-and-test which is used by most of the establishments using Alternative 2 and Alternative 3 (FSIS 2006). It looks effective in finding out the contaminated product lots and reducing the *Listeria spp.* on FCS by corrective action. However, two major flaws of this scenario would decrease the effectiveness of the hold-and-test sampling program. The products during the first FCS testing period have a great possibility to be contaminated with *Listeria* (if the FCS is positive) but they are released to the commerce without *Listeria* testing on the product lots. Although the second FCS positive may activate hold-and-test from that time on, the potential *L. monocytogenes* positive lots during the first FCS testing period are transferred to the market. Second, the occurrence of the contamination of *Listeria* on the FCS may result from the niches on the FCS, which is difficult to approach by the standard sanitation process. The niches may release *Listeria* periodically to the FCS and then contaminate the RTE foods. In this flowchart, the intensified cleaning and sanitizing are only applied on the day when the FCS was found positive. If there is no continued intensified cleaning and sanitizing, the FCS may be contaminated with *Listeria* on the next day because the harborage sites of *Listeria* continue spreading *Listeria*. To improve the effectiveness of this hold-and-test scenario, it looks reasonable to hold-and-test the product lots during the first FCS testing, and to continue intensified cleaning and sanitizing during the second FCS testing. This plant to consumption

model compared the difference between these two scenarios and suggested the optimal hold-and-test program in reducing the contamination of FCS and finding the positive FCS and product lots.

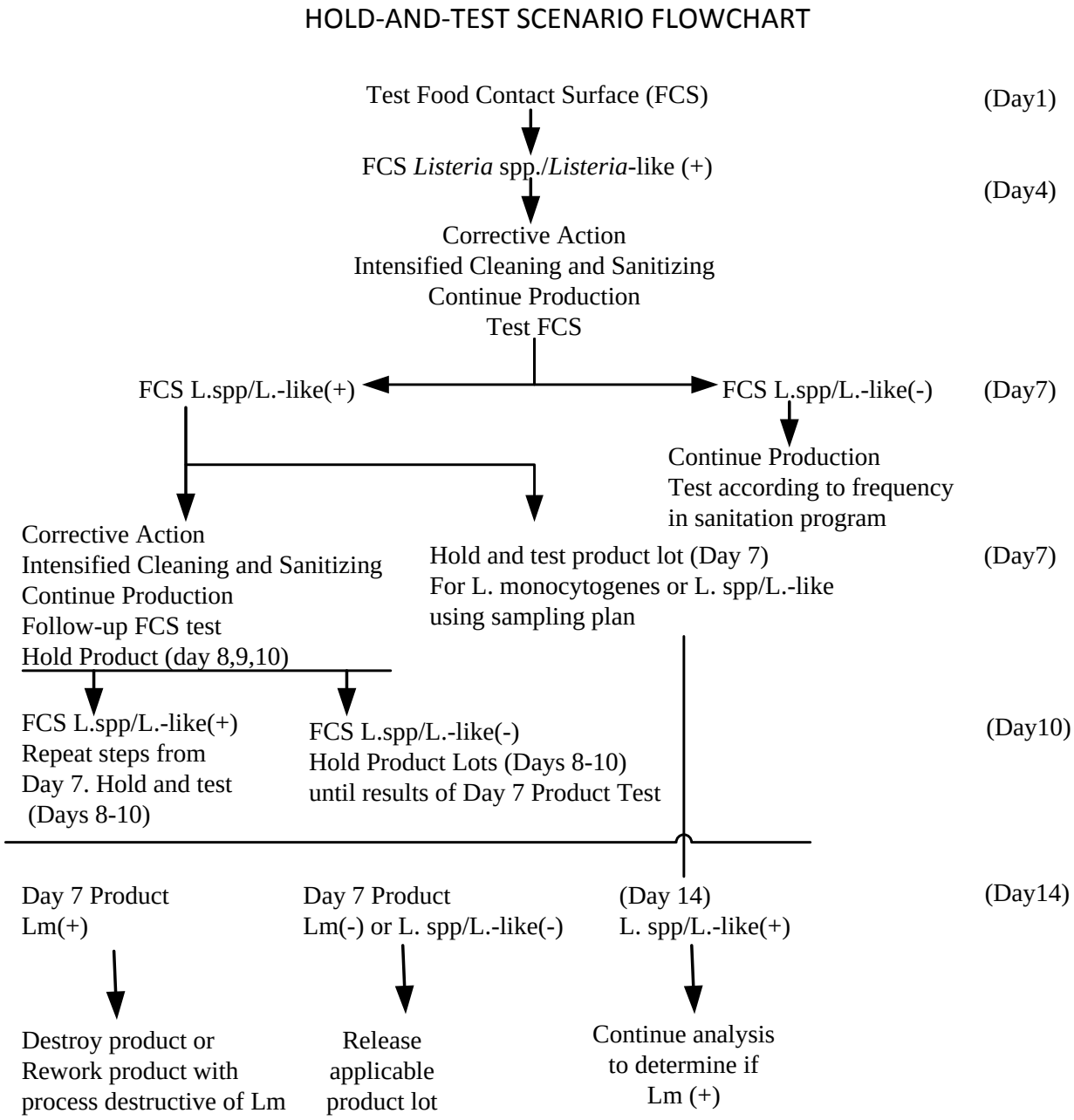

Figure 2-5. Hold-and-test scenario flowchart (FSIS 2006)

In order to provide effective and efficient mitigation strategies to reduce the risk of contracting listeriosis from the plant environment as the source, it is very important to understand the effectiveness of the current sampling and sanitizing program, and the effectiveness of the implementation of post-processing and growth inhibitors.

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Chapter 3 The Influence of Deli meats Sub-categorization on Risk Ranking for *Listeria monocytogenes* In Ready-To-Eat food

Abstract

In 2003, the Food and Drug Administration (FDA) and the USDA Food Safety and Inspection Service (FSIS) developed a quantitative assessment of the relative risk of *L. monocytogenes* in 23 ready-to-eat (RTE) foods to public health. Deli meats ranked as the riskiest food category, riskier than any other food categories. This study reassess the risk using newer concentrations data from a multi-state study and divides the deli meats category into four subcategories: prepackaged with growth inhibitor (GI), prepackaged without GI, retail-sliced with GI, and retail-sliced without GI. The relative risk based on the estimated deaths per annum and deaths per serving for each of the now 26 food category was ranked and compared with the previous risk ranking completed by FDA-FSIS (2003). The weighted median deaths per serving of the combined four deli meats was 74% of the median deaths per serving of the deli meats of original results, while those of non-deli meats foods increased by 191% to 537% because of the calibrated nature of the FDA-FSIS model. The median deaths per annum of deli meats decreased from 312 to 232 deaths per annum, while the median and mean deaths per annum for other food categories slightly increased with the use of new deli meats data but the relative ranking among non-deli meats food didn't changed. Deli meats still had the highest deaths per annum of any food category, but their rank for deaths per serving dropped for 1 to 3, below unreheated frankfurters and pate. Retail-sliced deli meats without growth inhibitors have the highest risk of *L. monocytogenes* based on both death per serving and death per annum, while other three deli meat categories pose much lower risk. The work illustrates the improvements that the deli meats processing industry has made and highlights the need for a more current and comprehensive risk ranking.

Keywords: risk assessment, listeriosis, risk ranking, deli meats, retail

3.1 Introduction

Since in the early 1980s, *Listeria monocytogenes* (*L. monocytogenes*) has been recognized as an important foodborne pathogen, most recently estimated to cause approximately 1455 hospitalizations, and 255 deaths each year in the United States (Scallan et al. 2011). According to the report by Centers for Disease Control (CDC) in 2000, *L. monocytogenes* has the second highest case fatality rate (21%) and the highest hospitalization rate (90.5%) among all the foodborne pathogens tracked by CDC (CDC 2000). Contaminated ready-to-eat (RTE) foods are the major vehicle of human listeriosis cases.

In order to understand the specific risk of foodborne *L. monocytogenes*, the Food and Drug Administration (FDA) and the Food Safety and Inspection Service (FSIS) developed a quantitative assessment of the relative risk to public health from *L. monocytogenes* among 23 categories of RTE foods in the United States. The results of the risk assessment, completed in 2003, indicated that deli meats cause the greatest risk for listeriosis, accounting for approximately 1,600 illnesses per year, approximately 89% of total listeriosis cases (FDA/FSIS 2003) believed to occur at that time.

Based on the results from this risk assessment, and in response to public comments on the FSIS proposed rule: Performance Standards for the Production of Processed Meat and Poultry Products (66 FR 12589), FSIS developed a risk assessment for *L. monocytogenes* in RTE meat and poultry products (FSIS 2003) that focused on federally inspected processing plants. The risk assessment model predicted that the use of antimicrobial growth inhibitors significantly lowered the public health risk of listeriosis. The addition of growth inhibitors has been adopted by significant parts of the RTE meat and poultry food processing industry (Endrikat et al. 2010). Additionally, improved equipment design and better sanitation programs have significantly reduced the *L. monocytogenes* prevalence at federally inspected facilities (Figure 3-1). Since about 2001, however, the incidence of listeriosis has remained relatively constant. Based on outbreak data from 1998-2008, Cartwright et al. have noted that the FSIS regulations and targeted sampling programs have reduced *L. monocytogenes* outbreaks associated with RTE meat and poultry over that time frame (Cartwright et al. 2013). More recent outbreaks have been due to FDA-regulated dairy products as well as fruits and vegetables, including sprouts, celery

and whole cantaloupes. Because of these industry changes, the results of the previous ranking may be out of date.

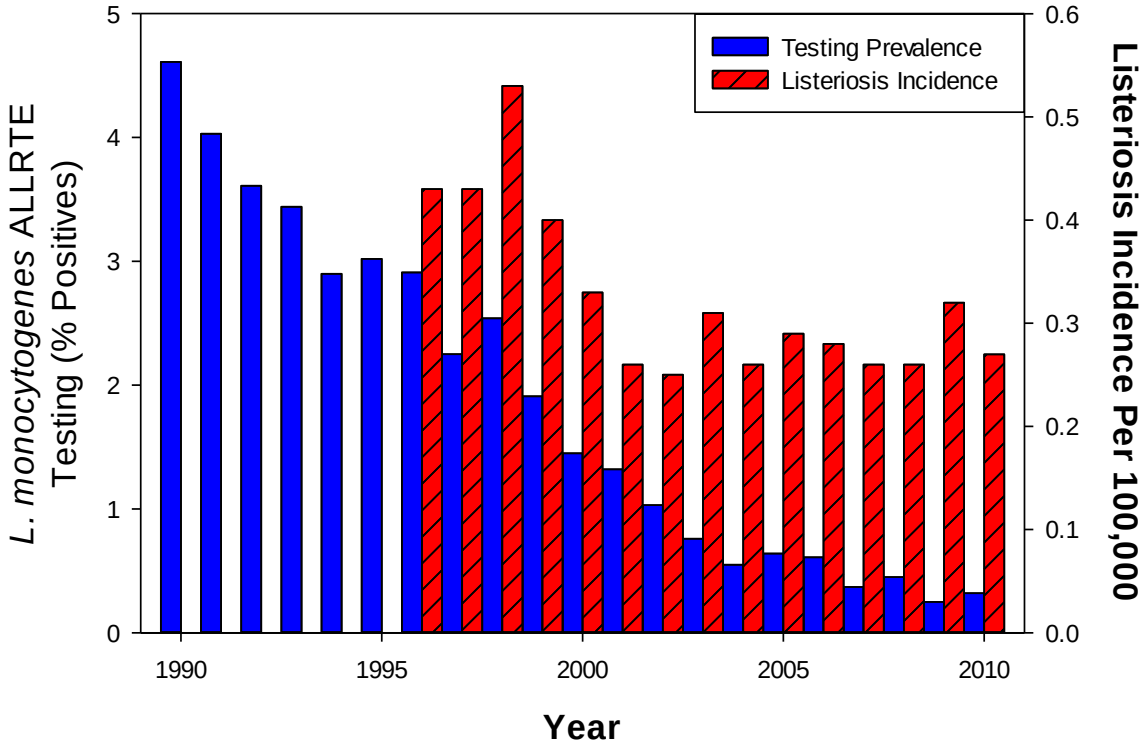

Figure 3-1. Percentage of RTE meat and poultry products testing positive for *L. monocytogenes* in FSIS inspected facilities compared to the incidence of listeriosis per 100,000 from CDC FoodNet surveillance.

(Source: http://www.fsis.usda.gov/Science/Micro_Testing_RTE/ and http://www.cdc.gov/foodnet/factsandfigures/2009/Table1b_all_incidence_96-09.pdf).

A preliminary analysis using *L. monocytogenes* contamination data for retail deli meats from California and Maryland by Gombas *et al*. indicated a significantly higher prevalence of *L. monocytogenes* in retail-sliced deli meats samples from retail markets compared with those prepackaged by the manufacturer, although the concentrations of *L. monocytogenes* were higher in prepackaged samples (Gombas et al. 2003). In order to enhance the reliability of this conclusion, a consortium of twenty-five research universities funded by the U.S. Department of

Agriculture (USDA) and Agricultural Research Service conducted an investigation involved with four states, analyzing the prevalence and level of *L. monocytogenes* for prepackaged deli meats and retail-sliced deli meats (Draughon 2006). Results of the risk assessment by Endrikat et al. indicates that of those listeriosis cases and deaths attributed to deli meats, approximately 83% are associated with deli meats sliced at retail (Endrikat et al. 2010).

Endrikat et al. conducted a comparative risk assessment for *L. monocytogenes* in prepackaged versus retail-sliced deli meats (Endrikat et al. 2010). By comparing the estimated deaths per annum and deaths per serving for four categories of deli meats (prepackaged with and without growth inhibitor, and retail-sliced with and without growth inhibitor), 69.8% of the estimated deaths occurred from retail-sliced deli meats that did not possess a growth inhibitor (Table 3-1).

Table 3-1. Use of Growth Inhibitor and Deli meats Associated Deaths for four Deli meats Subcategories.

Growth Inhibitor	Slicing Location, July 2007 (USDA personal communication based on form 10,240-1)			Percent of Deli meats Associated Deaths (Source: Data adapted from Endrikat et al. 2010)		
	Prepackaged	**Retail-sliced**	**Total**	**Prepackaged**	**Retail-sliced**	**Total**
With	32%	27%	59%	5.2%	13.2%	18.4%
Without	14%	27%	41%	11.7%	69.8%	81.5%
Total	46%	54%	100%	17.0%	83.0%	100.0%

While Endrikat et al. (2010) evaluated the impact subcategorization had on deaths from deli meats (Endrikat et al. 2010), the authors did not evaluate the broader context of how these deli meats subcategories compare to other RTE foods. The goal of this work is to conduct a revised risk ranking across all RTE foods by subdividing deli meats into four categories: with and without growth inhibitor and retail-sliced versus prepackaged, and compare the results to the previous model (FSIS 2003). The data for all other food categories remained unchanged.

Obviously, a subdivided category will have fewer deaths per annum than the parent food category. To evaluate deli meats as a single combined category in the newer model, the 4 deli

meat subcategories were also recombined using the fractions of slicing location and growth inhibitor use reported in Table 3-1.

3.2 Materials and methods

The basic risk assessment model was described in FDA-FSIS (2003), which follows the widely accepted framework that separates the assessment activities into four components: hazard identification, exposure assessment, hazard characterization (dose-response assessment), and risk characterization. The model was developed in Microsoft Excel using Visual Basic for Applications (VBA).

The overall model approach is illustrated in Figure 3-2. During the exposure assessment, a *L. monocytogenes* concentration at retail is developed for each food category. Growth during consumer storage is based on the category's specific growth rate, storage time and temperature, and maximum achievable concentration. Resulting concentrations at consumption are combined with serving sizes to calculate a dose. The dose-response portion of the model divides the population into neonates, intermediate age, and elderly to account for differing listeriosis susceptibility. After the dose distributions have been calculated for all the food categories, the total number of deaths for a given age group is estimated across all the food categories. An important aspect of the FDA-FSIS model that impacts the interpretation is the calibration of the scaling factor of the dose response model. The dose response curve is shifted by this scaling factor such that the total number of deaths within the age group meets a specified target. The elderly target, for example, was 307 deaths per year. Every uncertainty iteration met this target, i.e. there was no uncertainty in the number of deaths. The uncertainty was subsumed into the scaling factor distribution.

This calibration process is important because it requires that if one food category becomes less risky, the other food categories must become more risky to meet the targeted number of deaths. This may be appropriate for a risk ranking only, because in effect the ranking is based of dose in the food category. It implies, however, that it is difficult to truly consider the uncertainty in the public health impacts.

For this work, the results of the hazard identification were changed from 23 ready-to-eat categories to 26 categories by separating the deli meats into prepackaged deli meats with and without growth inhibitors and those sliced at retail with without growth inhibitors. Since categories and data for other foods were the same with those in FDA-FSIS (2003), only the revised model for deli meats were described in the following paragraphs.

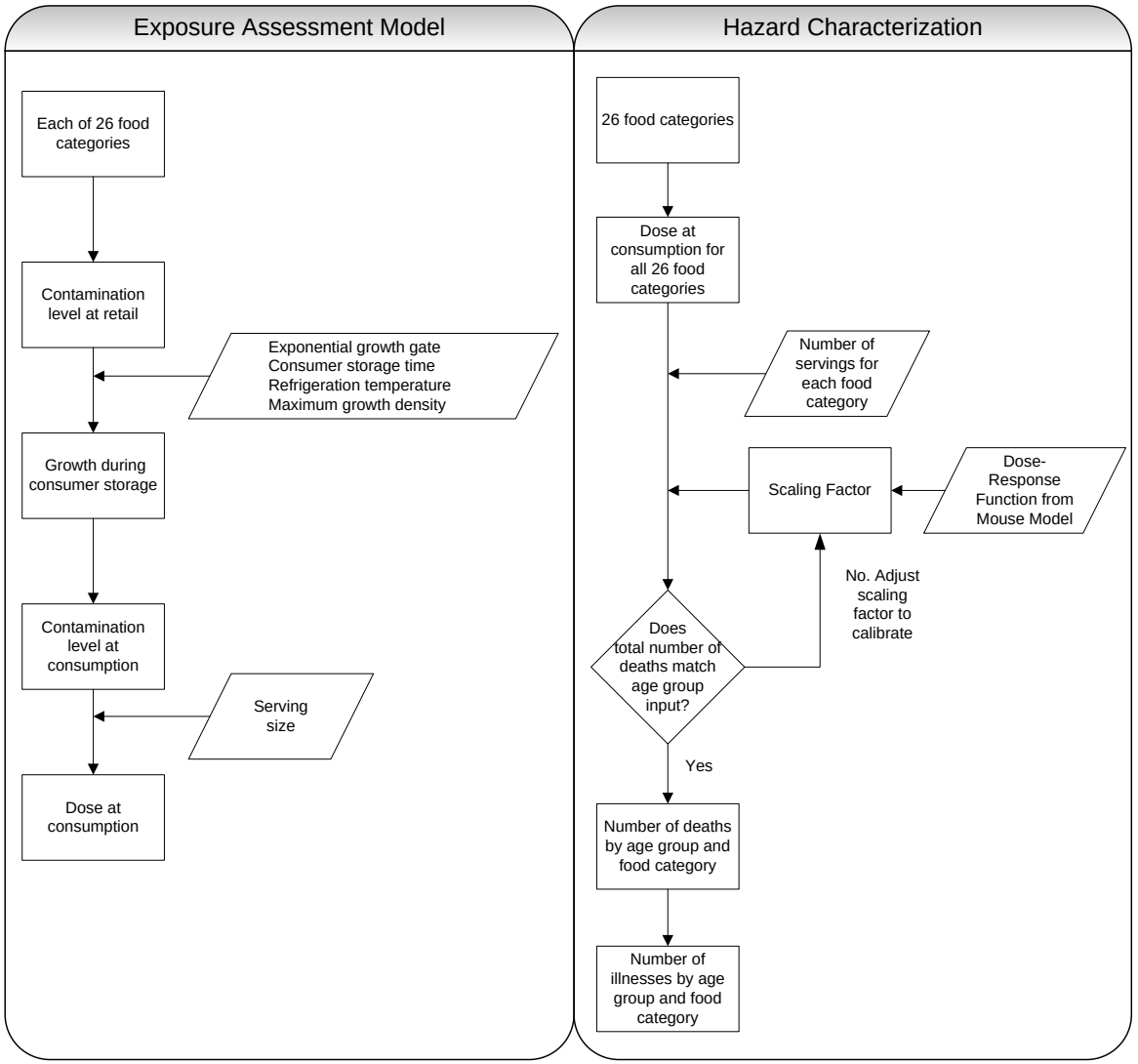

Figure 3-2. Flow chart for FDA-FSIS (2003) risk ranking model,

3.2.1 Retail occurrence

The retail stage determines the prevalence and level of *L. monocytogenes* in the two deli meat types (retail-sliced versus prepackaged) at the time they were transported into the retail stores. The prevalence and level of *L. monocytogenes* in deli meats at retail establishments were determined using data from a National Alliance for Food Safety and Security (NAFSS) study (Draughon 2006) in which 6 of 3,522 (0.17%) samples and 49 of 3,518 (1.39%) samples tested positive for *L. monocytogenes* from prepackaged and retail-sliced deli meats, respectively. In the above study, the samples were collected from four designated sites (northern California, Georgia, Minnesota and Tennessee) in the Foodborne Disease Active Surveillance Network (FoodNet). The two different *L. monocytogenes* concentrations at retail for prepackaged and retail-sliced were fitted to probability distributions, which then served as inputs to risk assessment model. The best-fit distributions were log normal. The retail-sliced product parameters were -11.1 ± 4.04 log MPN/g, while the parameters for the prepackaged product were -11.9 ± 3.39 log MPN/g.

