A MULTI-PRODUCT, MULTI-FACTOR THERMAL INACTIVATION MODEL FOR *SALMONELLA* IN MEAT AND POULTRY PRODUCTS

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ABSTRACT

A MULTI-PRODUCT, MULTI-FACTOR THERMAL INACTIVATION MODEL FOR *SALMONELLA* IN TURKEY, BEEF, PORK, AND CHICKEN

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No current modeling tool accounts for all the necessary factors to validate a thermal process for ready-to-eat (RTE) meat and poultry; some do not address *Salmonella* and/or important product/process attributes, and most have not been validated against industry-relevant data. Therefore, the objective of this project was to develop a multi-product, multi-factor thermal inactivation model for *Salmonella* in meat and poultry products. First, the effect of sublethal thermal injury on subsequent bacterial heat resistance was quantified by expanding the capabilities of a previously published path-dependent *Salmonella* inactivation model. *Salmonella* inoculated ground turkey, beef, and pork samples were subjected to multiple non-isothermal treatments. The resulting path-dependent model was validated against equivalent data, showing error reductions of 63 to 82%, relative to the state-dependent model, thus confirming the importance of accounting for sublethal injury in inactivation models. In the second part, thermal inactivation data for *Salmonella* in turkey, beef, and pork were selected from published sources (n_{obs}=411, 764, and 446 for each, respectively) and used to parameterize various versions of a multi-product, multi-factor model, using ordinary least squares and mixed-effects statistical methods. Validated against industry-relevant data, most models performed favorably when considering fat content, sublethal injury, and muscle structure. Overall, this project illustrated the current difficulties and positive outcomes of pooling thermal inactivation data from different sources, parameterizing models with them, and validating them against industry-relevant data.

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1. INTRODUCTION

1.1 Background/problem statement

Ready-to-eat (RTE) food products have become an important part of the American diet, due to their convenience, nutritional value, and palatability. However, because the production chain to make these goods is highly sophisticated, there is a high risk for physical, chemical, and biological contamination to occur. While it is easier to monitor physical and chemical impurities, it is harder to do so for biological elements, such as pathogenic microorganisms, because they are naturally occurring in the food materials.

Regarding meat and poultry products, the pathogens of concern are *Escherichia coli, Salmonella, Campylobacter,* and *Listeria monocytogenes.* Together with four other pathogens, these microbial agents are considered to cause most of the foodborne illnesses in the United States (CDC 2011). Therefore, to protect consumers, the United States Department of Agriculture (USDA) bases thermal processing regulations for these products on worst-case scenarios for *Salmonella* contamination, due to the pathogen's higher resistance to heat inactivation treatments (FSIS-USDA 1999b). The regulation requires that the products be cooked to reach a specific lethality of 6.5 or 7.0 $\log_{10}$ reduction in *Salmonella* population for beef or poultry, respectively (FSIS-USDA 1999b). To fulfill these requirements, processors have two options: to follow pre-established cooking conditions, also known as "safe harbors", or to choose their own cooking conditions. The safe harbors prescribe how long to hold a product at a certain temperature to comply with the regulation (e.g., 12 minutes at 60°C). The problem with this option is that it can cause over-processing, which in turn leads to higher energy costs and a decrease in product yield and quality. On the other hand, if processors use their own cooking

schedules, they have to prove “based on scientific rationale [and] experimental data” that they comply with the lethality performance standard (FSIS-USDA 1999b). The drawback in this case is that most establishments do not have the financial or scientific resources to provide the evidence specific to their process, and the scientific information available is related almost exclusively to laboratory studies, and likely have not been validated for industrial processes.

Essentially, the regulation delegates the problem of validating process lethality to industry, which in turn does not have the necessary tools to achieve this. Nonetheless, there are microbial inactivation computer programs available to help processors validate their own cooking conditions. These are the product of the combined effort of several food safety agencies and concerned food industry groups, including the USDA, the American Meat Institute (AMI), the Food Standards Agency (FSA) and the Institute of Food Research (IFR) in the United Kingdom, and the Food Safety Centre (FSC) in Australia. The federal regulation only mentions the program developed by the USDA’s Agricultural Research Service (ARS), the Pathogen Modeling Program, and states that “[o]ther programs may be available commercially”. However, as explained in more depth in the next chapter, they present several drawbacks and fall short from the tool industry needs to provide the scientific evidence required in the federal regulation.

1.2 Goals and objectives

Given the importance of assuring food safety for consumers, it is imperative that industry obtain the necessary tools to achieve this. A model applicable to any meat or poultry cooking process, that could provide processors a documented scientific means to comply with federal regulations, would be an important step forward. Therefore, the main goal of this project was to develop a multi-product multi-factor model that could be used by industry for thermal

inactivation of *Salmonella* in different meat products. Specific objectives were: (i) to test an improved secondary model for thermal inactivation, accounting for enhanced thermal resistance resulting from sublethal injury of *Salmonella* in multiple meat products; (ii) to determine the most relevant parameters that should be included in a multi-product multi-factor thermal inactivation model for *Salmonella*; (iii) to propose and parameterize multiple options for a multi-product multi-factor thermal inactivation model; and (iv) to validate model performance when applied to independent pilot-scale data.

2. LITERATURE REVIEW

2.1 *Salmonella* and foodborne illness

According to the Center for Disease Control (CDC), there are approximately 48 million cases of foodborne illness cases each year in the United States (CDC 2011). These in turn cause an estimated 128,000 hospitalizations, and 3,000 deaths (CDC 2011). Among the most common bacterial pathogens in food, the CDC lists *Campylobacter*, *Salmonella*, and *E. coli* O157:H7 (CDC 2011). *Salmonella*, the leading bacterial cause of foodborne illness, and the pathogen of concern for this project, is naturally found in the intestinal tract of birds, reptiles, and mammals (USDA-FSIS 2010), and reaches food for human consumption via fecal contamination during processing, especially in the case of meat and poultry (Adams and Moss 2008). When infected, a person can develop non-bloody diarrhea, abdominal cramps, and fever, which characterize salmonellosis (USDA-FSIS 2010). The illness is not considered life-threatening if treated promptly. However, small children, the elderly, and individuals with weak immune systems can be more susceptible to the infection and develop additional long term complications, such as Reiter's syndrome and chronic arthritis (USDA-FSIS 2010).

2.2 Federal regulations regarding heat processing in RTE products

Effective March 8, 1999, there is one regulation governing the performance standards for the manufacture of certain ready-to-eat (RTE) meat and poultry products: USDA-FSIS, 9 CFR Parts 301, 317, 318, 320, and 381 (FSIS-USDA 1999b). The regulation encompasses three parts: lethality, stabilization, and handling. Relevant to this project are the lethality performance standards. According to the regulation, the kill can be accomplished with several antimicrobial

methods, but inclusion of a cooking step is mandatory, hence the importance of the heating process. In addition, there are no documented means of assessing the cumulative effect of several antimicrobial methods. Therefore, if this path is chosen, processors must scientifically prove that the lethality levels are reached (FSIS-USDA 1999b).

2.2.1 Safe Harbors

One of the options available to producers to comply with federal regulations regarding RTE meat and poultry products is to follow the pre-established time/temperature cooking schedules developed by the FSIS, also known as “safe harbors”. These indicate how long to cook a certain product once a minimum specified temperature is reached to achieve the regulatory standard (FSIS-USDA 1999a). For example, a beef roast would be deemed safe if cooked for 12 min once 60°C is reached throughout the product. The full table can be found in “Compliance Guidelines For Meeting Lethality Performance Standards For Certain Meat And Poultry Products” (FSIS-USDA 1999a). The safe harbors are the easiest way to comply with the regulation, and while not required, are given as an option. However, the use of these cooking schedules may limit a processor from manipulating product attributes only acquirable during the cooking step, such as moisture retention or crust-layered roasts.

2.2.2 End-point lethality

When concerned about the final quality of the product and total energy expenditure in cooking, processors may prefer to customize their own cooking schedules. In this case, they must prove “based on scientific rationale [and] experimental data” that these special cooking conditions comply with the regulatory standards (FSIS-USDA 1999b). Currently, processes need to accomplish a 6.5 $\log_{10}$ reduction and 7.0 $\log_{10}$ reduction in *Salmonella* population for RTE

meat and poultry products, respectively (FSIS-USDA 1999b). Even though this choice clearly gives processors more flexibility in their cooking options, the challenge is proving that their time/temperature choices reach the required lethality levels. This is because there currently there is no scientifically proven, generalized tool available to present this evidence, and producing proof for each specific process is economically burdensome.

A new regulation involving 9 CFR Parts 301, 303, 317, 318, 319, 320, 325, 331, 381, 417, and 430 was proposed by the FSIS on February, 2001 (USDA-FSIS 2001). If finalized, the new regulation would require that the processing method for *all* RTE and partially-cooked meat and poultry products comply with the 6.5 and 7.0 log reduction currently applicable to only certain products.