3.2.2 Growth

The growth stage uses a modified exponential growth rate to account for antimicrobial growth inhibitor usage to predict growth of *L. monocytogenes* in deli meats between retail and consumption. An exponential growth rate of *L. monocytogenes* was used in the growth stage to simulate growth from retail to consumption. Within the exposure assessment model, the growth rate was treated as a stochastic input parameter. It was adjusted for stochastic temperature by using a square-root model (Ratkowsky et al. 1982; FDA/FSIS 2003). More current manufacturer production volume data were used to calculate the fraction of deli meats in each category (Table 3-1).

Exponential growth rates for *L. monocytogenes* were calculated for product with and without antimicrobial growth inhibitors by using data from the 2003 FDA/FSIS risk assessment and the estimated fraction of deli meats with and without inhibitors in 2003, i.e., before Interim Final Rule 9 CFR 430 (FSIS 2003). The 2003 ranking used a mean exponential growth rate at 5 ℃ (EGR5) of 0.282 log cfu/g/d. Based on the regulatory requirement to qualify as a growth inhibitor, Endrikat et al. (2010) used a weighted log linear equation to estimate the EGR5 for deli

meats with and without growth inhibitor to be 0.143 and 0.311 log cfu/g/d respectively (Endrikat et al. 2010). The overall growth rate of *L. monocytogenes* is lower after the implementation of the Interim Final Rule, because the composition of the product is different—a greater fraction of product contains antimicrobial growth inhibitors.

Consumers store deli meats for different time periods depending on whether they are prepackaged or retail-sliced. Storage times for each category were taken from Pouillot et al. (2010). Storage temperature was assumed the same for all deli meat subcategories, and also taken from Pouillot et al. (2010).

3.2.3 Consumption and dose-response

The consumption stage predicts the *L. monocytogenes* exposure dose consumed in servings of deli meats, which results from the serving size and the number of servings. The dose-response stage predicts the probability of illnesses and death from *L. monocytogenes* per serving by integrating the predicted exposure distribution with a dose-response relationship. It is generated by relating the effects observed in mice to the effects of *L. monocytogenes* in humans by using an appropriate scaling factor. The dose-response model was conducted on the populations of three age groups: neonatal (16 weeks after fertilization to 30 days after birth), intermediate (older than 30 days and younger than 60 years) and elderly (60 years old or older).

3.3 Results and discussion

Using the new data of the prevalence and the EGR5 of *L. monocytogenes* in four subcategories of deli meats, risk assessments for *L. monocytogenes* in the 26 categories (deli meats was treated as four separate categories according to where they were sliced and whether growth inhibitors were used) of RTE food were conducted on the model of FDA-FSIS (2003). The estimated median deaths per annum and deaths per serving across the various food groups for the new simulations are displayed in Figure 3-3, which illustrates the values for both the individual deli meats subcategories and the combined value weighted by the number of servings.

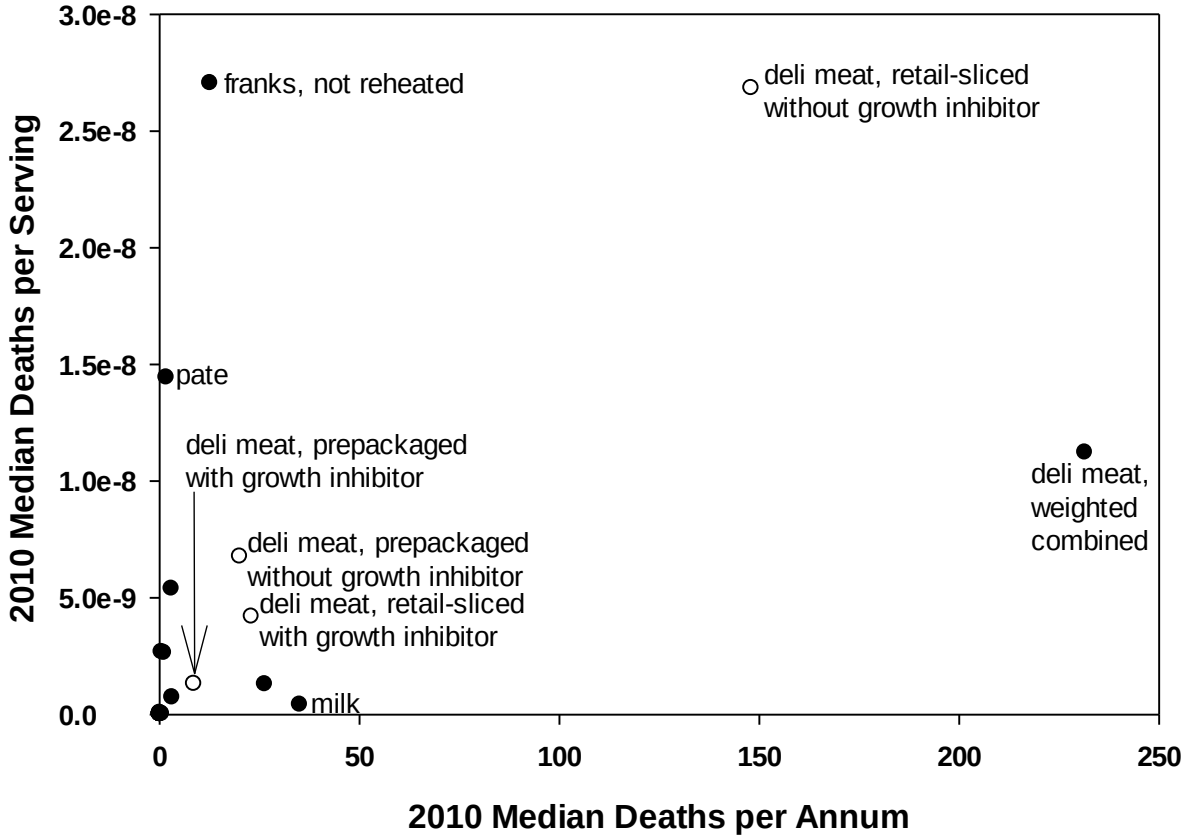

Figure 3-3. The comparison of estimated median deaths per annum and median deaths per serving across the various food groups. Open circles indicate the individual deli meats subcategories.

Overall, the combined deli meats category caused the most *L. monocytogenes*-related deaths per annum of any RTE foods. When deli meats were considered as four subcategories, the retail-sliced deli meats without growth inhibitors were associated with significantly higher deaths per annum and per serving than the other three subcategories. Despite accounting for only 27% of deli meats servings and 1.6% of all RTE servings, this subcategory accounted for 47% of deli meats associated deaths and 42% of deaths across all 26 categories. The death per serving was approximately equal to franks, not reheated. The risk for the other three deli meats fell below the pasteurized milk and high fat dairy based on the deaths per annum and below not reheated frankfurters and pate based on the deaths per serving. On a subcategory basis, the per annum risk

rankings (from greatest to least) were 1: retail-sliced deli meats without growth inhibitors, 2: milk, 3: high fat dairy, and 4: retail-sliced deli meats with growth inhibitors. The per serving risks rankings (from greatest to least) were: 1: unreheated frankfurters, 2: retail sliced deli meats without growth inhibitors, 3: pate, and 4: prepackaged deli meats without growth inhibitors.

3.3.1 Comparison of risk rankings between this study and FDA-FSIS (2003)

Figure 3-4 compares the risk ranking of this study with that of FDA-FSIS (2003) on a per annum and a per serving basis. Some apparent changes can be found. The total mean number of deaths across all food categories was constant for both versions at 414 deaths because of the calibration. The median number of deaths, in contrast, decreased from 351 to 316 between the two runs. The median deaths attributed to deli meats dropped even more, from 311 to 231 deaths. Median deaths for the non deli meats category increased by 45 between the two models, even though data for these food categories did not change.

The median per serving risk of the combined four deli meats was 74% of the median deaths per serving of the deli meats of original results, while those of non-deli meat foods increased by 190% to 530% because of the calibrated nature of the FDA-FSIS model. While deli meats ranked highest for deaths per annum in both models, the risk per serving actually changed. Deli meats had the highest risk per serving in the original model, while the newer model ranked deli meats third on a per serving basis, below unreheated franks and pate. This demonstrates that the new risk ranking implied the effectiveness of food safety regulatory agencies and the manufactures on deli meats in recent years.

Since the data used in the model for other food categories were the same with those in the FDA-FSIS (2003) and only recent data for deli meats has been changed, the incorporation of new deli meat data decreased the risk of deli meats but increased the risk of other RTE foods because of the calibration within the model. This is illustrated by all the food categories except deli meats falling above the 1:1 reference line in Figure 3-5a and b.

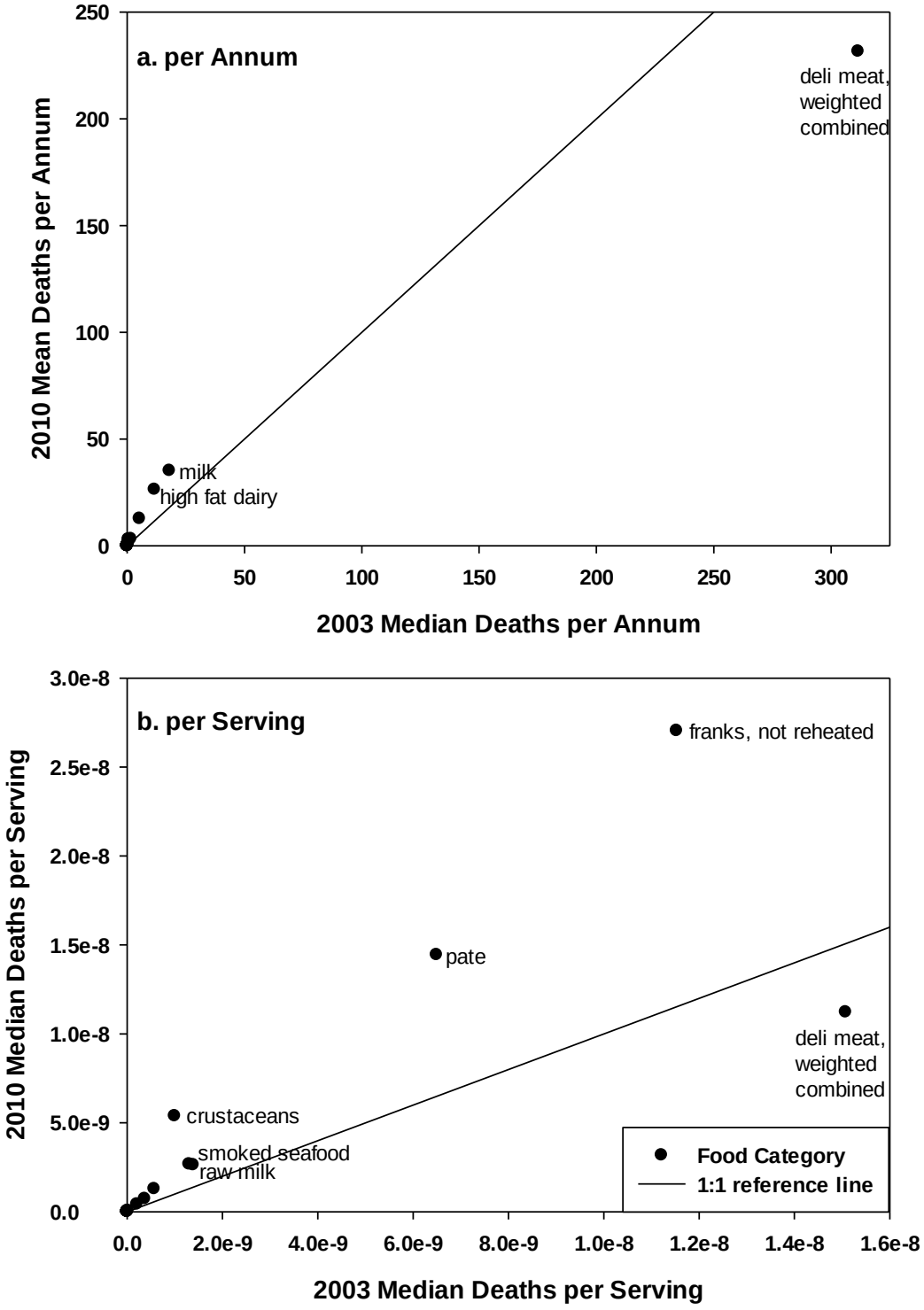

Figure 3-4. The comparison of median deaths per annum (a) and median deaths per serving (b) of this study with that of FDA-FSIS (2003).

3.3.2 Comparing the uncertainty distributions

In both 2003 and 2010 risk assessment, 4000 uncertainty runs were carried out and the means of the death per annum for all the food categories were calibrated to 414 by adjusting the scale factors in the dose-response model. However, the distribution of the uncertainty runs and the mean of deaths per annum for each food category would be different between the two runs. Figure 3-5a illustrates the probability density of deaths per annum for deli meats for the 4000 uncertainty runs of 2003 and 2010 risk assessments. The 2003 distribution is left skewed, but sharply peaked near the target total deaths. The curve of 2010 is also left skewed but has two peaks at about 70 and 250 because it is a sum of the 4 smaller categories, 3 of which have much lower risk than the retail-sliced without growth inhibitor. The drop in medians discussed above can clearly be seen.

In contrast, the uncertainty distributions for milk are shown in Figure 3-5b. Since the total numbers of deaths per annum were the same for both assessments, the deaths caused by *L. monocytogenes* in other food categories would be higher in this study due to the decrease of deaths caused by *L. monocytogenes* in deli meats. The uncertainty distribution for milk is shifted rightward, indicating a higher number of deaths in the more modern ranking. The actual median deaths increased from 18 to 35 between the two runs.

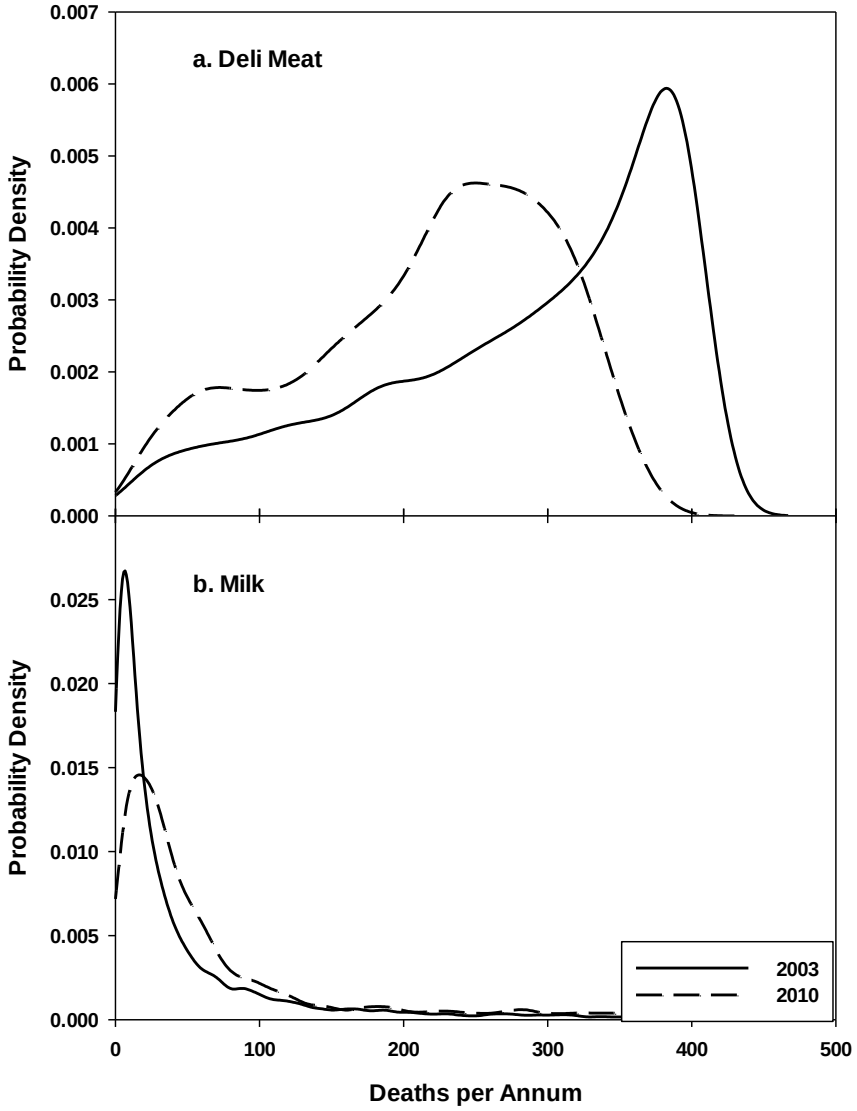

Figure 3-5. The 2003 versus 2010 uncertainty distribution of deaths per annum caused by combined Deli meats (a) and Milk (b) for 4000 uncertainty runs.