2.3 Factors affecting bacterial inactivation

There are numerous methods to inhibit bacterial growth in foods; these include addition of chemical agents (preservatives), freezing, drying, controlled atmospheres, high pressure systems, among others. On the other hand, current methods for complete pathogen inactivation via processing are mostly limited to heating and irradiation. Even with the application of these two technologies, food processors commonly use the “Hurdle concept”, which means several of these methods are used to conjointly prevent microbial growth in foods (Jay and others 2005; Adams and Moss 2008). For example, in a broad perspective, the ingredients for a RTE chicken dinner would come from a high quality source, the meal would be prepared with the addition of preservatives, and then cooked to kill any possible incident pathogens, be packaged under sanitary conditions, and finally frozen and kept at freezing temperatures until consumed. The combination of controls is designed to help ensure product safety. In the case of certain RTE

meat and poultry products, because the use of an inactivation method is imperative for the destruction of pathogens, a heating step is always included. While irradiation is approved for fresh meat and poultry products, regulations regarding these RTE products require the inclusion of a heating step (FSIS-USDA 1999b). For that reason, heating is used as the preferred method for bacterial destruction in manufacturing RTE meat and poultry products.

Bacterial behavior under heating conditions is well documented for a variety of extrinsic and intrinsic factors. Extrinsic factors refer to environmental conditions, which include relative humidity, temperature, and gaseous atmosphere – the most important of these for thermal inactivation being temperature (Adams and Moss 2008). Intrinsic factors denote properties of the food that affect bacterial response, such as nutrients, pH and buffering capacity, oxidation-reduction potential, moisture or water activity, antimicrobial constituents, food structures, and fat in the product (Adams and Moss 2008). The most relevant for heat processing would be moisture, food structure, and fat (Juneja and Eblen 2000; Juneja and others 2001; Juneja and others 2000a; Tuntivanich and others 2008; Mogollon and others 2009; Velasquez and others 2010; Orta-Ramirez and others 2005; Carlson and others 2005).

Other critical factors cover the characteristics of the microorganisms, which refer to specific growth rate, physiological state of the cells, mutualism, and antagonism (Adams and Moss 2008). For thermal processing, the factor of main interest among these would be the physiological state of the bacterial cell, specifically referring to sublethal injury. Factors most significant for heat inactivation are described in detail in the following sections.

2.3.1 Temperature

Thermal processing inherently involves an increase of temperature in the processing environment and consequently in the food product. Subjecting bacterial cells to high temperatures for a certain period will injure them and eventually kill them. There is no cut-off temperature at which bacterial cells will instantly deactivate; rather, it is a gradual process. This is because naturally occurring bacterial populations contain cells in different stages of growth, and log-phase cells are more susceptible to heat than their stationary-phase counterparts (Adams and Moss 2008). Nonetheless, higher temperature causes faster pathogen inactivation. For example, cooked beef is deemed equally safe if held for 71 mins at 55°C or 54 s at 66.1°C (FSIS-USDA 1999a). While longer cook times also have an influence on bacterial inactivation, temperature is the most determining factor (Jay et al. 2005).

2.3.2 Fat

Studies conducted by Juneja et al. (Juneja and Eblen 2000; Juneja et al. 2000a; Juneja et al. 2001) showed that fat percentage (%) in different meat and poultry products significantly increased *Salmonella* thermal resistance. The same conclusions were reached by Ahmed et al. (1995) with regard to *E. coli* O157:H7. However, the goals of these studies did not include investigating the reason behind this. While it is presumed that fat globules present in food can act as a shield for bacterial cells against heat (thus increasing their heat resistance) (Adams and Moss 2008), it can be argued that from a biological standpoint, the increased resistance could be due to biochemical interactions between the pathogens' cell membrane (a lipid bilayer) and the fats in the product. Regardless of the real cause, this means that a high-fat product would need to

be cooked for longer times and/or higher temperatures to achieve the same log reduction than a low-fat product.

2.3.3 Muscle structure

Food structures have been proven to affect bacterial growth and inactivation (Adams and Moss 2008). For the case of meat and poultry products, this is relevant to ground vs. whole-muscle products. Although the exact mechanism by which cells increase their thermal resistance in these two different environments is not completely understood, it may be due to the different internal structures in the meat and/or available water (Tuntivanich et al. 2008). Orta-Ramirez et al. (2005), Tuntivanich et al. (2008), and Velasquez et al. (2010) reported that *Salmonella* had significantly higher thermal resistance (~double) in whole muscle meat and poultry products when compared to their ground muscle counterparts. Mogollon et al. (2009) tested whole muscle, coarsely ground, finely ground, and pureed beef. *Salmonella* was significantly more resistant in whole-muscle beef than in the other products, but there was no effect of the degree of grinding.

2.3.4 Media moisture content

Studies from Carlson et al. (2005), McCann et al. (2009), Goepfert et al. (1970), and Reichart (1994) found that pathogens present in a dry environment portray a higher thermal resistance than those residing in a moist medium. For example, Carlson et al. reported that the thermal inactivation for an 8-servorar *Salmonella* cocktail decreased 64% ($p<0.01$) when water activity (a_w, a measure of available water in a product) in ground turkey was decreased from 0.99 to 0.95 (2005). In Goepfert et al.'s study, media with different a_w were prepared by using sucrose, fructose, glycerol, and sorbitol; results showed that the cells in environments with the

lower a_w yielded D-values 25 to 75% lower than those tested in the media with higher a_w (1970).

Moisture as a percentage of product composition is also commonly related to fat percentage, meaning that a high-fat product will more likely have lower moisture content than a low-fat product. Consequently, the same principle for inactivating pathogens with longer cooking times for high-fat products applies to low-moisture products. In addition to the biological effect, a secondary consequence of a dry system is that with less water present, the heat transfer process is less efficient, making the cooking less lethal to the bacterial cells.

2.3.5 Sublethal injury

Cell injury results when a process affects the bacterial cell in a negative way, but fails to kill it. Pathogens are inevitably injured when exposed to heating, freezing, and starvation environments (Wesche and others 2005). In any of these cases, cells can either become more susceptible to further inactivation procedures, or react to the changing environment by adapting, thus becoming more resistant to the processes (Mackey and Derrick 1987b; Wesche et al. 2005).

Under sublethal heating conditions, which occurs for example when slowly cooking a beef roast, a portion of the bacterial population adapts to the gradual increase in temperature by developing heat-shock proteins (Xavier and Ingham 1997a; Jorgensen and others 1996), or by other unknown mechanism (Mackey and Derrick 1990). These physiological changes allow cells to resist heat inactivation at higher temperatures, thereby affecting cooking time needed to inactivate the microorganisms (Jorgensen et al. 1996; Xavier and Ingham 1997a).

Many studies agree that heat-shocking bacterial cells increases the population resistance to heat. For example, Knabel et al. (1990) reported that the thermotolerance of *Listeria monocytogenes* increased when cells were heat shocked at 43°C for 5, 30, and 60 min. Farber et al. (1990) subjected the same pathogen to four different heat shock temperatures (40, 44, 48, and 52°C), and reported the same conclusions. Pagan et al.'s (1997) results agree with these findings with respect to time, but their study also analyzed the effect of heat shock temperature on *L. monocytogenes*. They found that just before lethal temperatures were reached, the higher the temperature, the more thermotolerant the cells became. Therefore, in processing conditions where sublethal heating is expected, microbial adaptation should be taken into account when predicting subsequent lethality.

A preliminary model addressing this issue was developed by Stasiewicz et al. (2008) for *Salmonella* inactivation in turkey thigh. The new "path-dependent" model incorporated a term that accounted for sublethal injury, which considered the amount of time the cells remained in the "heat shock region", which was determined to be between 38°C and 52°C (Stasiewicz et al. 2008). The study fitted data from an 8-serovar *Salmonella* cocktail at different residence times and hold temperatures within the heat shock region (thus yielding different degrees of sublethal injury) to the path-dependent model. Results showed that the use of the latter reduced prediction error by 56% when compared against a traditional "state-dependent" model, which did not account for sublethal injury (Stasiewicz et al. 2008).

Cold shock of cells can also lead to a change of bacterial thermal tolerance. This can happen in the processing environment when raw food materials are stored in chilled settings prior to the heat treatment. The effect of cold shock on cells is not fully documented, and different studies have reached contradicting conclusions. For example, Leenanon et al. (2001)

found in a first study that heat tolerance for *E. coli* O157:H7 was decreased with cold shock (TSB for 1 week at 5°C), while a second study (Elhanafi and others 2004) revealed that heat tolerance increased, although the cold shock treatment was different (tryptic soy broth (TSB) for 4 weeks at 4°C). Also, Bang et al. (2002) observed minimal heat tolerance increase in *Vibrio vulnificus* after a cold-shock treatment. On the other hand, Wesche et al. (2005) did not find a significant difference between the thermal tolerance of cold-shocked and control *Salmonella* cells in turkey. These conclusions, in addition to limited inactivation data on this area, make it difficult to account for this type of bacterial injury in heat inactivation models. Results are similar for the case of starvation stress. For example, Bang et al. (2002) reported a slight increase in thermotolerance for one of the three strains of *Vibrio vulnificus* tested, but Wesche et al. (2005) found no significant difference between control and starved-cell heat tolerance of *Salmonella*. In addition, because different pathogens experience starvation under many different conditions (e.g., phosphate buffer, peptone water, nitrogen or carbon starvation), the lack of data availability makes this phenomenon extremely difficult to characterize and introduce in heat inactivation models.