3.4 Discussion

From the above results, the prepackaging and application of growth inhibitor in deli meats played important roles in the risk assessment of *L. monocytogenes* in RTE foods. This agrees with Pradhan et al.'s finding that reformulation of deli meats with growth inhibitors could reduce listeriosis by a factor of 2.8 to 7.4 in the number of human listeriosis cases for a given deli meats

(Pradhan et al. 2009). Either control measure in the producing of deli meats, prepackaging or growth inhibitor, would reduce the risk by at 75% in the estimated deaths per serving or deaths per annum, with prepackaged deli meats with GI much lower risk than other three sub-categories of deli meats, especially retail-sliced deli meats without GI. Prepackaged deli meats have lower risk than retail-sliced deli meats, no matter with GI or without GI, so the process at retail level increased the population of *L. monocytogenes* and risk associated with *L. monocytogenes*. Cross-contamination between contaminated products to uncontaminated products by slicers was found to be the main reason for the spreading of *L. monocytogenes* (Lin et al. 2006; Vorst et al. 2006). Since retail-sliced deli meats without GI ranked the top in all the food categories, more attention should be paid to the deli meats sliced at retail stores and the transportation pattern of cross-contamination in retail store should be identified in order to control the prevalence of *L. monocytogenes* in retail-sliced deli meats and reduce the risk of *L. monocytogenes* associated cases caused by deli meats.

As with previous studies (Pradhan et al. 2009; Endrikat et al. 2010), this analysis clearly indicates the importance of growth inhibitors and the need to control cross contamination for retail-sliced product. It also reinforces the need for continued regulatory encouragement for growth inhibitor usage. The ability to subdivide broad food categories into more internally homogenous subcategories allows limited regulatory efforts to be better focused.

Clearly there are limitations to this analysis. The time frame for data collection between deli meats and other food categories is different, and non deli meats products may have undergone similar improvements. But the near constant incidence rate for listeriosis would imply that either this has not happened or that new RTE food categories need to be considered. The results do indicate the need for a revised ranking analysis that incorporates additional food categories using newer data for all RTE foods.

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Chapter 4 Plant-to-consumption Model on the Effectiveness of *Listeria monocytogenes* Control Interventions in RTE Plants

Abstract

The Centers for Disease Control and Prevention (CDC) have estimated that up to 1,455 cases of listeriosis, resulting in 255 deaths, occur each year in the United States associated with the consumption of contaminated food. A previous risk ranking found that consumption deli meats posed the greatest risk of listeriosis in the US, accounting for approximately 89% of all foodborne listeriosis cases per year. Because *Listeria* can survive and grow at storage temperature (<4 ℃), source control of *L. monocytogenes* in the food processing plant environment is a critical step in risk management. FSIS has developed Interim Final Rule 9 CFR Part 430 for *L. monocytogenes* in ready-to-eat (RTE) meat and poultry products in federal establishments according to a plant-to-consumption risk assessment. The food establishments that product post-lethality exposed RTE meat and poultry product must choose one of the three process alternatives to reduce the risk of *L. monocytogenes* contamination and growth. These alternatives emphasize post-processing lethality and the use of growth inhibitors. Although the introduction of Interim Final Rule played an important role in controlling the prevalence of *Listeria* in RTE food, there is still room for improvement, including more effective sampling processes and a more adequate sanitizing program. This work developed a plant-to-consumption model for *L. monocytogenes* that incorporates and compares these alternatives as well as other control interventions. This plant-to-consumption model analyzed the effectiveness of the three alternatives on reducing the *Listeria* in the RTE food products and also investigated the optimal sampling and sanitizing program. Results showed that formulation of food products with a growth inhibitor has the greatest impact on reducing the risk of *L. monocytogenes*, followed by post-processing lethality and sanitation programs. There is great potential to reduce the risk of *L. monocytogenes* by changing production practices: according to the model, using growth inhibitors results in a 70% reduction in listeriosis and using growth inhibitor along with post-processing treatment result in 91% reduction in listeriosis.

Keywords: *Listeria monocytogenes*, sampling, growth inhibitor, ready-to-eat deli meats, risk assessment

4.1 Introduction

Foodborne illness caused by *Listeria monocytogenes* is a serious issue to public health due to its high hospitalization rate (90.5%) and mortality (20%) (CDC 2009; Rocourt 1999). Since *L. monocytogenes* can be easily eliminated during high temperature processes, ready-to-eat (RTE) foods consumed without further cooking become the main vehicle for the spread of this pathogen. According to the risk assessment performed by the FDA and FSIS/USDA, deli meats result in the highest risk of *L. monocytogenes* to public health among all other RTE food categories (FDA/FSIS 2003). *L. monocytogenes* is a pyschrotrophic pathogen that can grow in the food-processing environment and consumer home under low temperatures (less than 4℃), contamination of *L. monocytogenes* in RTE food from the plant environment could result in extremely high concentrations of pathogen cells in the food at consumption. So the processes before packaging of RTE meat and poultry products are the critical control point for *L. monocytogenes*.

Many predictive and quantitative microbial risk assessment models have been developed in order to guide the decision making on food safety and risk management on *L. monocytogenes* (Haas et al. 1999; Augustin et al. 2000; Bovill et al. 2000; FSIS 2003b). Schaffner developed a mathematical framework based on simplified parameters (transferability, persistence and cross-contamination rate) for modeling *Listeria* cross-contamination in food processing plants (Schaffner 2004). However, limited data available for fitting into the model restrained application of this model. During the processes at retail such as slicing and packaging, Endrikat et al. found there was significant difference in the *L. monocytogenes* prevalence between the prepackaged and the retail sliced deli meats (Endrikat et al. 2010). To assess the cross-contamination at the consumers' home, Yang et al. used one-dimensional Monte Carlo simulation to develop the risk assessment focusing on the consumer handling practices in the home (Yang et al. 2006) and Zhao et al. developed the cross-contamination model in the kitchen (Zhao et al. 1998).

Listeria species (spp.) are more likely to be transferred to food workers through contact with raw foods in the food processing plant (Kerr et al. 1993) and then to other food contact surfaces (FCS). It is important to maintain sanitary condition of the FCS through adequate sanitation

processes. In 2003, FSIS developed a preliminary model to evaluate the effectiveness of FCS testing. The report concluded that post-processing treatment was more effective, and, while not fully analyzed, growth inhibitors might prove the most effective. FSIS used this to develop Interim Final Rule, 9 CFR Part 430, which required all establishments that produce post-lethality exposed RTE products must choose one of the three alternatives to maintain sanitary conditions (Figure 4-1). For high risk foods such as RTE meat, Alternative 1 is preferred which combines a post-lethal decontamination step along with formulation using growth inhibitor. In Alternative 2 the establishments is offered the option of applying a post-lethal decontamination step (Alternative 2a) or formulation with growth inhibitor along with *Listeria* sanitation program (Alternative 2b). Finally, Alternative 3 relies on having an effective sanitation program along with end-product testing. This is generally recommended to low risk foods (for example, salad vegetables), however, if used for RTE meats a hold-and-test policy must be enforced. FSIS regulates large, small, and very small plants differently, and the testing requirements thus vary by plant size.

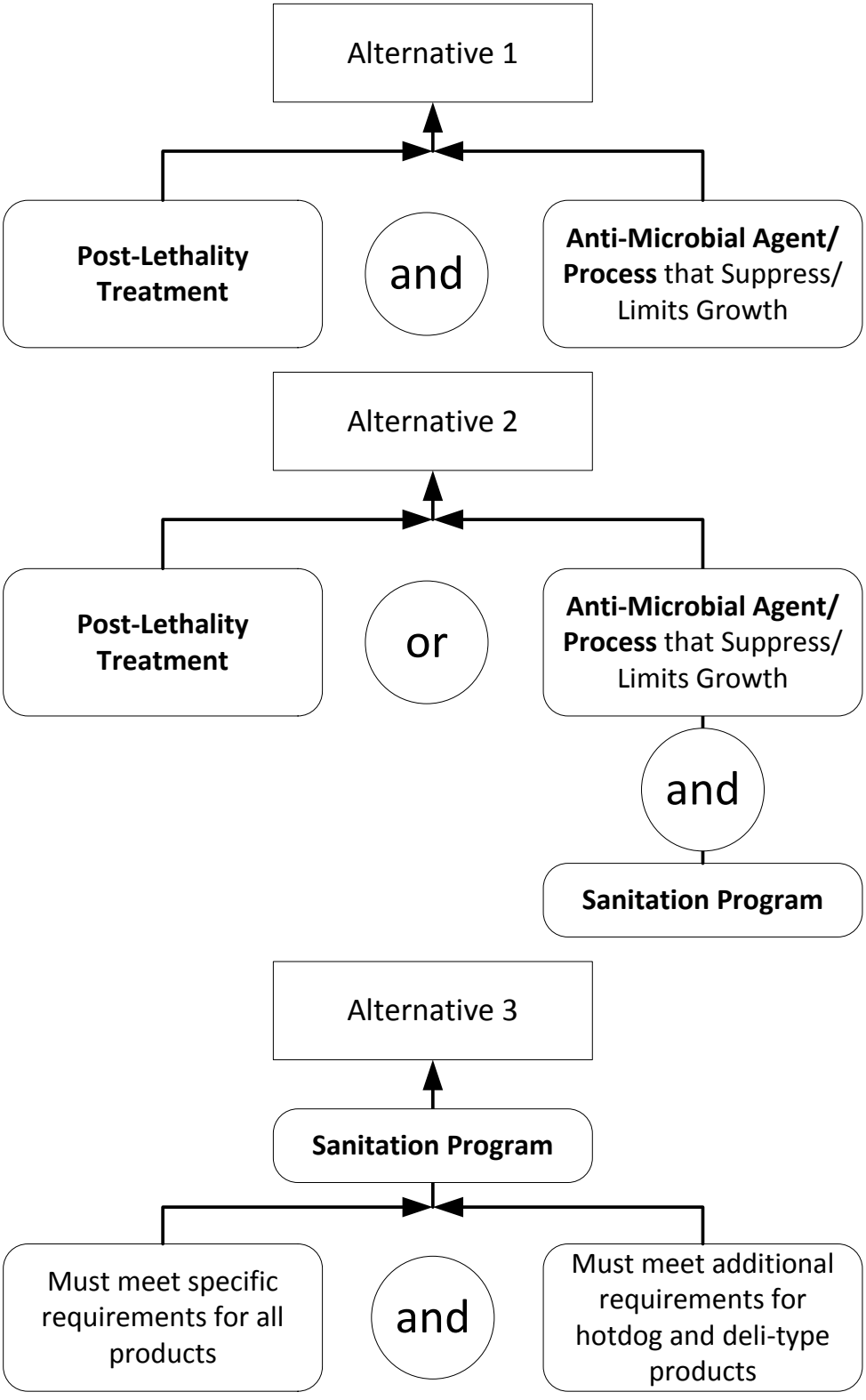

Figure 4-1. Alternatives to control *L. monocytogenes* in ready-to-eat food processing operations as recommended by the USDA.

Until now, no single complete model has been developed integrating all the steps from the food processing plant to the time of consumption. This study developed a comprehensive model that incorporated considerable components in the food production processes, including contamination at plants, post-processing intervention, growth inhibitor treatment, sampling and sanitation at plants, cross-contamination at retail and growth during storage time.

The objectives of this work are to: 1) develop a comprehensive risk assessment model of *L. monocytogenes* in RTE meat and poultry product from the plant to the time of consumption; 2) determine the effectiveness of the three alternative interventions in reducing *L. monocytogenes* contamination in finished RTE product, and the subsequent risk to public health; 3) analyze the most influential factors on the effectiveness in the interventions.

4.2 Model development

4.2.1 Model features

The plant-to-consumption model was developed in the statistical programming language R (R Core Team, 2011). It is based on a dynamic model that predicts *L. monocytogenes* concentrations at different stages in the food distribution chain, including after post-processing, arriving at retail, leaving retail and at the time of consumption (Figure 4-2). The bacterial concentrations on the food contact surface and in each lot of RTE product over time, as well as the resultant risk caused by these bacterial represented by illness per serving, are predicted in the dynamic model. The model is based on a first order Monte Carlo simulation, in which many of the parameters used in the model are stochastic random variables based on the distributions of these variables derived from the literatures or reasonable assumptions.

A mass balance approach is used as the basis of the plant-to-consumption model. The number and disposition of *Listeria* organisms are tracked for both food contact surface area and the product over time. *Listeria* organisms originate from the harborage sites that serves as sources, move on to the food contact surface, transfer to the product, grow during storage and transportation, cross-contaminate at retail and finally are consumed at home. Deli meats were treated as the weighted combination of the three largest deli meats by sales: turkey, ham and beef. Each had their own specific growth rates and lag times, which were influenced by whether

the product contained growth inhibitor or not. During the process, the organisms may die-off or be removed by sanitation, grow at different growth rates, or be discarded by the consumers when concentration reaches the maximum limit (spoilage). The plant-to-consumption model incorporates food contact surface testing, product testing, sanitation, pre- and post-packaging interventions, growth inhibitors, cross-contamination at retail and growth during storage. Risk estimation of *L. monocytogenes* is represented by the illnesses per serving of RTE product and the annual illnesses from the dose-response model. The resulting predicted risk of *L. monocytogenes* thus incorporates the comprehensive effects of all the possible interventions.

Because *L. monocytogenes* is considered an adulterant, any positive finding at a food processing plant has regulatory implications. Many plants prefer to test for environmental and FCS contamination based on *Listeria* species, (*L. spp.*) instead. These results can indicate the need for enhanced sanitation by the plant without regulatory implications, and the FCS testing proposed by FSIS is based on *L. spp.* rather than *L. monocytogenes*. Thus the model tracks *L. spp.* within the plant and switches to *L. monocytogenes* only at retail.

The key input parameters and data sources for to the model are provided in Table 4-1.

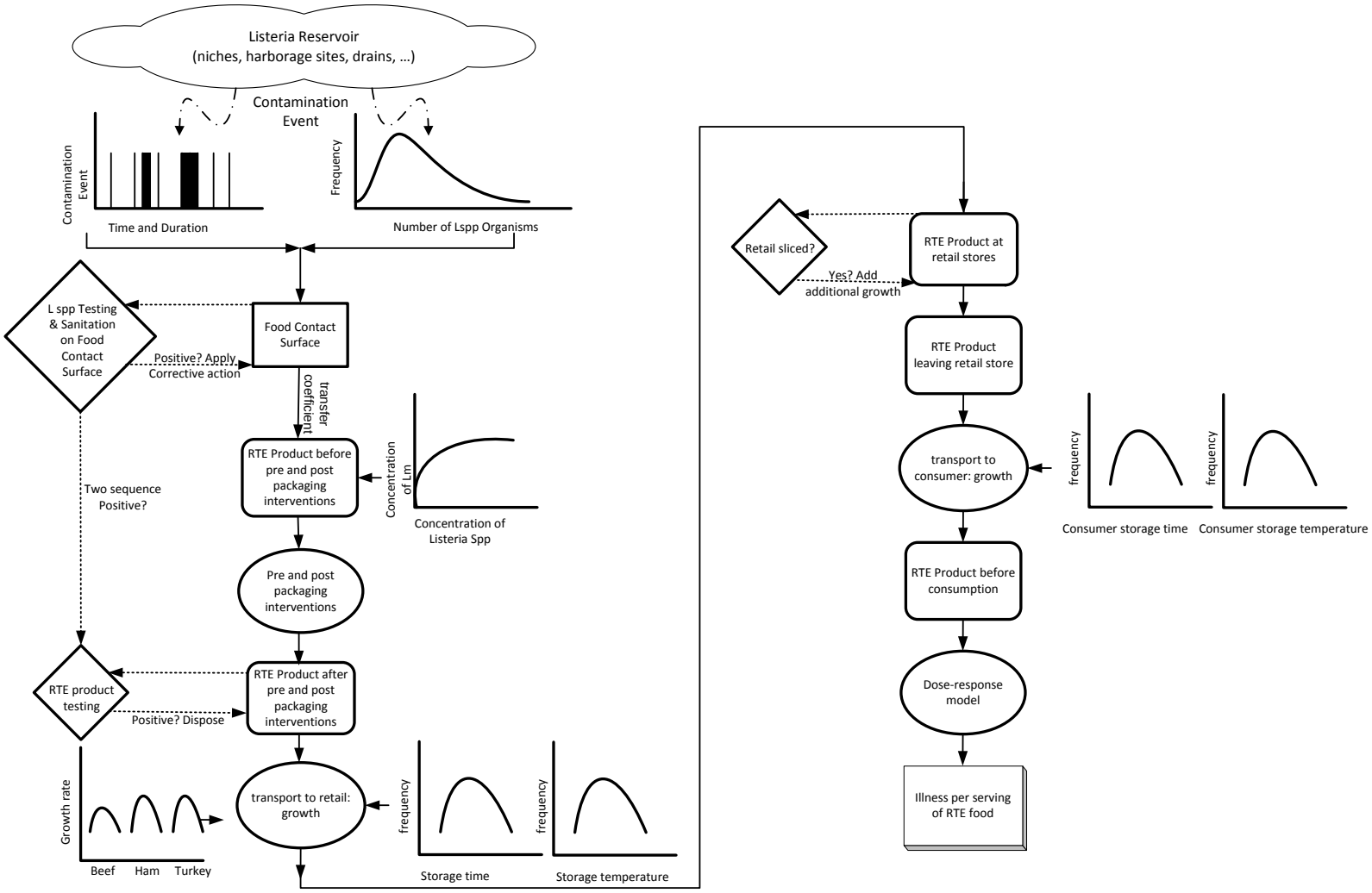

Figure 4-2. Flowchart of the plant-to-consumption model

Table 4-1. Key input parameters in the model

Parameter and Data Source	Value	Units
Contamination event frequency (*Listeria* species prevalence data taken from an FSIS in-depth verification)	normal(1.077, 0.456)	$\log_{10}$ days
Contamination event duration (Tompkin (2002) - number of plants with successive weekly positive *Listeria* food contact surfaces.)	normal(0.602, 0.573)	$\log_{10}$ days
Daily added concentration during contamination event (Calibrated by FSIS plant data. See Methods section)	normal(-6.3, 2.6)	$\log_{10}$ cfu/cm^2
Lot mass (FSIS RTE survey results (FSIS 2003b)	Large: normal(8787, 6350) Small: normal(3221, 4808) Very small: normal(1270, 4309) Truncated to minimum of 454	kg
Sanitation timings (Assumed)	between shift and at the end of the day	
Sanitation effectiveness (Assumed)	0.9, 0.95, 0.999 for between lots, between days and enhanced sanitation respectively	
Transfer coefficient (Hoelzer et al. 2012)	$\log_{10}$ normal (-0.28, 0.20) Truncated to maximum of 1	
Ratio of *L. monocytogenes* to *Listeria spp*. (Tompkin 2002)	N(0.52, 0.26) Truncated to minimum of 0 and maximum of 1	
Post processing lethality efficiency (Food Code 2009)	0.99	
EGR @ 5^{o}C product without GI (Pradhan et al. 2009)	Turkey: logistic(0.2755, 0.0723) Ham: logistic(0.1941, 0.0472) Beef: logistic(0.2722, 0.0646)	$\log_{10}$ cfu/g/day
EGR @ 5^{o}C product with GI (Pradhan et al. 2009)	Turkey: logistic(0.0975, 0.0253) Ham: logistic(0.1065, 0.0282) Beef: logistic(0.1258, 0.0517)	$\log_{10}$ cfu/g/day
Lag times, product without GI (Pradhan et al. 2009)	Turkey: triangular(0.46, 0.46, 5.55) Ham: triangular(0.40, 0.40, 16.94)	days

	Beef: triangular(2.68, 2.68, 22.81)	
Lag times, product with GI (Pradhan et al. 2009)	Turkey: triangular(2.39, 2.39, 23.87) Ham: triangular(6.11, 6.11, 34.62) Beef: triangular(1.12, 1.12, 13.06)	days
Fraction of deli meats (IDDBA 2009)	Turkey: 0.45 Ham: 0.41 Beef: 0.14	
Sampling frequency (FSIS's minimal frequency under Interim Final Rule, by alternatives)	4, 2, 1 times/shift/plants for large, small and very small plants, respectively	
Sample mass	25	grams
Consumer storage time (Pouillot et al. 2010)	Retail-sliced: weibull(1.830, 7.777) Prepackaged: weibull(1.137, 18.39)	days
Consumer storage temperature (Pouillot et al. 2010)	logistic(40.15, 3.193)	^{o}F
r parameter (FAO/WHO risk assessment)	Healthy: 2.41e-14 Susceptible: 1.05e-12	
Proportion of susceptible and non- susceptible population (FAO/WHO risk assessment 2004)	Susceptible: 0.175 Healthy: 0.825	
serving size (FDA-FSIS 2003)	Empirical cumulative serving size from 0.00 to 648	grams

4.2.2 Contamination in plant

Contamination frequency. This model assumes that *Listeria* species move from the reservoir in the environment onto the food contact surface during a "contamination event" in the plants. The key parameters defining a contamination event are composed of the frequency of the event, the duration of the event, and the amount of *Listeria spp* transferred from the reservoir to the food contact surface.