2.3.6 Inactivation media (liquid vs. meat and species)

Most of the initial laboratory studies measuring bacterial thermal inactivation were carried out in laboratory media, such as tryptic soy broth (TSB). However, it has been demonstrated that bacterial inactivation rates vary with the medium in which the cells develop (Shah and others 1991; Sergelidis and Abrahim 2009; Smith and others 2001; Murphy and others 2000). Therefore, if the results of any thermal inactivation study are to be applied to industry processes, it is imperative to consider the media in which the tests were carried out. This would

mean, for example, using data from ground beef when evaluating ground beef, but not for other products.

2.4 Quantifying bacterial inactivation

Given the impossibility of taking samples from every single RTE product leaving the processing line to test for pathogen presence, food processors and academics alike utilize mathematical models that describe the inactivation kinetics of these microorganisms. Another option considered in industry is "challenge studies" specific to the manufacturing plant and cooking process. These will be explained in detail in the following sections.

2.4.1 Mathematical models

Generally, thermal inactivation models are developed and tested on laboratory-scale data. This fact poses a difficulty when the model is applied to an industrial setting. This is because industrial conditions (i.e., product characteristics, process conditions, etc) rarely resemble a laboratory setting. As a result, due to the aforementioned reasons, the bacterial response may be different. Nonetheless, a recent study (Breslin 2009) tested these models on pilot plant data, which have a closer resemblance to an industry plant, thus producing better estimates of model performance.

A significant difficulty that arises when trying to apply laboratory data to industrial settings is that not all scientific studies use the same experimental methods or the same model (except for maybe the traditional log-linear model); every researcher has preferences, and specific reasons are typically not reported for choosing a certain model (Murphy et al. 2000);

(Quintavalla and others 2001; Smith et al. 2001). As a result, it is difficult for processors to decide which data and/or model best applies to their own processes.

Another obstacle processors face when choosing models is that multiple studies focus primarily on developing mathematically improved models (Vaidya and Corvalan 2009; Corradini and Peleg 2009; Corradini and others 2010) . Although they may describe certain inactivation data sets extremely well, or are able to account for multiple environmental factors, their usefulness in industry can be hindered because of the extensive experimental data needed to characterize them, or due to the lack of sufficient technical expertise or resources. Furthermore, numerous reports simply develop the models mathematically and only show theoretical results; there is no parameterization with real data, and the crucial validation against independent data is not carried out (Vaidya and Corvalan 2009; Corradini and Peleg 2009; Corradini and others 2009). As a result, the scientific literature contain numerous models, but very few reach industry or have significant implications on the food safety system.

The following section attempts to describe the different types of thermal inactivation models along with the characteristics that affect their utility for diverse applications. Notice that, as stated by McKellar et al. (2004), “the most appropriate model would be the simplest model possible for a given purpose and the given data quality, provided that it is validated and precise”. Although there are multiple studies dedicated to the use of these models, few books have been devoted to their categorization and description, and most of these contain in turn more information and detail about bacterial growth than about inactivation methods (McKellar and Lu 2004; Brul and others 2007; McMeekin and others 1993).

2.4.1.1 Primary models

Primary models define a relationship between bacterial behavior and time at a single environmental condition (Whiting and Buchanan 1993) . The most used primary inactivation model follows log-linear kinetics. However, multiple studies have proven that shoulders and tails in deactivation curves (log N/N_0 vs. time) are clearly not well described by this linear model (Peleg 2006; Juneja and Marks 2003). Therefore, several other models have been studied. These include those following log-normal, Weibull, or log-logistic distributions, and those based on probabilistic models, sigmoidal, or semi-logarithmic survival curves, among others.

2.4.1.1.1 Log-linear kinetics (D-value)

The log-linear model describes the most basic form of relationship between pathogen cells, time, and thermal inactivation. It assumes that a bacterial population present in food will decrease exponentially with time at a constant temperature. This is normally referred to as the "log-linear model" (or first-order kinetics), and can be expressed as D-values to characterize pathogen thermal kinetics. The D-value is the time it takes at a specified temperature to reach a decimal reduction in a bacterial population. A variety of D-values for different products, pathogens, and applications can be easily found in literature. Although this model is widely accepted and used, in part because of its simplicity, in part because it is the most widely studied, it presents one, but very important weakness. The drawback when trying to fit the model to inactivation data is that bacteria will often not show a log-linear decay. This is because the log-linearity assumption does not account for any type of bacterial adaptation, heating and cooling lag times, cell growth stage, among other natural factors. Van Boekel (van Boekel 2002) collected inactivation data from 55 different studies and found that the bacteria presented the

behavior characteristic of the log-linear model in only 2 of them, proving that this traditional model represents “the exception rather than the rule”. As a result, researchers have in recent years increasingly sought and studied other models with better prediction abilities.