Duration. The frequency of a contamination event was estimated based on time series *Listeria* species prevalence data taken from an FSIS in-depth verification conducted in a plant that was associated with an *L. monocytogenes* outbreak in humans. The data were analyzed using survival analysis and distribution fitting using NCSS statistical software. Based on this analysis, the data was found to best fit the lognormal distribution with the estimated mean and standard variation. The duration of a contamination event was estimated based on sequential weekly *Listeria* species testing results from Tompkin (2002). This data provided the number of consecutive weeks that *Listeria* species positives persisted during the weekly testing, allowing the duration of a contamination event to be estimated. This data was also fit to a lognormal distribution for model simulation based on the maximum likelihood fit as determined using survival analysis and distribution fitting.

Contamination levels. As there was no reported literature available to estimate the *Listeria spp.* transferred from a harborage site to a food contact surface during a contamination event, the model was calibrated so that the distribution of *Listeria spp.* concentration on food contact surfaces matched FSIS surveillance data (LaBarre, personal communication) of the concentration of *L. monocytogenes* on the products in plants. During a contamination event, the plant-to-consumption model increases the concentration of *Listeria spp.* on the food contact surface by a stochastic amount for each RTE lot simulated to account for the transfer of organisms from the harborage site to the food contact surface.

4.2.3 Contamination from FCS to Lots

Transfer coefficient. The amount of *Listeria species* transferred from the food contact surface to the RTE product were assumed to be mainly influenced by the transfer coefficient for *Listeria species* and the effectiveness of plant-to-consumption sanitation procedures. The transfer coefficient ranges from 0 to 1 and indicates the fraction of *Listeria species* transferred from the food contact surface to the product lot being processed. Many studies have been done on the investigation of transfer coefficient of *Listeria species* from various food contact surface to the meat, generating a great deal of data. Hoelzer et al. (2012) summarized and analyzed this transfer coefficient data from literature and the transfer coefficient of *Listeria* from stainless steel to meat was used in our model as the transfer coefficient from FCS to RTE products.

***Sanitation*.** Sanitation effectiveness measures the proportion of bacteria on the food contact surface that is removed through sanitation procedures. Hoelzer et al. summaried the effectiveness of two typical sanitizers (hypochlorite and quaternary ammonium compounds) and found that the effectiveness reduced dramatically for these two sanitizers when protein was present. This model assumes that protein was present for sanitation between lots but protein was absent for sanitation at the end of the day (more intensified cleaning at the end of the day) (Hoelzer et al. 2012). No growth of *Listeria* was assumed on the FCS during the contamination events.

Ratio of L. monocytogenes to L spp. No reference available in the literature about the ratio of *L. monocytogenes* to *L. spp*, so the ratio used in this model was estimated by the prevalence of *L. monocytogenes* to *Listeria* species available from the published literature (Tompkin 2002), which indicated whether or not a food contact surface was positive for *L. monocytogenes* when a surface was found positive for *Listeria species*. The mean ratio of *L. monocytogenes/L. spp.* was found to be 52% and the standard deviation was 26%.

Post-lethality treatment. The model considers the effect of post-lethality (also called post-processing) treatments and growth inhibition in controlling the *L. monocytogenes* concentration during the shelf life of the RTE food products. Post-processing treatments (Pasteurization, ultraviolet treatment etc.) reduce the concentration of *L. monocytogenes*

in the product and growth inhibitors limit the growth of *L. monocytogenes* during storage from plant to consumers. The regulation requires minimum 1 log (10%) kill of *L. monocytogenes* and 2 log kill of *L. monocytogenes* is recommended, so the post-processing effectiveness is set to 0.99 (2 log).

4.2.4 Growth from plant to consumer

Growth of L. monocytogenes. *L. monocytogenes* has been shown to grow at temperatures ranging from -0.4 to 45 ℃ (Keskinen et al. 2008; Jordan et al. 2010). It is considered a psychrotolerant organism as its optimum growth temperature is in the range of 30 to 37 ℃, while it has the ability to grow at temperatures <15 ℃ (Keskinen et al. 2008; Sauders et al. 2009; Jordan et al. 2010). Previous researchers found that the *L. monocytogenes* can grow at refrigeration temperatures for 3 days to 3 months (Gray et al. 1948) and *L. monocytogenes* can survive at cold temperatures in soil, cattle feces, pond water and animal silage for up to 6 years (Fenlon, 1999). A large number of studies have shown that *L. monocytogenes* can proliferate in many refrigerated ready-to-eat (RTE) foods (Dufour 2011). Bacterial growth is one of the important basic processes that leads the exposure and the risk to *L. monocytogenes* (FDA/FSIS 2003). This model considered the growth of *L. monocytogenes* during the storage of RTE product at retail and in consumers' refrigerators by the methods in predictive microbiology. The exponential model predicts the evolution of the size of the bacterial population according to time in a given environment. The growth model used is the exponential "trilinear" model

$$y_t = \begin{cases} Log\, y_0 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots t<\lambda \\ \min(Log\, y_0 + \mu \cdot (t-\lambda), Log\, y_{\max}) \ldots\ldots\ldots t \geq \lambda \end{cases}$$

where y_t (cfu/g) is the bacterial concentration at time t (d), λ (d) is the lag time, y_{max} (cfu/g) is the maximum achievable concentration in the media and μ is the specific growth rate (log cfu/g/d). Growth only occurs once the cumulative time from leaving the establishment for each serving exceeded the respective lag phase (Figure 4-3).

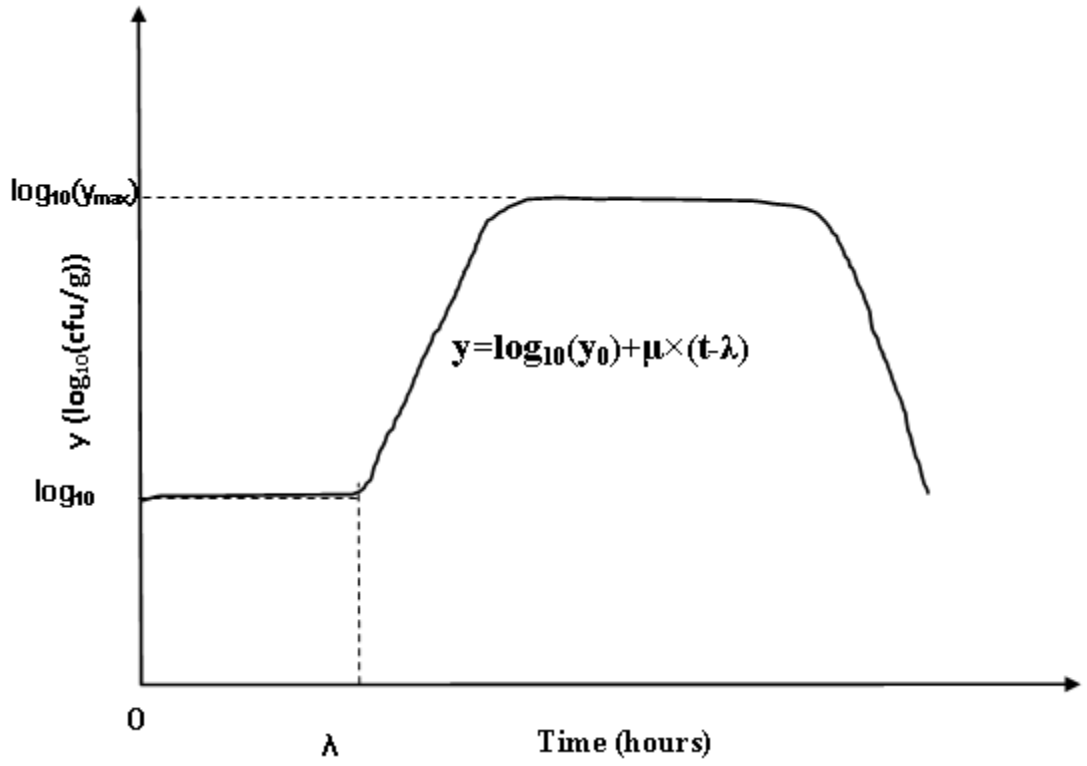

Figure 4-3. The microbial growth model in the media with limited nutrition sources.

The growth of *L. monocytogenes* during shipment from the plant to retail depends on the growth rate of *L. monocytogenes* and the storage time from plant to retail. The Food Code 2009 requires the shelf life of RTE food less than 14 days (FDA 2009). Considering the consumers' storage time, shelf life at retail of 5 to 10 days was assumed. The lag time and the growth rate of the three deli meats (ham, beef and turkey) were taken from the literature (Pradhan et al. 2009) and the growth rate is adjusted for product that undergoes growth inhibitors (Pradhan et al. 2009) and by the storage temperature using (FDA/FSIS 2003). Although the lag time was related to storage temperature and pH of deli meats (Ransom 2005), this model ignored this relationship because it is difficult to monitor the pH and these relationships were not quantitatively well-established.

$$EGR_t = (\frac{T+1.18}{6.18})^2 \times EGR_5$$

Cross-contamination. Cross contamination at retail is found to increase the prevalence and concentration of *L. monocytogenes* in deli meats sliced at retail, compared to deli meats sliced and packaged at the processing establishment without further processes when sold at retail (Gombas et al. 2003; Draughon et al. 2006). Within the model, product entered retail is split into prepackaged (i.e., sliced at the processing establishment) or retail-sliced based on the ratio of these two categories. We assumed retail-sliced products were subject to cross contamination while prepackaged product *L. monocytogenes* concentrations remained unchanged during the retail stage of the model.

This study took a simplified approach to modeling the increase in concentration due to retail-cross contamination. Retail-sliced concentrations are adjusted by the mean and standard deviation of the retail distribution, such that the z-score (or normalized cumulative percentile) of each serving is maintained before and after retail slicing. A z-scaling approach was applied to retail-sliced product according to the following equation:

$$z = \frac{\mathrm{Lm}_{\text{leaving}} - \mu_{\text{retailsliced}}}{\sigma_{\text{retailsliced}}} = \frac{\mathrm{Lm}_{\text{arrive retail}} - \mu_{\text{prepackaged}}}{\sigma_{\text{prepackaged}}}$$

$$\text{then } \mathrm{Lm}_{\text{leaving}} = \mu_{\text{retailsliced}} + \sigma_{\text{retailsliced}} * \frac{\mathrm{Lm}_{\text{arrive retail}} - \mu_{\text{prepackaged}}}{\sigma_{\text{prepackaged}}}$$

Where $\mathrm{Lm}_{\text{leaving}}$ is the $\log_{10}$ concentration of retail-sliced product leaving retail, $\mu_{\text{retail-sliced}}$ and $\sigma_{\text{retail-sliced}}$ are the mean and standard deviation of the retail-sliced product, and $\mu_{\text{prepackaged}}$ and $\sigma_{\text{prepackaged}}$ are the mean and standard deviation of the prepackaged product as reported by Endrikat et al. (Endrikat et al. 2010).

Consumer handling. Consumer storage time and temperature are based on an analysis of a web survey conducted by Pouillot (2010). The analysis found that consumers tend to use retail-sliced product more quickly than prepackaged product. Storage temperatures did not vary by product type.

The variability distribution of serving size (i.e., the grams of RTE deli meats that a consumer ingests in a single meal) was adapted from a previous risk assessment of deli

meats (FDA/FSIS, 2003). The same serving size distribution applies to both healthy and susceptible populations.

4.2.5 Sampling procedure

Sampling of FCS. For both food contact surface testing and product testing, the modeled concentration of the organism was multiplied by the sample size to estimate the mean of a Poisson distribution, a probability distribution that is appropriate for modeling such concentrations. For food contact surfaces, the concentration is measured in cfu/cm^2 and the sample size is measured in cm^2. For RTE product, the sample size is measured in cfu/gram, and the sample size in grams. A random number was generated from this distribution that represented the number of cfus in the sample itself. Once the number of organisms in the sample was known, the probability that a test to detect the presence of the pathogen would yield a positive or negative result could be determined by using a binomial distribution: $1-(1-p)^n$, where p is the probability of detecting 1 cfu in the sample, and n is the number of cfus in the sample from the Poisson calculation. The p probability is based on the detection limit and microbiological test sensitivity, and is the input parameter to the risk assessment model. The sampling frequency of FCS was analyzed from 0 sample per line per month (sampling no FCS) to 60 samples per line per month (sampling all FCSs).

Sampling of Lots. Lots are tested for *L. monocytogenes* during either routine lot testing or additional testing as a result of the *Listeria species*-positive food contact surface testing. Under the baseline scenario, product lots are not normally tested. The lot testing response is lagged by the time it takes to analyze and get the results of a food contact surface testing. The model assumes the reporting time of two days for *L.spp.* and four days for *L. monocytogenes*. The model also assumes that product lots of RTE product that test positive for *L. monocytogenes* are removed from the food supply, accomplished by reprocessing the lot for human food, converting of the lot into products not intended for human consumption, or disposing of the lot.

The lots simulated in this model are allocated to different plants categorized by the plant size. Three different processing plant sizes were modeled in this research. The fraction of

the deli meats food supply produced by large, small and very small plants and the pounds per shift per line for each plant size were estimated. A survey among RTE processors of deli meats as reported by FSIS (2003b) found that for deli meats, about 48% of the food supply is produced by large plants, 48% by small plants, and the remaining 4% by very small plants. The estimated fraction of production volume is shown in Table 4-2.

Table 4-2. Baseline allocation of deli meats production and FCS testing by plant size and alternative.

Alternative	Plant Size	Percent of deli meats production, baseline, %	Fraction of number of plants (N=1981)	FCS Samples per year	Product lot samples per year
1	Large	3.32	0.66	2	0
1	Small	1.14	3.13	2	0
1	Very Small	0.03	2.17	2	0
2a	Large	19.94	0.66	4	0
2a	Small	16.09	1.06	4	0
2a	Very Small	1.53	1.16	4	0
2b	Large	19.94	6.71	48	0
2b	Small	16.09	8.88	24	0
2b	Very Small	1.53	4.44	12	0
3	Large	4.80	2.78	48	0
3	Small	14.68	25.09	24	0
3	Very Small	0.9	43.26	12	0

The fraction allocations within the alternatives indicate that 42% of production includes growth inhibitors, 42% receives a post processing lethality, and 20% receive neither. (The values sum to more than 100% because approximately 4% includes both growth inhibitors and post processing lethality.)

The survey found that the average mass of a lot of RTE product varied by plant size, there is no evidence that there is a difference in the occurrence of *L. monocytogenes* in RTE product by plant size. To account for the variation in lot mass, the model adjusted the food contact surface area by plant size.

The model generates the requested number of lots for each plant size determined by the fraction of production for each plant size in Table 4-2, and then combines them to form a continuous distribution that is tracked through retail and consumption.

4.2.6 Dose-response model

The dose-response model used in the model is the exponential approach developed in the FAO/WHO (2004) risk assessment. The model assumes that each pathogen cell acts independently and the distribution of organisms from serving to serving follows a Poisson distribution (Haas et al. 1999). The model is expressed by $P(illness) = 1 - e^{-rD}$, where P(illness) is the probability of illness for a given dose D. The model parameter r is the probability that 1 pathogen cell initiates illness to the target population.

The FAO/WHO model separates the population into two groups: a healthy population that is generally resistant to listeriosis and a susceptible population consisting of immune-compromised, elderly, or pregnant individuals. Based on susceptibility information available from the United States of America, it was determined that the elderly (60 years and older) were 2.6 times more susceptible relative to the general healthy population, while perinatals were 14 times more susceptible (FAO/WHO 2004). The susceptible fraction of the population was set at 17.5% of the overall population and accounts for 80-98% of the listeriosis illnesses.

The dose response model, along with the 95% confidence intervals, is shown in for both the susceptible and healthy populations (Figure 4-4). Only the median curves are used in the current model. Note that the median infectious dose for the susceptible population is on the order of $10^{11} – 10^{12}$ cfu, and illnesses are unlikely for population if the dose is less than 10^{10} cfu.

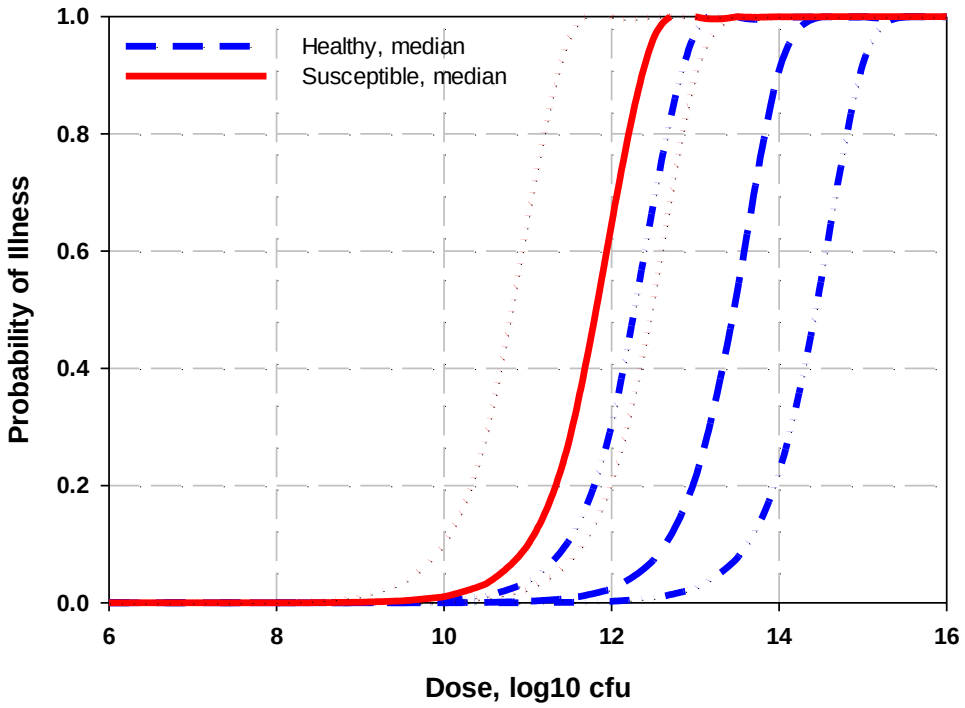

Figure 4-4. FAO/WHO dose response model for listeriosis. Median and 95% confidence intervals shown.

4.3 Results and discussion

4.3.1 Effect of sampling frequency on Listeria concentrations at retail

For the baseline simulation, the current allocation of plants were used, but no FCS or product lot testing was applied. The variation from leaving the plant through leaving retail and finally at consumption is shown in Figure 4-5. The growth between the various stages can be seen. Note also the extended upper tail at consumption. This is caused by consumer abuse – storing the product at high temperatures for extended periods.

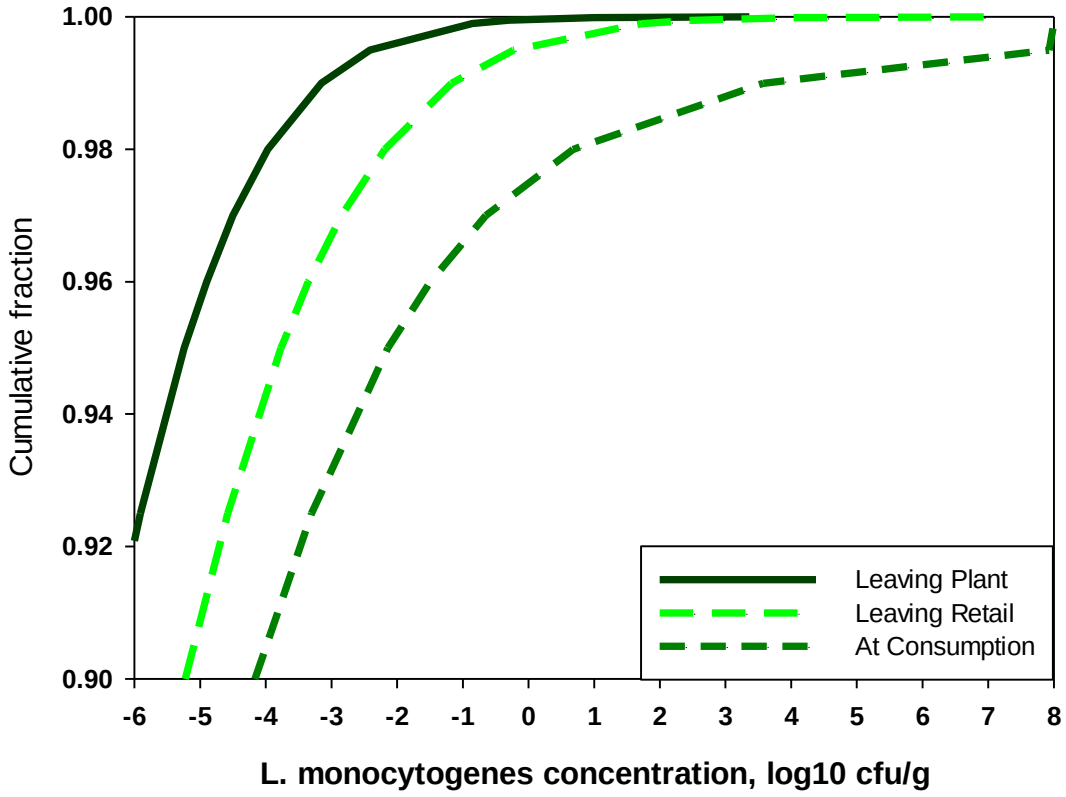

Figure 4-5. CDFs of *L. monocytogenes* concentrations at various stages of the food chain for the baseline scenario.

The simulations of different testing and intervention scenarios with 1,000,000 lots were conducted and the quantiles of *L. monocytogenes* concentration in food products leaving the plant are displayed in Figure 4-6. The 4-2-1 in the figure means the sampling frequency (samples per month) on food contact surface (FCS) at large, small and very small plants. The maximum sampling frequency was 60 samples per month, given that two lots produced per day in each plant, while 60-60-60Lot means every lot was also sampled in each plant. The suggested sampling frequency on FCS by FSIS ("Updated Compliance Guidelines", May 2006), 4, 2 and 1 samples per month in large, small and very small plants, seemed ineffective compared with the baseline, in which no samplings were involved. The concentrations of two scenarios, the scenario with both post-processing and growth inhibitor, and the one with post-processing alone, were significantly less that these of other scenarios, indicating that the post-processing was

much more effective in reducing the initial concentration of *L. monocytogenes* in RTE food.

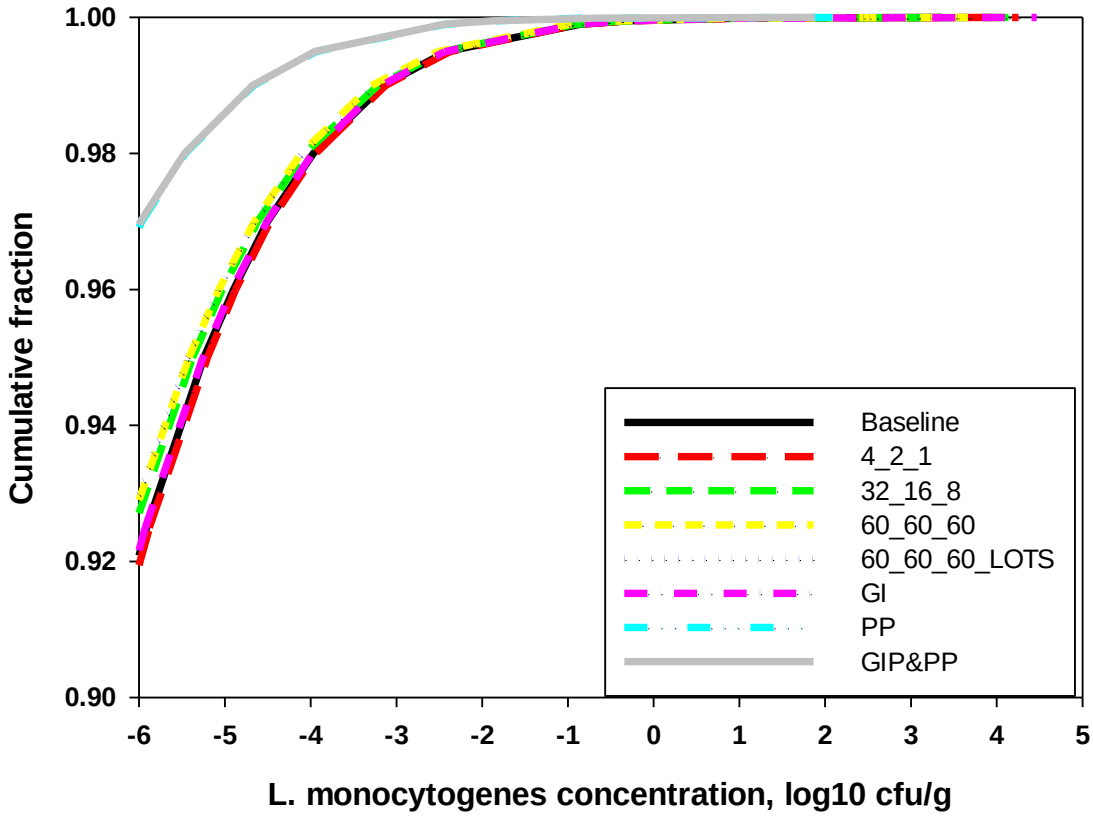

Figure 4-6. CDFs of the *L. monocytogenes* concentration leaving the plant for various testing and intervention scenarios.

A similar plot of the concentration distribution at retail is shown in Figure 4-7. Growth and cross contamination have increased the concentrations in all the scenarios. As at the plant, the major distinction is between the scenarios that included a post-processing lethality and those that did not. A slight separation occurs between GI&PP and PP alone because of the lower growth rate with inhibitors.

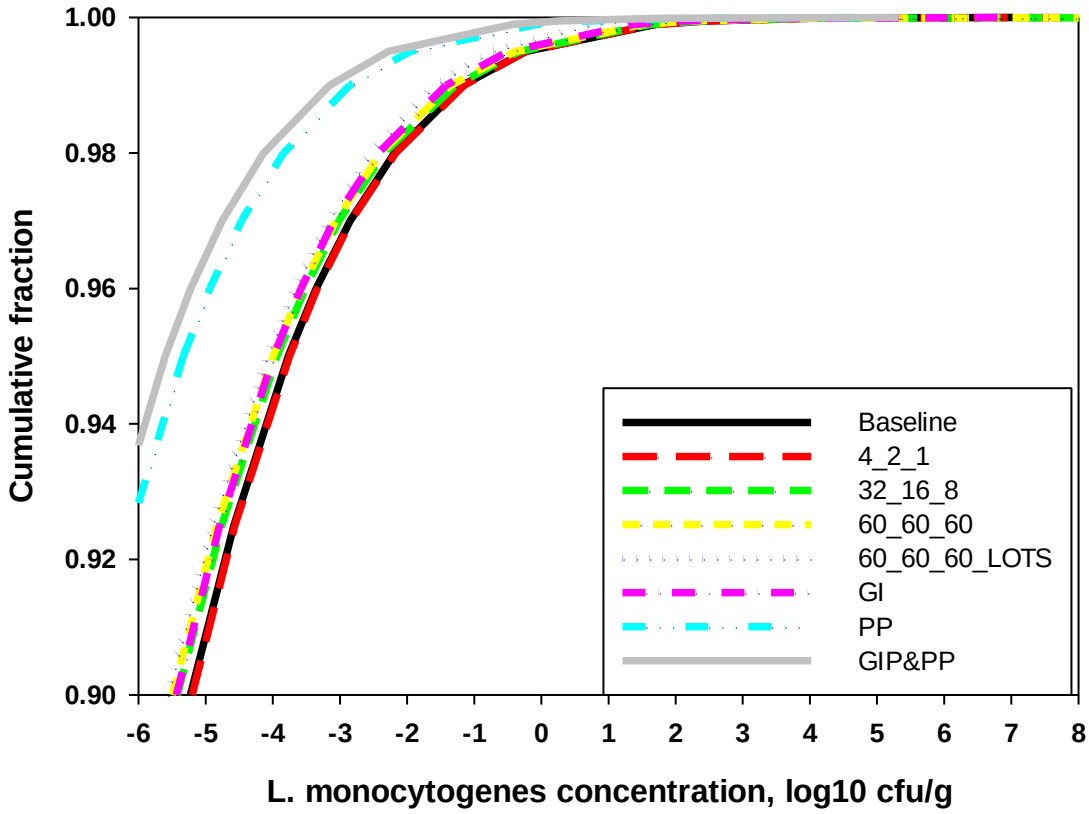

Figure 4-7. CDFs of the *L. monocytogenes* concentrations leaving retail for various testing and intervention scenarios.

Finally, the concentration distributions at the time of consumption are shown in Figure 4-8. Further growth and longer tails are apparent. The baseline and any FCS or product lot testing are still grouped together, indicating that testing alone is not an efficient strategy for reducing *L. monocytogenes* exposure. More frequent testing does reduce the exposure very slightly – compare the 60-60-60 and 60-60-60Lot with the reduced testing frequency results. The exposure reductions are generally less than half a log unit.

The interventions that involve post-processing lethality and growth inhibitors are more effective and more complex. As expected, the combined interventions are more effective than either alone. When just one of the interventions is applied, post-processing lethality results in lower concentrations for the majority of the distribution. The curves cross,

however, at about 1×10^{-2} cfu/g. Beyond this concentration, the GI distribution is actually lower than the PP distribution. Recall that illnesses even for the susceptible population require doses of at least 1×10^{10} organisms. Since most deli meats serving sizes are between 100 and 500 grams, only the most extreme portion of this upper concentration tail is capable of causing illness. In this region, growth inhibitors are a more effective intervention than post-processing lethality alone.

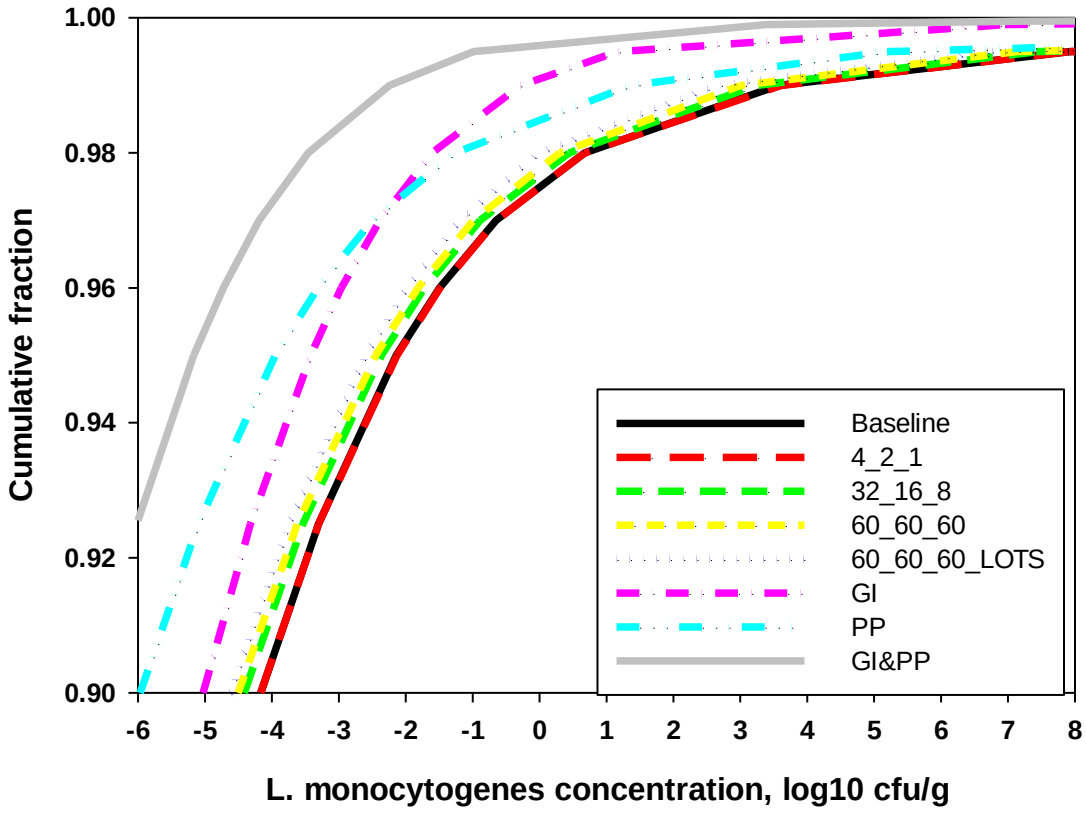

Figure 4-8. CDFs of the *L. monocytogenes* concentrations at consumption for various testing and intervention scenarios.

4.3.2 Effect of post-processing and growth inhibitor on Listeria concentrations at retail

The implementing of 100% post-processing (PP) and 100% growth inhibiting packaging (GIP) indicates effective reduction of *L. monocytogenes* at consumption compared with the baseline as well as most of the scenarios with FCS sampling. The scenario with post-processing lethality shows great decline in the *L. monocytogenes* level, especially at lower concentrations, but growth inhibitor reduces the highest concentration levels..

The implementation of post-processing only acts at the beginning, but the growth inhibitor plays a more important role during the whole lifetime of RTE food. Although the reducing in concentration of *L. monocytogenes* at retail from the implementing of 100% post-processing is greater than that from 100% use of growth inhibitor, with the increasing of storage time, the growth inhibitor would be more and more important on controlling the levels of *L. monocytogenes*. Figure 4-9 shows this with the comparison between two additional simulations that extended the storage time from plant to retail from U(5,10) days to U(10,20) days, where U(5,10) means the uniform distribution between 5 and 10 days. When the storage time from plant to retail doubled, the concentrations both increased, but the concentrations with the use of GIP increased much slower than the concentration with the use of PP. As discussed previously, the GI CDF curve crosses the PP curve for the baseline scenario at about 1e-2 cfu/g (approximately the 97th percentile). With the extended storage time, the CDFs cross at about 1e-4 cfu/g (approximately the 90^{th} percentile). The disparity between the use of post-processing and growth inhibitor would be more obvious when the storage time in consumers' refrigerator also increases. Overall the performance of the growth inhibitor would be more effective compared with the post-processing intervention when the shelf-life increases.

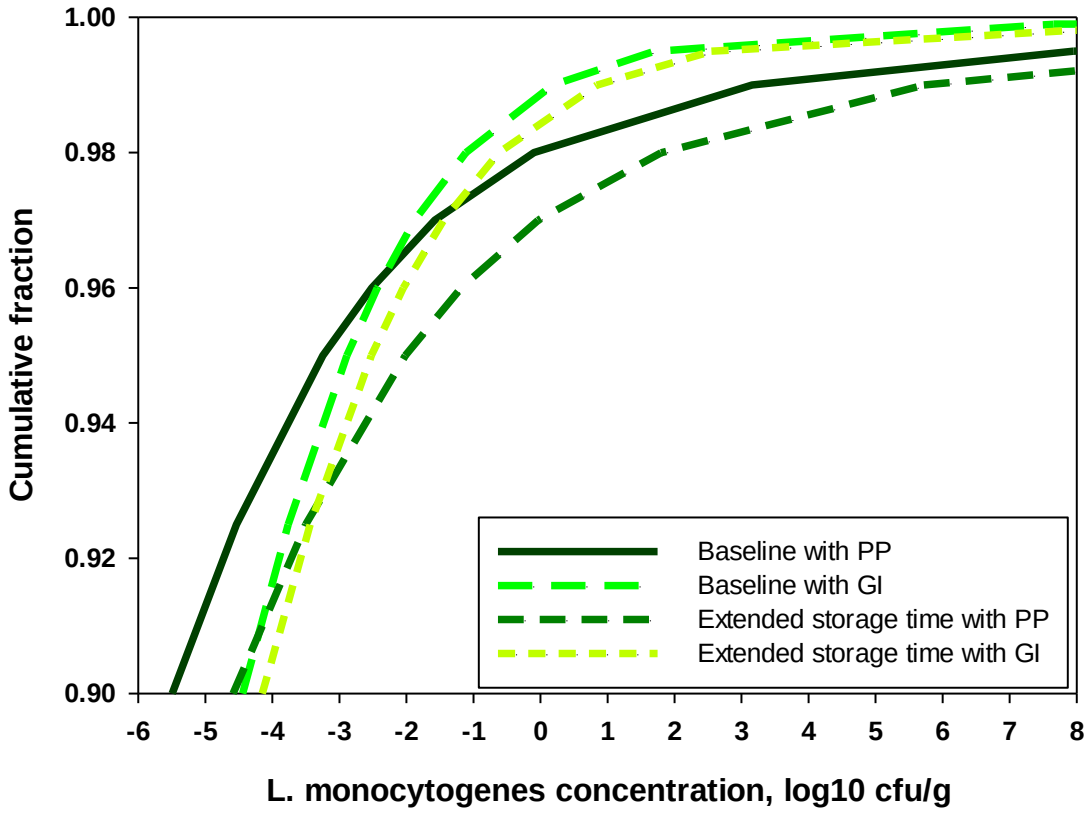

Figure 4-9. The effectiveness of post-processing and growth inhibitor at consumption when storage time is extended.

The most common recipe of the growth inhibitor by the RTE processors is 1.5% ~ 3% lactate alone or in combination with 0.125% ~ 0.25% diacetate (wt/wt formulations) so that they qualify for the lower testing frequencies (Glass et al. 2002; Tompkin 2002). The long-term effectiveness of these concentrations for extended periods needs further research. Additionally, Alternative fractions are currently based on self-reported industry data. FSIS monitoring of their actual GI application is quite limited. Either potential problem could lessen the GI benefits shown here.