2.4.1.1.2 Weibull distribution

The Weibull model is commonly used to describe failure phenomena. In the case of inactivation kinetics, it can be interpreted as the failure of microorganisms to survive lethal environmental conditions after a certain time (van Boekel 2002). The solved form of the equation that is relevant for inactivation microbiology is (Peleg 2006):

$$\log S = \log \frac{N}{N_0} = -bt^n \qquad (1)$$

where S is the survival ratio N/N_0, N is the current bacterial population (CFU/g), N_0 is the initial population (CFU/g), b is a inactivation rate parameter, and n is the shape of the survival curve (Peleg 2006). Depending on the bacterial survival curve, the shape will be either described as concave upward or concave downward, yielding values of $n<1$ and $n>1$ respectively (Peleg 2006; van Boekel 2002). Common speculations as to why the curvature is upward or downward refer to the bacterial population characteristics. When the curve shows an initial “shoulder”, it might mean that the cells are hardy and are adapting or strongly resisting thermal kill; in this case the curve is said to be concave downward and $n>1$. On the other hand, there might be a swift decrease in microorganism numbers with “tailing” as time increases, which may mean that most of the population was weak enough to be deactivated fast (Peleg 2006; van Boekel 2002). Note that when $n=1$, the model becomes log-linear, a special case of the Weibull model.

This model has become increasingly popular among the research community for its simplicity, and the versatility to describe different inactivation data sets. Van Boekel (van Boekel 2002) applied the model to 55 different data sets and obtained acceptable 95% confidence intervals for each case.

2.4.1.1.3 Logistic distribution function

The survival curve described by this model always has a prominent "shoulder", a section in the inactivation curve where there was heating, but negligible pathogen inactivation. Short shoulders are often the result of thermal lag times, which is the time delay it takes for the cold spot of a food product to reach lethal temperatures. This is usually due to the size and mass of the food, and the processing equipment. In such a case, the shoulder is an experimental artifact, not a biological phenomenon. However, when such an effect is observed, and with longer times than commonly expected, it might be due to characteristics of the bacterial population. This effect can also be described by the Weibull distribution with n>1 or any unimodal distribution. However, the main difference with the Logistic distribution function is that it will portray a log-linear behavior once the shoulder disappears. In the case of the other models, the inactivation lines are significantly more curved (Peleg 2006; McKellar and Lu 2004).

2.4.1.1.4 Sigmoidal survival curves

In a way, sigmoidal curves present both aspects of the Weibull distribution, because they contain both the concave upward and the concave downward behaviors. In terms of cell characteristics, this means that populations are a mixture of highly-resistant and highly-sensitive individuals. There are two types of these curves; the first ones initially show the concave downward behavior, and then, as the pathogens are deactivated, the behavior switches to concave

upward. The second group of curves is the exact opposite as the first: they initially show concave upward trends and then concave downward (Peleg 2006). Although less used than the Weibull distribution, several studies (Miller and others 2009; Feng and others 2011) cite these curves and show experimental data that are adequately described by this model.

There is no unique equation that describes sigmodial survival curves; what groups them are the observed characteristics described above. Rather, these curves are represented by different empirical models (Peleg 2006).

2.4.1.2 Secondary models

Secondary models describe the relationship between primary model parameters and the conditions in which the bacteria reside, such as temperature, pH, salt concentration, among others (Whiting and Buchanan 1993). Just like industry has a preference for the D-value model, the secondary model most commonly used and studied is the z-value, which is generally used in conjunction with the D-value. However, just as with the log-linear model, the z-value presents several drawbacks, which has led scientists, to search for a better model.

2.4.1.2.1 Z-value

The z-value model follows the same log-linearity assumption the D-value model does, and describes the temperature dependence of the D-value (Van Boekel 2008). Basically, it assumes that the D-value decreases exponentially with temperature, and so it can be defined as the change in temperature it takes for 10-fold change in D-value. Most studies in which D-values have been determined also include a z-value, and so it is common for the scientific community and industry to associate one parameter with the other, making together the traditionally used

complete Bigelow-type model (Bigelow 1921). However, as stated by van Boekel, for this widely accepted model to be valid, *both* the rate of bacterial change with time and the rate of D-value change with temperature have to be semilogarithmic- something that rarely happens, because when either the D-value or the z-value deviate, the predictions become questionable (Van Boekel 2008). For these reasons, research is continuously being carried out to develop better inactivation models.