4.3.3 Public health impacts

With the input of the dose at consumption of the RTE product for the dose-response model, the mean numbers of illnesses caused by every serving were calculated for

susceptible population and non-susceptible population. The risk of illnesses caused by RTE products for the baseline simulation was 3.75×10^{-07} and 8.61×10^{-09} illnesses per serving for the susceptible and non-susceptible population. Given that approximately 20.6 billion servings of RTE food consumed in US each year, with 17.5% consumed by susceptible population and 82.5% by non-susceptible population, the risk estimated by this model corresponds to approximately 1220 illnesses per year. This number matches well with the estimated number of illnesses in US across all food groups each year (1,600) reported by Scallan et al. 2011, indicating that the selection in baseline parameters was reasonable and practical.

The objective of both the sampling program and interventions was to reduce the risk of *L. monocytogenes* in RTE to public health. Figure 4-10 depicts the influence of the sampling frequency on FCS as well as the use of post-processing and growth inhibitor on the predicted number of listeriosis cases under each scenario. Only the 60-60-60 and the 60-60-60Lot testing strategies showed any substantial improvement over the baseline, at 85% and 88% of the baseline illnesses respectively.

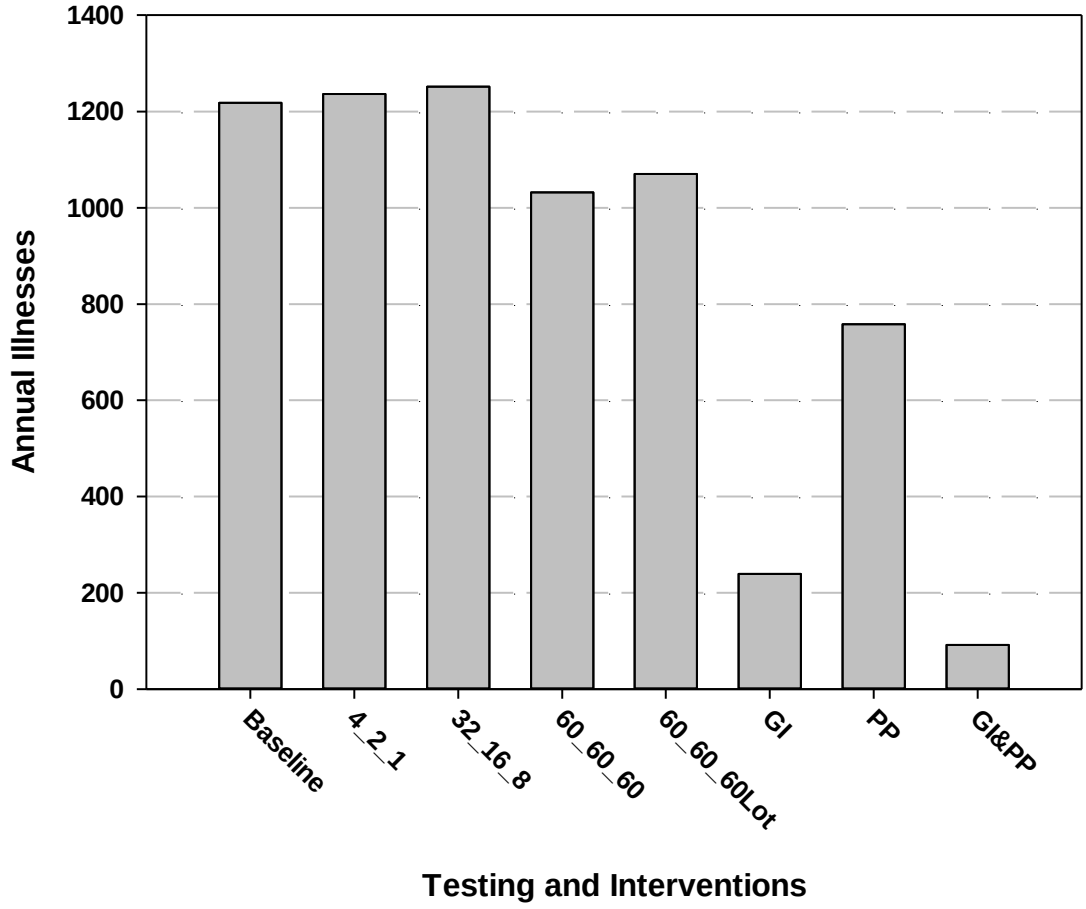

Figure 4-10. Estimated illnesses for various testing and intervention scenarios.

The implementation of post-processing and growth inhibitor performed substantially better at reducing the risk of listeriosis. Post-processing lowered the number of illnesses to about 62% of the baseline, while growth inhibitor alone reduced the illnesses to 19% of the baseline and the combination to 7.5% of the baseline. These results support the previous exposure analysis that indicated that control of the upper tail of the concentration is critical to reduce listeriosis cases.

4.3.4 Detection of L. monocytogenes in lots with/without food contact surface testing.

Considering that the cost of *L.spp* testing is much lower than that of *L. monocytogenes* testing, and the fact that *L.spp* was a good indicator of *L. monocytogenes*, testing the

existence of *L.spp* on the FCS was considered before any sampling on food production lots for *L. monocytogenes*. Table 4-3 shows the prevalence of *L. monocytogenes* on FCS and Lots in the baseline simulation. Overall RTE product lot prevalence for *L. monocytogenes* is 2,371/1,000,001 (0.24%) and the food contact surface prevalence for *L. spp* is 68,926/1,000,001 (6.9%). The lot prevalence when the food contact surface is positive is 2,368/68,926 (3.43%), which means knowing that the food contact surface is positive increases the likelihood of finding a positive lot by 14 times. Pearson's Chi-squared test for independence (with p far less than 2.2×10^{-16}),) indicated that the relationship between positive FCS and positive Lots is extraordinary high, which also means the testing of *L. spp* would be very effective on finding the *L. monocytogenes* positive lots, i.e. majority of positive lots would be detected if all the positives FCS were identified.

Table 4-3. The prevalence of *L.spp* on Food Contact Surfaces and *L. monocytogenes* in RTE product Lot

	Lot positive	**Lot negative**	**Sum**
FCS positive	2,368	66,558	68,926
FCS negative	3	931,072	931,075
Sum	2,371	997,630	1,000,001

Pearson's Chi-squared test with Yates' continuity correction: X-squared = 32002.65, degree of freedom = 1, p-value < 2.2×10^{-16}

These results are based on simultaneous testing possible during the simulation. In practice, the lags from reporting times reduce the effectiveness of FCS testing without a well-designed test and hold strategy.

4.3.5 Public health policy

All the establishments that produce RTE food are required to select one of the three alternatives during the production processes to reduce the risk of *L. monocytogenes*. The risk associated with the three alternatives was different, with a trend increasing from alternative 1 to 3, according to FSIS's report. By separating lots by alternatives from the

results of the baseline simulation, Table 4-4 shows the number of positive lots in the plants after post-processing and the percentage in the total lots under the three different alternatives. There was also an ascending trend from Alternative 1 to 3, which matched well with FSIS's report. This also agreed with FSIS's report in 2007, which indicated that the rate of *L. monocytogenes* positive samples in alternatives 3 was higher than alternative 2a, 2b and alternative 1.

Table 4-4. The positive fractions in products with three alternatives for the baseline scenario.

	Alternative 1	Alternative 2a	Alternative 2b	Alternative 3
Positive	11	147	6082	4322
Negative	32161	360129	354194	242955
Total	32172	360276	360276	247277
% of positive	0.034	0.041	1.688	1.748

based on analysis of 1,000,000 lots

Figure 4-11 showed the estimated annual illnesses caused by consuming the RTE food under different scenarios. The risk under the scenario with both post-processing and growth inhibitor was the lowest, followed by that with growth inhibitor and sampling program, while that with Alternative 3 was the highest among all the scenarios. Different from the results of the concentration at plants and at retail, post-processing alone did not have a good performance in reducing the risk of *L. monocytogenes* to public health. If all the food establishments select Alternative 2a, the estimated annual illnesses was even greater than the baseline, even though the baseline contains just over 20% of production from Alternative 3. The reason is because the baseline also has 42% of production with growth inhibitors, and this reduces the predicted illnesses. This result demonstrates the great impact growth of *L. monocytogenes* during storage has on public health. Although post processing in these runs resulted in a 2 log kill, any remaining organisms can rapidly grow to high numbers if temperature abused and in the absence of growth inhibitors. Given that the mean exponential growth rate (in log10) from plant to consumption was 2.69 (for the baseline simulation), 500 *L .monocytogenes* units would be in the food at the points of consumption if only one single cell survived during the post-processing.

Alternative 3 had the worst performance among all the alternatives according to Figure 4-11. Even if all the product lots were sampled in the scenario with solo sanitation program, the estimated annual illnesses were still more that other alternatives and doubled that in the baseline simulation.

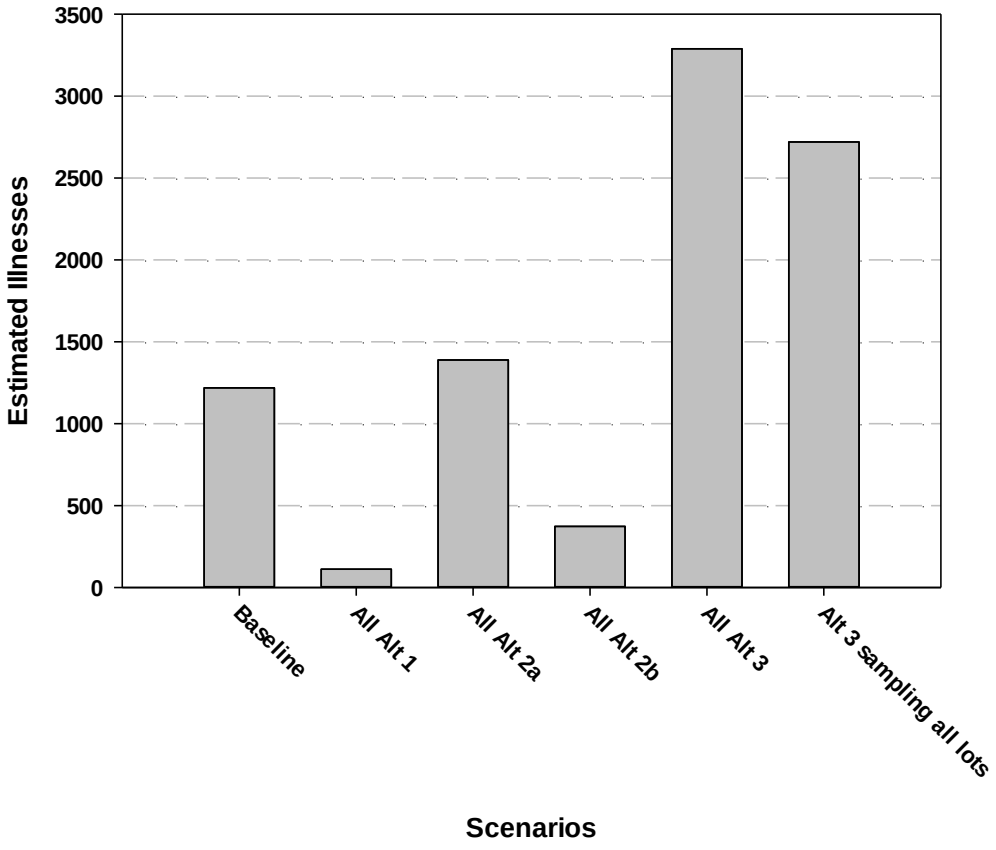

Figure 4-11. The estimated annual illness caused by *L. monocytogenes* under different alternative scenarios.

Switching from Alternative 3 to Alternative 2b or 1 would be very helpful in reducing the risk of *L. monocytogenes* to public health. Table 4-2 included a summary of the percentage of establishments in US that selected each of the three alternatives in 2007 (FSIS 2007), which demonstrated that more than 70% of the RTE food processing plants, especially the very small plants, 20% of production volume were still relying on solo sanitation processes to control the incidence of *L. monocytogenes* contamination. From the result in Figure 4-11, there is great potential to reduce the risk of *L. monocytogenes*

by changing among the alternatives, 70% reduction if all are switched to Alternative 2b and 91% reduction if all are switched to Alternative 1.

4.4 Limitations

Due to the lack of data in the literature, this model has the following limitations:

(1). The model only considers food contact surfaces as the source of *Listeria species*/*L. monocytogenes* in product.

(2). The model assumed that *L. monocytogenes* are evenly distributed on the FCS and the food product lots.

(3). FCS was simply treated as an integral entirety, without individual components, such as the prep table, dicing machine and convey belt.

4.5 Summary

Listeriosis is a significant food safety issue and the continuing outbreaks have underlined the need to review current regulatory legislation. According to the FDA/FSIS's risk assessment, deli meats posed the greatest risk of listeriosis in the U.S. *Listeria species* extensively exist in the environment of food processing facility and were reported to have great potential transferring from the environment to food contact surface and then to the RTE food. The Interim Final Rule 9 CFR Part 430 requires that all food processing plant must implement one of the three alternatives: (1) post-lethality treatment and growth inhibitor, (2) post-lethality treatment or growth inhibitor plus sanitation program, and (3) sanitation program with specific requirements, to reduce the contamination of *L. monocytogenes* during the production processes. This proposed model investigated the effectiveness of sanitation and sampling program including the current hold-and-test process, by simulating the food producing, transportation and storage, and tracking the transfer from the environment in plant to the RTE food, the growth and die off of *Listeria species* on RTE food. Results showed that Alternative 1 reduced the risk of *L. monocytogenes* the most, followed by Alternative 2b, Alternative 2a and Alternative 3.

Although the post-processing greatly reduced the *L. monocytogenes* concentration at plant and retail, the use of growth inhibitor has the greatest impact on reducing the estimated annual illnesses caused by *L. monocytogenes*, because of the continuous effect of the growth inhibitor on the growth of *L. monocytogenes* during storage.

4.6 Reference

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Chapter 5 Optimization of the Current Sampling Program for *Listeria monocytogenes* in Ready-to-eat Food Production Facility

Abstract

Listeriosis caused by *Listeria monocytogenes* has raised great public health concern in recent years due to its high hospitalization rate and mortality. Studies show that ready-to-eat (RTE) meat and poultry products are responsible for majority of the listeriosis cases. Since *L. monocytogenes* can propagate under typical storage temperatures, the initial contamination of *L. monocytogenes* on RTE food results in great risk to public health. In order to reduce the *L. monocytogenes* contamination in the food processing plants and mitigate the growth during storage, FSIS developed the Interim Final Rule (9 CFR 430) for *L. monocytogenes* in ready-to-eat meat and poultry products at federal establishments, which required RTE food establishments to self-classify among three alternatives. Alternative 3, which only requires sanitation processes without post-processing intervention or growth inhibitor treatment, results in the highest risk of *L. monocytogenes* exposure. It is necessary to improve the effectiveness of the current sampling and sanitation program in the Alternative 3 as many establishments are applying this relative low-cost *L. monocytogenes* controlling plan. Using a plant to consumer risk model, this study investigated several important factors in the hold-and-test program, analyzed the sensitivities of these factors, and proposed the reasonable improvement of the hold-and-test strategies. Holding all lots during the food contact surface (FCS) testing period instead of holding lots after finding the positive FCS would increase the detection rate of positive lots by about three times. The sensitivity analyses indicated that increased FCS or lot testing was capable of finding contaminated product, which was removed from the food supply. However, the results of such testing did not lead to public health improvements. Only parameters that directly impacted growth or contamination levels in the products had any measurable effect. Based on these results, FSIS should continue to focus on encouraging more plant to use growth inhibitors and post processing lethality. These results may help the food establishments make the right choose from the three alternatives.

Keywords: *Listeria monocytogenes*, ready-to-eat meat, Hold-and-Test, sanitation, sampling

5.1 Introduction

Responsible for approximately 1455 hospitalizations and deaths per year in the United States (Scallan et al. 2011), *Listeria monocytogenes* (*L. monocytogenes*) is often present and persists within ready-to-eat (RTE) food processing environments, such as drains, dicing machines, floors and food contact surfaces (Lawrence et al. 1995; Autio et al. 1999; Aarnisalo et al. 2006). During the late 1990's and early 2000's, several major outbreaks of *L. monocytogenes* associate with RTE deli meats and frankfurters led to increase efforts by both industry and regulatory agencies to control this organism (FDA/FSIS 2003).

Since *L. monocytogenes* can grow under refrigerator temperatures, the RTE products like cooked ham and turkey, which do not go through additional cooking prior to consumption, become a major vehicle for exposing consumers to the pathogen. By tracking the *L. monocytogenes* ribotypes in the environment and products of smoked fish plants, Thimothe et al. found that *L. monocytogenes* prevalence correlated with the prevalence in finished fish product samples (Thimothe et al. 2004). This correlation can be weakened by applying specific control strategies such as employee training and targeted sanitation procedures (Lappi et al. 2004). Another study conducted by Lunden et al., who analyzed the successive *L. monocytogenes* contamination which happened at three sequential plants as a dicing machine was transferred from plant to plant, provided evidence on the existence of *L. monocytogenes'* long term harborage site in the plant environments (Lunden et al. 2002). So it is critical to reduce the *L. monocytogenes* level in the plant environmental and the RTE food during the production process, especially in the processes before packaging.

FSIS/USDA issued 9 CFR 430, the Interim Final Rule to lower the *L. monocytogenes* contamination in RTE meat and poultry processing plants. All of the establishments producing post-lethality exposed RTE meat and poultry product must choose one of the

three alternative interventions to reduce the incidence of *L. monocytogenes*. Alternative 1 requires the food establishments to apply both post-processing intervention and growth inhibitors; Alternative 2 requires the food establishments use either post-processing intervention or growth inhibitor with additional sampling program. Alternative 3 only relies on the sanitation process, with the help of additional sampling program on the food contact surface and products. According to the FSIS risk assessment for risk-based sampling of *L. monocytogenes*, more than 70% of the establishments choose Alternative 3, that with the highest risk of *L. monocytogenes* to public health among all the three alternatives (FSIS 2007).

An establishment under Alternative 3 must provide FCS testing on all the identified sites that could contaminate product at a frequency no less than the recommended minimum sampling frequency. The minimum sampling frequency varies with the size of the plants, with 4, 2, and 1 samples per month per line for large volume plant, small volume plant and very small volume plant, respectively. FSIS recommends higher frequency of FCS testing in order to accumulate supportable data faster to ensure that the establishment's sanitation program is effective and appropriate to keep *L. monocytogenes* out of the production environment. The extra data would also further support that a plant is not producing an adulterated product and may help the plant to decide to reduce its FCS testing frequency at some point in the future.

In the FSIS's interim final rule in 2003 on the control of *L. monocytogenes* in ready-to-eat (RTE) meat and poultry products, most processors of RTE products are required to conduct microbiological testing of product contact surfaces. The rule states that establishments using antimicrobial agents or processes under Alternative 2 and establishments producing frankfurters or deli products under Alternative 3 must identify the conditions under which they will implement hold-and-test procedures. "Hold-and-test" is a procedure that identifies the conditions under which the establishment will hold product pending test results following an *L. monocytogenes* or an indicator organism (usually *L. species*) positive FCS test result. The rule describes the hold-and-test procedures to be followed by establishments producing hotdog and deli products under Alternative 3. If such an establishment obtains a positive for *L. monocytogenes* or an

indicator organism such as *Listeria spp.* in follow up testing on food contact surfaces, it must hold lots of product that may have become contaminated by the food contact surface and must sample and test these lots before their release into commerce.