2.4.1.2.2 Arrhenius relationship

The Arrhenius model found its way to microbiology from its common and useful application in chemistry and other sciences, in which chemical reactions are continuously studied. The Arrhenius equation can be presented in several ways, a common one being (McKellar and Lu 2004):

$$k = Ae^{\left(-\frac{E_a}{RT}\right)} \qquad (2)$$

where k is the reaction rate constant, A is a constant related to the reaction, E_a the reaction activation energy, R the Universal gas constant (8.314 J/molK), and T the absolute temperature. Because it is common that the Arrhenius equation yields high deviations at extreme temperatures, for microbiology it is generally depicted with the use of a reference temperature (T_{ref}) (Nunes and others 1993):

$$k = k_{ref}\, e^{\left[-\frac{Ea}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)\right]} \qquad (3)$$

where k_{ref} is the corresponding reaction rate at T_{ref}, T is the temperature of interest, and k the reaction rate at T. Notice that with the inclusion of a reference temperature, A is replaced by k_{ref}, making this form of the model to behave less erratically in the region around T_{ref}, and giving a physically relevant meaning to this parameter, rather than using A. For that reason, in thermal inactivation kinetics for *Salmonella* in meat and poultry products, T_{ref} is commonly chosen as 60°C (333 K) or similar.

Peleg (2006) discredits the use of the Arrhenius equation by mentioning that its predictions include high errors in low or high temperatures. However, he fails to mention that choosing an adequate T_{ref} significantly lowers the risk for erroneous predictions in the temperature range of interest. Similarly, he states that the Arrhenius equation is a second logarithmic transformation of the inactivation data (the first being $\log N/N_0$), which presumes the primary model parameters to be linear. According to him, this is not a common case in inactivation kinetics, and so the Arrhenius relationship should be invalid. However, he does not consider the fact that the parameters for this equation can be estimated via global, non-linear regression. If this is the case, the model parameters are free to be linear or non-linear as they fit best (vanBoekel 1996; Van Boekel 2008) . In addition, Dolan et al. (Dolan and others 2007) have demonstrated that when estimating model parameters for this equation, it is possible to choose an optimum T_{ref} so that the correlation coefficient between the equation's parameters is minimized and so all parameter estimates from the regression are the best possible achievable. As stated above (McKellar and Lu 2004), a model is useful if it has been appropriately validated and

fittingly describes a given set of data. For the case of the Arrhenius model, it has proven to be the best fit for several heat inactivation studies (Stasiewicz et al. 2008; McQuestin and others 2009; Murphy et al. 2000).

2.4.1.2.3 Log-logistic model

The log-logistic secondary model describes the relationship of primary model parameters to environmental factors by marking a specific environmental "set point" where the primary model parameter will change, so that it significantly affects bacterial inactivation (Peleg 2006). For example, if using the Weibull distribution as primary model, temperature as the environmental factor, and b as our parameter of interest, the log-logistic model will have the following form (Peleg 2006):

$$b(T) = \exp\{1 + \exp[k(T - T_c)]\} \quad (4)$$

Where T_c is the "set point" that marks when b(T) will drastically change, and k is the rate of change for b with respect to temperature after T_c is reached (Peleg 2006). This means that once the system reaches a high enough temperature (T_c), b(T) will make bacterial inactivation significant, otherwise, it is insignificant.

2.4.1.3 Tertiary models

Tertiary models bring together primary and secondary models and make them available to users through a computer interface (Whiting and Buchanan 1993). Not until a tertiary model is established do primary and secondary models become useful to industry. Currently, the only available tertiary models, which are relatively useful for heat inactivation processes, are the

Pathogen Modeling Program (PMP) (USDA-ARS 2007), the American Meat Institute (AMI) Lethality Spreadsheet (American Meat Institute Foundation 2010), and the ComBase Predictor (ComBase 2012); all described in the following sections. Apart from their respective advantages and disadvantages, the one main flaw present in all tools is that they do not provide the user with confidence intervals and prediction intervals for the estimated lethality. Therefore, the user is unable to know the prediction reliability. As a result, even if the process predicted lethality is over the regulatory target, it might be that the actual outcome is outside of the confidence and/or prediction intervals, still yielding an inadequately processed product.

2.4.1.3.1 Pathogen Modeling Program (PMP)

The Pathogen Modeling Program (USDA-ARS 2007) is a tool provided by the USDA's Agricultural Research Service (ARS). This is the only program mentioned in the federal regulation as an option to provide "scientific evidence" (FSIS-USDA 1999a). Although the tool is designed primarily for pathogen growth, it includes a few heat inactivation models. However, there is none available to predict *Salmonella* lethality, which is the target pathogen in the regulation. In addition, those available do not include any secondary model relationships, so that the tool cannot account for different product or processing conditions that affect the thermal treatment. Furthermore, the program is not customized to work under non-isothermal conditions, an inherent feature of industrial processes. It is evident that although widely known, this tool cannot be used at all to help processors comply with the specific regulations for meat cooking.