FSIS provided the hold-and-test scenario flowchart which the establishments can directly use or develop their own hold-and-test scenario. This flowchart illustrates what an establishment could do in case of a FCS testing positive for *Listeria spp.* or *Listeria-like* organisms, and what actions to take when a follow-up FCS test is positive. The repeated positive FCS testing would imply an inadequacy of the sanitation system indicating that the establishments should investigate and reassess the sanitation program and the equipment layout to determine the cause of the contamination. When one FCS is tested to be positive, the establishment will take corrective action such as intensified cleaning and sanitizing, and test the FCS again. If another positive FCS occurs during the follow-up testing, the establishment must hold the applicable product lot if positive for L. spp. or L.-like species, or destroy or rework with a process destructive of *L. monocytogenes* if positive for *L. monocytogenes*, and test the FCS until the establishment corrects the problem as indicated by the test result. If second FCS is found to be positive, the product on that day that the second FCS results are available would be tested for *L. spp.* or *L. monocytogenes*; while the products during the testing period should be held. Then, if the lots were positive for *L. monocytogenes*, destroy the tested product or rework products, and test the held products.

Figure 5-1 demonstrates the general process of hold-and-test which is used by most the establishments using Alternative 2 and Alternative 3 (FSIS 2006). It looks effective in finding out the contaminated product lots and reducing the *Listeria spp.* on FCS by corrective action. In this hold-and-test program, the hold procedure is activated after finding the second sequential *L. spp.* positive sample for the FCS. This hold-and-test process lowers the cost of storage for the holding of RTE food, by double checking the FCS sample, but ignores the possible contamination which might have happened during time between the beginning of the first FCS testing and the end of the second FCS testing. This gap can be more than 3 days based on the time needed for the *L. spp.* testing.

To improve the effectiveness of this hold-and-test scenario, it looks reasonable to hold-and-test the product lots during the first FCS testing, and to continue intensified cleaning and sanitizing during the second FCS testing. This plant-to-consumption model compared the different between these two scenarios and suggested the optimal hold-and-test program in reducing the contamination of FCS and finding the positive FCS and product lots. There are also some other improvement in the hold-and-test program including the sampling size, sanitation frequency and efficiency.

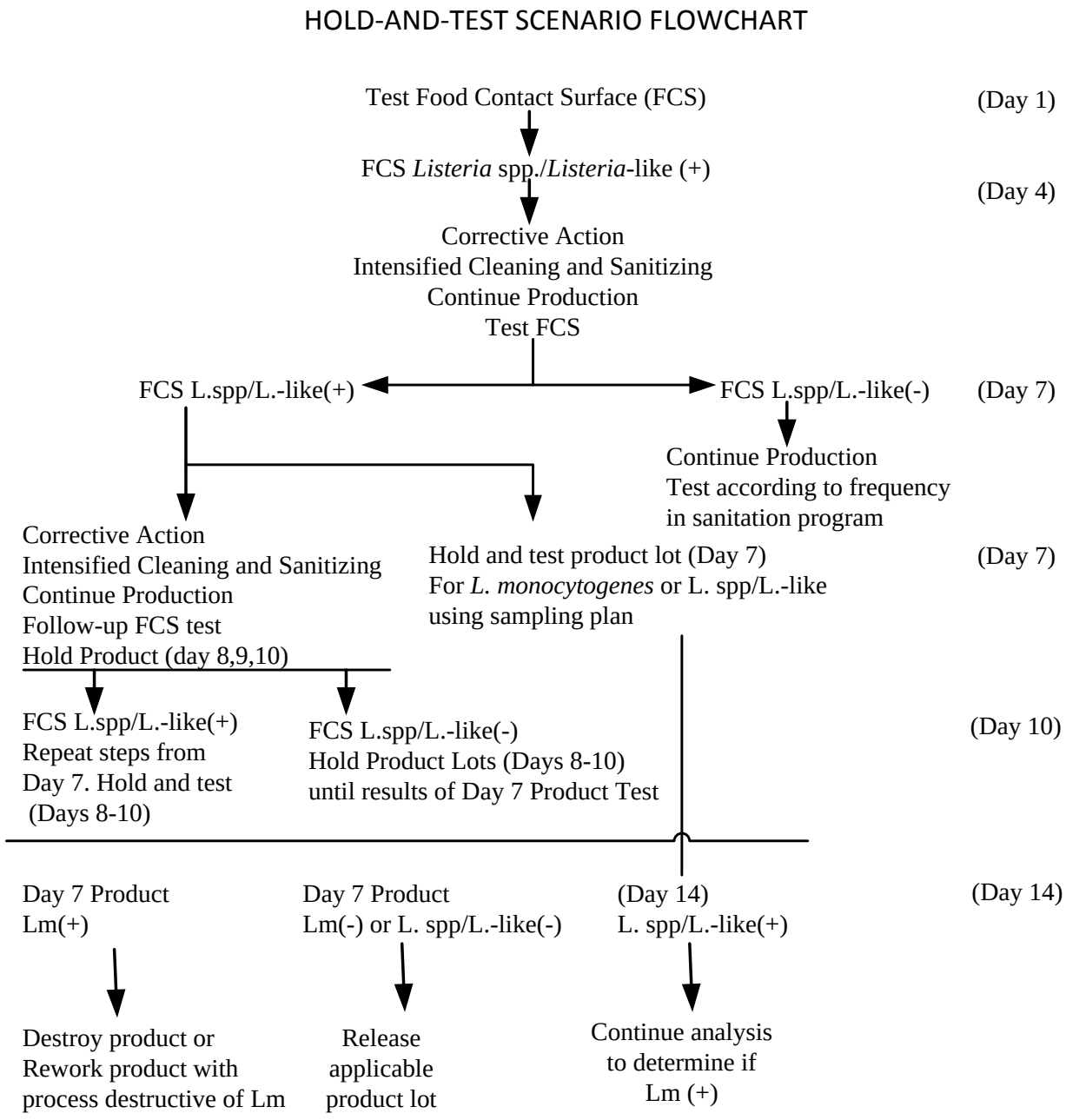

Figure 5-1. Hold-and-test scenario flowchart (FSIS 2006).

The goal of this study is to analyze the important factors that impact on the effectiveness of the hold-and-test program in controlling the risk of *L. monocytogenes* in RTE meat and poultry products at the food facility plants. This work would help the food regulation

agencies to make proper food safety policies related *L. monocytogenes* and aid the RTE meat and poultry food establishments choosing the best hold-and-test procedures.

5.2 Methods and materials

This study provided a comprehensive model tracking the prevalence and number of *L. monocytogenes* cells in the food contact surface and the RTE food products throughout the entire food supply continuum from plant to retail and then to home consumption. Figure 5-2 illustrates the flow diagram and the main components of this model.

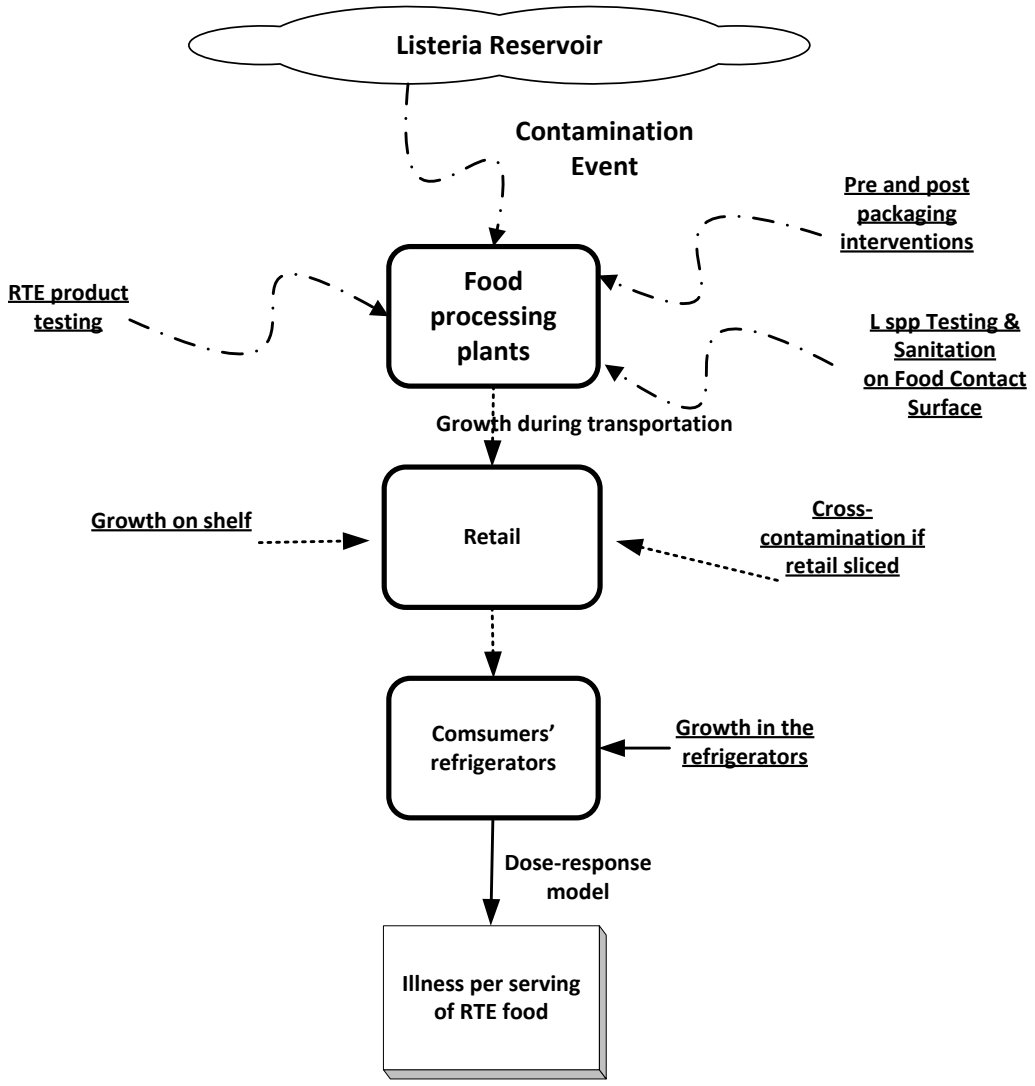

Figure 5-2. The major components of the plant-to-consumption model

This first-order Monte Carlo dynamic model tracks the *L. spp* and *L. monocytogenes* cell number (concentrations) on the FCS in the food processing plants and in the RTE food product, following the sequential processes of contamination of FCS from the harborage site, transfer from FCS to RTE food lots, post-processing intervention, growth during storage at retail, cross-contamination at retail, growth in home refrigerators and final consumption by the customers. During these steps in the plant, the FCS and lots were routinely tested for the existence of *L. spp* or *L. monocytogenes*, the results of which would determine the intensity of sanitation and the testing frequencies. At the end of the complete *L. monocytogenes* pathway, the risk caused by these *L. monocytogenes*

represented by illness per serving is predicted by the dose-response model (FAO/WHO 2004) and it serves as an important criterion for the analysis of the key parameters in this model. The basic components of this model were described in the previous chapter with detailed introduction on each parameter. In this study, the following factors of the model were primarily discussed.

Sampling frequency. FSIS provided the recommended minimum frequencies for FCS sampling to be met by the establishment in the Compliance Guideline of Controlling *Listeria monocytogenes* in Post-lethality Exposed Ready-to-Eat Meat and Poultry Products (FSIS 2012) (Table 5-1). The food establishments should develop routine FCS testing on *L. spp.* following these minimum frequencies and continue additional testing once a positive sample is found. In this research, higher frequencies of FCS testing were modeled to achieve higher detecting rate of the positive FCS.

Table 5-1. FSIS suggested minimum verification testing frequency of a food contact surface under Alternatives 1, 2, and 3.

Alternatives		Minimum FCS testing frequency
Alternative 1		2/year/line
Alternative 2a and 2b		4/year/line
Alternative 3	Large	4/month/line
	Small	2/month/line
	Very small	1/month/line

Holding timing. The FSIS suggests the food establishments holding the products when the second L. spp. positive in FCS sample is found. This model also simulated the scenarios with earlier and later holding timing for the RTE food lots in the hold-and-test procedure and the resultant probability of reducing the risk of *L. monocytogenes* was analyzed.

***L. spp.* testing time.** The time needed for the *L. spp.* testing on the FCS and *L. monocytogenes* in the RTE food lots impacted how soon and how long the RTE food product should be held. Technological improvements have reduce the reporting time for

L. spp testing from 3 days to 2 day, and for *L. monocytogenes* from 7 days to 4 days. With the development of the laboratory condition and testing methods, the required testing could be greatly reduced (Bang et al. 2013). Thus the testing time was treated as another optional factor in this model.

Sampling size. Sampling size influences the possibility of detecting a positive *L. spp.* or *L. monocytogenes* samples. In the FCS testing and product testing, the numbers of pathogens cells on the FCS or in the product were calculated by a Poisson distribution, the mean of which is the multiplication of the sample size by the concentration of *L. spp* on the FCS or *L. monocytogenes* in the product. The probability that a test would detect the presence of the pathogen would be determined by the binomial distribution: $1-(1-p_{detect})^n$, where p is the probability of detecting 1 cfu in the sample, and n is the number of colonies in the sample from the Poisson calculation. For area-based FCS testing, the baseline model assumes 1000~3000 cm^2 were swabbed and tested. For mass base product testing, the baseline model assumes 25 grams were tested. The impact of both variables was evaluated through a sensitivity analysis.

Sanitation efficiency. The cleaning efficiency of FCSs is a highly uncertain model input. It depends on the type of sanitizer used, the nature of the FCS bacterial contamination (biofilm versus dispersed), the presence of other material on the FCS (e.g. proteins) and the actual proficiency. Most studies have been conducted under laboratory conditions where the operators knew that sanitation was being evaluated. The actual efficiency during day-to-day plant operations is an open question. Hoelzer et al. (2012) reported on sanitation efficiencies, and these values were used for the baseline model with a sensitivity analysis to evaluate the importance.

Time of transport. As previously discussed, growth is a key requirement for *L. monocytogenes* concentrations to reach a level that might cause illness in the susceptible population. Growth depends on a combination of time and temperature, as well as the product-specific growth rate and lag time. To evaluate the impact of growth, the time from plant to retail was also evaluated through a sensitivity analysis.

Post-processing lethality. Plants in Alternative 1 or Alternative 2a apply a post-processing lethality such as steam pasteurization to reduce the *L. monocytogenes* concentration after the product has been bagged and sealed. Available equipment can achieve a wide range of log kill. The baseline assumption is a 2 log reduction based on FSIS recommendations, but a range of reductions were evaluated through a sensitivity analysis.

5.3 Results and discussion

5.3.1 Hold timing

The current hold-and-test program ignored the possible positive lots generated during the testing periods of the FCS. Thus, in order to have a good understanding in how many positive lots had been released from the plants to the market due to the lagging reporting time of FCS sampling, a simulation was carried out with all the food products being held until the results of FCS samplings came out. The benefit of this scenario was the opportunity to test all the lots associated with detected positive FCS. The number of sampled lots and detected positive lots were in Table 5-2. for the baseline simulation and the improved hold-and-test program. In order to focus on the impact of holding timing, all the FCSs were sampled and testing in the baseline and improved hold-and-test program simulation. Although more lots were sampled during the scenario with all product holding during the time period of FCS testing, much more positive lots were found by testing. The detection rate of positive product for *L. monocytognenes* increased about three times, from 0.82% to 2.33%.

Table 5-2. Effectiveness of detecting the positive lots by changing the hold timing.

	Hold after Positive FCS	**Hold while FCS testing**
Total positive lots	3453	3453
Actually sampled lots	14421	44001
Detected lots	118	1027
Detection rate (%)	0.82	2.33

5.3.2 Model stability

Since the simulations were based on only 1,000,000 total lots across all large, small and very small plants, another 20 baseline simulations with different random seeds were run to validate the stability of this model. The variability of the major results, including the annual illnesses in US, the risk per serving for both healthy and susceptible population, the 80%, 99% and 99.99% percentile of the *L. monocytogenes* concentration at retail were in Figure 5-3 as a box plot. These graphs indicated central tendency (the median), spread (both the interquartile range and the 95th percentiles), and an indication of symmetry/skewness (the location of the median within the box). The results indicate very little spread among the 20 replicate model runs, proved that the model was stable enough with 1,000,000 sequential simulations.

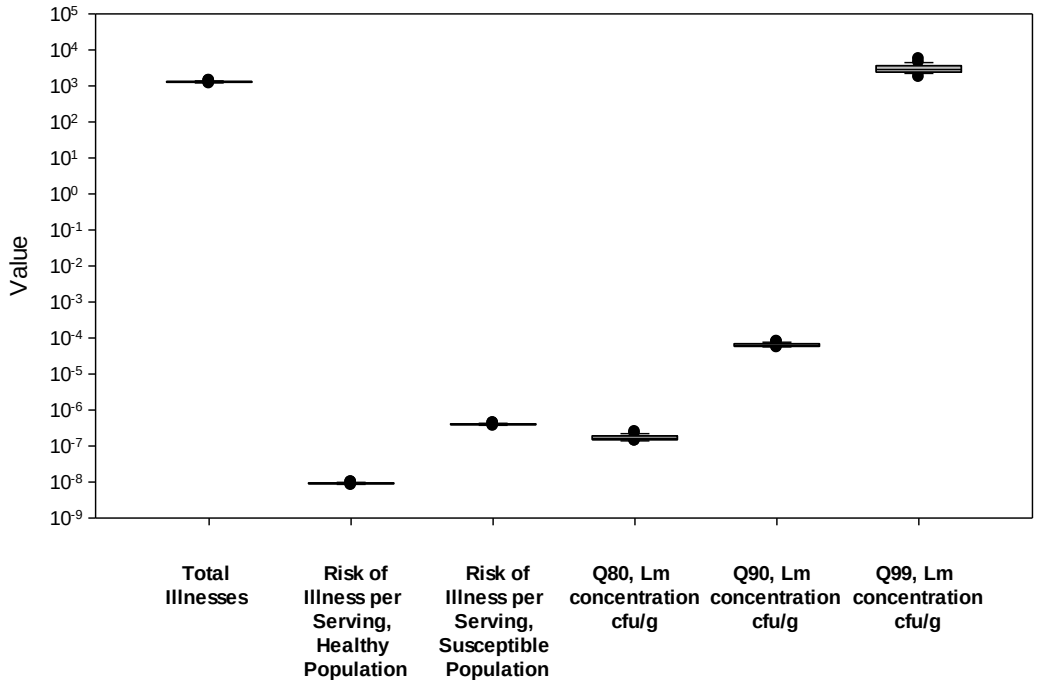

Figure 5-3. Results of 20 random simulations indicating degree of model stability based on 1,000,000 lot simulations.

The mean and standard deviation for the number of illness based on the 20 simulations was 1302 ± 59. The minimum and maximum were 1165 and 1407 respectively. While

this indicates some run-to-run variability, the variability was deemed low enough to conduct a sensitivity analysis based on 1,000,000 simulated lots.

5.3.3 Sensitivity analysis

As there were variations in most of the parameters that being used in this model, there is uncertainty in the statistical modeling. To assess the magnitude of this uncertainty, new sets of simulation with varied selected input parameters, including post-processing effectiveness, storage time from plant to retail, FCS sampling area and Lots sampling size, were performed, as shown in Table 5-3.

Table 5-3. Parameters in the sensitivity analysis.

Parameters	*FCS Sampling Area (cm^2)*	*Sample size (g)*	*Sanitizing efficiency between lots*	*L. spp. testing time (day)*	*Time from plant to retail (day)*	*PP** efficiency*
Varied values	U*(500,2500)	5	0.8	1	U(2.5,5)	90%
	U(1000,3000)	10	0.85	2	U(5,10)	99%
	U(1500,3500)	15	0.9	3	U(10,15)	99.9%
	U(2000,4000)	25	0.925	4	U(15,20)	99.99%
	U(3000,5000)	50	0.95	5	U(20,25)	99.999%
	U(4000,6000)	75	0.975	6	U(25,30)	99.9999%
	U(5000,7000)	100	0.99			99.99999%
		125				

*U(500,2500) means the parameter is uniform distribution between 500 and 2500.

** post-processing intervention

FCS Area Sampled. The FCS surface area sampled was varied from 500 to 5000 cm^2. The larger areas would have a higher probability of a positive detection for a given FCS concentration. The results are shown in Figure 5-4 for illness, number of positive FCS tests, and number of positive lots detected and discarded. In each case, the baseline, 60-60-60 FCS tests, and 60-60-60 Lot tests, i.e. the baseline and all possible tests for FCSs and lots, were analyzed.

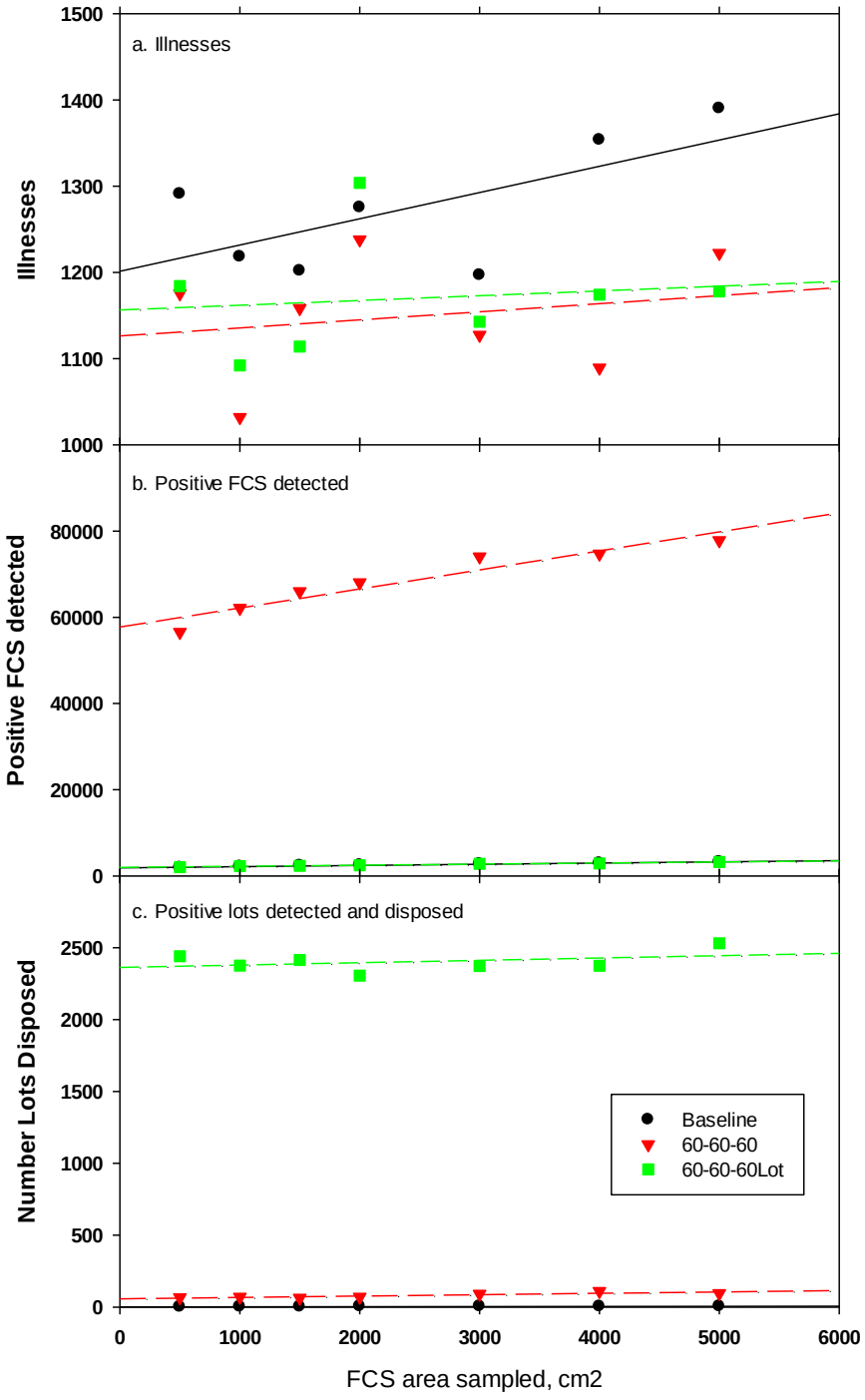

Figure 5-4. Sensitivity analysis of FCS area sampled.

None of the illness slopes were statistically different from 0 ($p>0.05$). Increasing the sampled area did not reduce the risk of illness. The only noticeable shift occurred when all possible FCS tests were performed, which led to an increase of the number of positive FCS detected. But this increase was not sufficient to reduce illness.

Product mass sampled. When a product lot is tested, a sample mass typically 25 grams is used to conduct the analysis. Lin et al. conducted a study analyzing the effect of sample size on the efficacy of the BAX-PCR and USDA/FSIS enrichment culture assays in detecting *L. monocytogenes*. It was found that increasing the sample size taken improved the detection of *L. monocytogenes* (Lin et al. 2006). For the sensitivity analysis, the mass was varied from 5 to 125 grams. The results are shown in Figure 5-5.

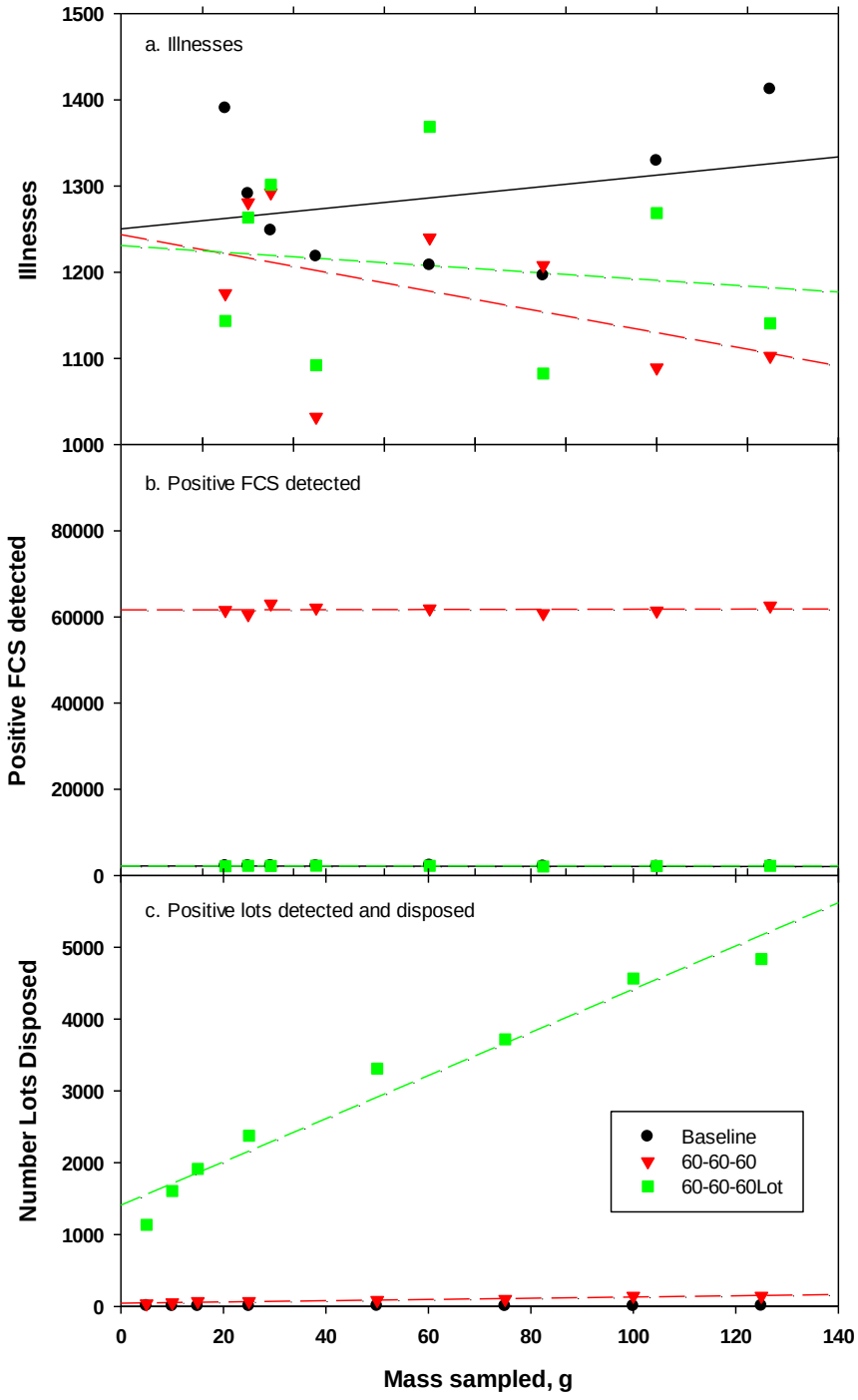

Figure 5-5. Sensitivity analysis for product sample mass.

Again, no statistically significant trends in the number of illnesses were present. The increased in FCS positives when all FCS tests were performed is visible. There was a statistically significant linear increase in the number of positive lots detected and disposed as the sample mass increased, but these additional lots dispose were not sufficient to reduce illnesses.

Post-processing lethality efficiency. FSIS requires a 1 log reduction to qualify in Alternative 1 or 2a, and recommends a 2 log reduction, which was used for the model baseline. To evaluate other efficiencies, the log kill was varied from 1 to 7, and the results are shown in Figure 5-6.

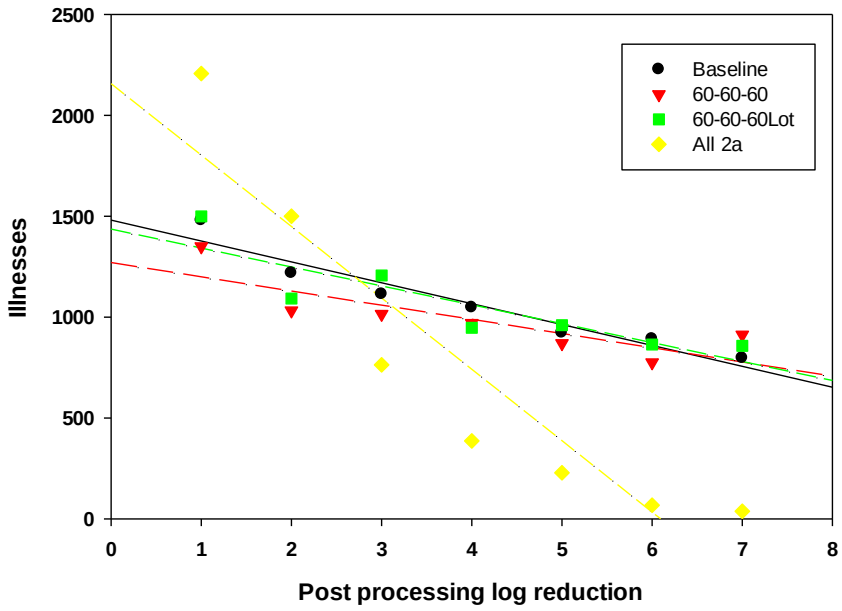

Figure 5-6. Sensitivity analysis of post-processing lethality efficiency.

There was an initial reduction in illness for the baseline and testing scenarios, but this reduction seemed to level off at about 900 cases. Recall that for these scenarios, approximately 60% of production did not undergo post processing treatment and is unaffected by this parameter. An additional scenario was evaluated where all the plants were moved to alternative 2a, so that all products received a post-processing treatment. For this scenario, increasing efficiency significantly reduced illness, to the point that

almost no cases appeared. Thus high post-processing efficiency can be valuable, but only if a sufficient fraction of production uses this treatment.

Sanitizing efficiency. The sensitivity for sanitizing efficiency is shown in Figure 5-7. None of the trends are statistically significant.

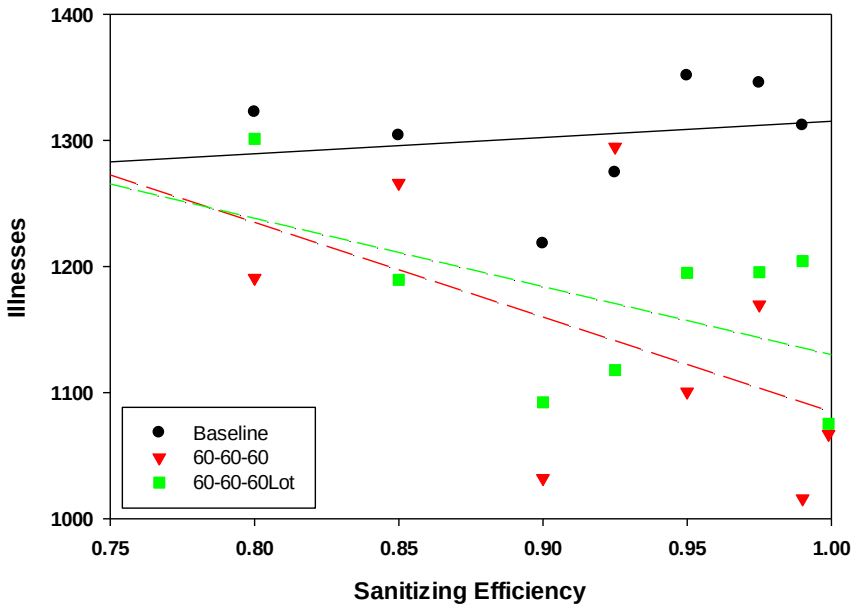

Figure 5-7. Sensitivity analysis for sanitizing efficiency.

***Listeria spp* testing time.** The sensitivity for *Listeria spp.* testing time is shown in Figure 5-8. The results with varied *L.spp.* testing time from 1 day to 6 days are not significantly different with a slight increase for scenarios of the 60-60-60FCS and 60-60-60Lot testing.

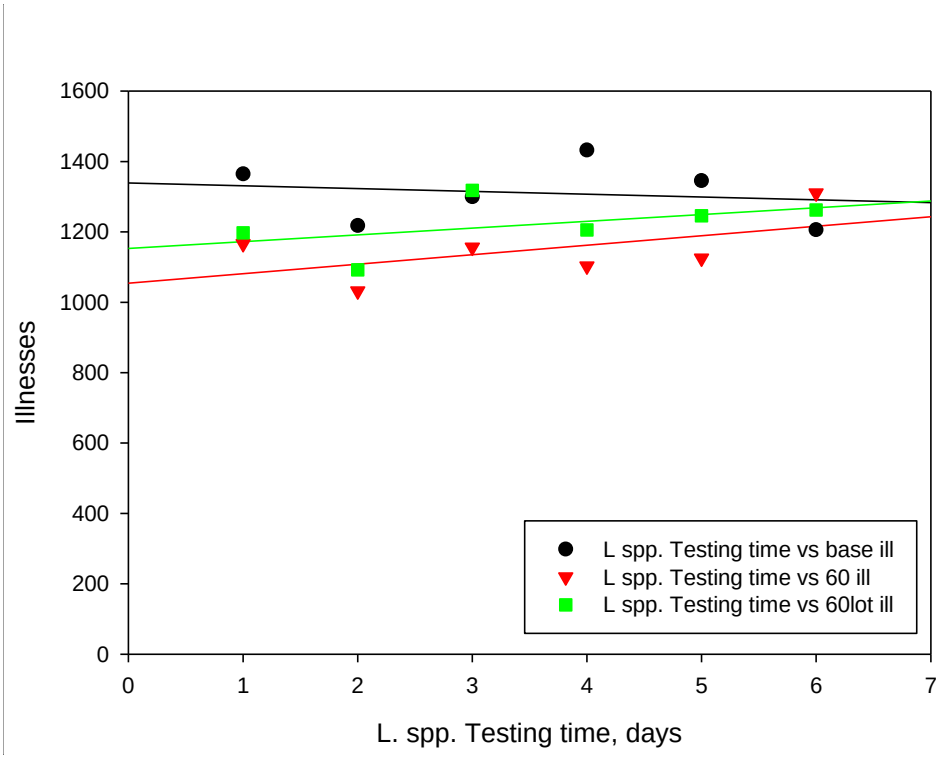

Figure 5-8. Sensitivity analysis of *L. spp.* testing time

Time from plant to retail. The sensitivity of the time from plant to retail is shown in Figure 5-9. This is a highly uncertainty parameter. The current model uses a uniform (5-10) day time period. Pradhan et al. (2009) used a uniform (10-30) day range for the same parameter.

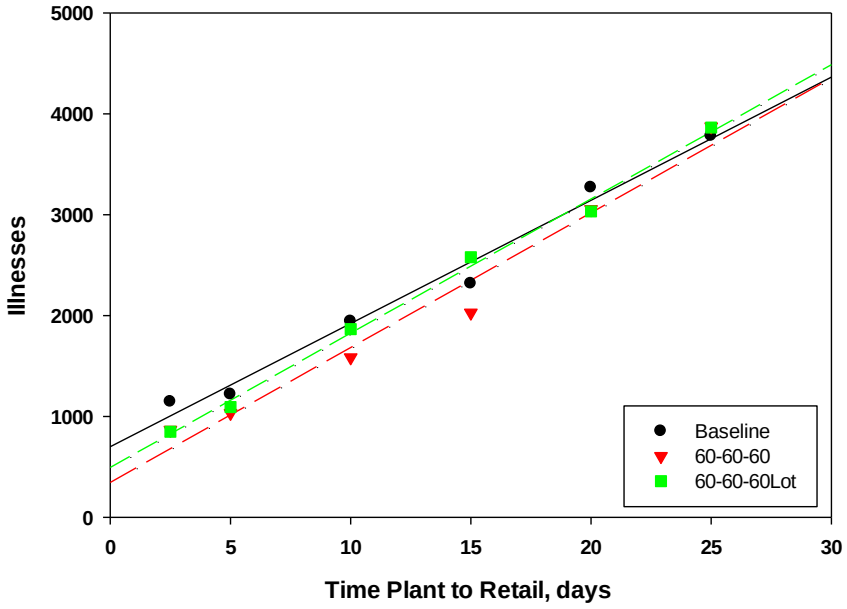

Figure 5-9. Sensitivity analysis of time from plant to retail.

A clearly increased trend in annual illnesses is found for all 3 scenarios. The increased storage time allows additional growth, which leads to more illnesses. By conducting a hazard analysis of refrigerated RTE food, Ransom investigated the scientific parameters for establishing safety-based use-by date labels (SBDLs). The estimated annual mortality was relative with many factors including intrinsic and extrinsic environments, and use-by date and the storage temperature have the highest impacts (Ransom 2005).

5.4 Summary

Holding all the products during FCS testing increased the probability of finding out the positive lots by 3 times for the establishments that implemented hold-and-test program did not help in reducing the annual illnesses. The sensitivity analyses indicated that increased FCS or lot testing was capable of finding contaminated product, which was removed from the food supply. However, the results of such testing did not lead to public health improvements. Only parameters that directly impacted growth or contamination levels in the products had any measurable effect. The hold-and-test intervention helped in removing the contaminated product with concentration higher than the detection limit but

growth of *L. monocytognenes* outside of the plants determined the major risk of *L. monocytogenes* to public health due to the temperature and used-by date abuse. Based on these results, FSIS should continue to focus on encouraging more plant to use growth inhibitors and post processing lethality.

5.5 References

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