2.4.1.3.2 AMI Lethality Spreadsheet

The American Meat Institute (AMI) is an association that represents most of the red meat and turkey processors and suppliers in the US. The Institute revises and distributes to its

members up-to-date information relevant to the meat and poultry industries. Through the AMI Foundation, research is carried out to improve processing methods and products (AMI 2010). One of the most important outcomes of the research related to food safety is the AMI Lethality Spreadsheet (American Meat Institute Foundation 2010). This program can calculate total process lethality for any pathogen using the log-linear model, as long as the adequate D- and z-values are given. Values for *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* are listed for certain products. However, they are only examples from specific laboratory studies, which cannot adequately describe an industrial process (Breslin 2009). According to the spreadsheet instructions, the users must obtain those figures from their company's "challenge study data, from scientific literature, or other reliable sources" (American Meat Institute Foundation 2010). For most producers this is as problematic as proving a process meets the required lethality. Also, just like the PMP, this spreadsheet is not adapted to include secondary models that account for other product characteristics that can alter bacterial behavior in food. Therefore, this is tool is only useful to a certain extent if processors have their own D- and z-values.

2.4.1.3.3 ComBase Predictor

ComBase is the result of the combined efforts of several food safety agencies across the globe: the USDA's Agricultural Research Service (ARS) in the United States, the Food Standards Agency (FSA) and the Institute of Food Research (IFR) in the United Kingdom, and the Food Safety Centre (FSC) in Australia (Baranyi and Tamplin 2004). Initially, it was a compilation of microbiological data published in the UK, collected data from scientific literature at IFR, data from European research institutions, and data from members of the USDA-ARS Center of Excellence in Microbiology Modeling Informatics (CEMMI) (ComBase 2012). Today,

researchers are encouraged to submit their data to the database, and so the ComBase Browser has grown to be the biggest source for food microbiological data, containing 50,474 data records from all over the world (ComBase 2012).

Equivalent to the US's PMP, the UK's agencies developed the Food MicroModel, which eventually evolved to become the ComBase Predictor, by incorporating all the available data in ComBase (ComBase 2012). Although highly oriented to predict microbial growth with 23 models, the tool also has 6 thermal inactivation models. A variety of foodborne pathogens are targeted on both types of models, while spoilage microorganisms are also included in the growth models (ComBase 2012). Differing from the PMP and the AMI Lethality Spreadsheet, the ComBase Predictor does include secondary models, allowing the user to adjust the prediction to several environmental factors such as salt concentration, changing temperature, pH, CO_2 concentration, etc (ComBase 2012). Also, predictions for up to four microorganisms can be carried out simultaneously (ComBase 2012). However, because the data used for the models comes from those compiled by ComBase, and most such data were developed in non-food media, the Predictor warns the user that the "growth models represent fail-safe, conservative predictions" (ComBase 2012). This statement is consistent with the results of the study by Tamplin et al. (2005), where it was determined that "the absence of an appreciable lag period at 6, 8, and 10°C suggest that more occurrences of growth at refrigeration temperatures should be expected than are typically assumed in risk assessment models". In addition, the specific models used for each prediction are not documented, and the exact sources of the data used to develop model parameters are not listed. Therefore, the quality of the tool's predictions can easily be questioned.

An additional drawback is that the Predictor asks for data within the temperature range of 54.5 and 65°C, and the "first time-point must be [zero]" (ComBase 2012). This represents a problem for processors, because normally time/temperature data are logged once the products enter the heating instrument and until the end of cooling, meaning that there would have to be manual manipulations of the data to select the appropriate points that the model can use. In addition, some processes may reach temperatures higher than 65°C, in which case the Predictor does not accept the data to carry out the prediction.

While the ComBase Predictor may have several useful characteristics for growth predictions, the reasons stated above are sufficient to deem this tool inadequate for thermal processing validations in meat and poultry applications.

2.4.2 Challenge studies

Challenge studies involve monitoring a product process from beginning to end and assessing the bacterial growth and inactivation throughout all its stages. This inevitably involves actually inoculating a product with the pathogen of concern and running it through all the steps in the production process, then analyzing the lethal effects the complete process had on the bacterial population (Adams and Moss 2008). This option may seem appealing from the point of view of obtaining results that are specific to the product and process in question and thus unequivocally assessing the effectiveness of the procedure. However, deliberately introducing a pathogen in an industrial setting is not feasible, due to obvious contamination concerns. For that reason, this method is not highly considered by processors. However, pilot-plant challenge studies, such as those carried out by Breslin (2009) and Wiegand (2012) come closer to resembling an industrial setting and give good approximations of bacterial death kinetics.

3. MODELING SUBLETHAL THERMAL INJURY

3.1 Introduction

Although important to certain industrial processes, the potential effects of sublethal injury on foodborne pathogens have not been quantified in a manner applicable to prediction models. Currently, most secondary models (whether Arrhenius-type, Bigelow-type z-value, or other empirical form) assume that the rate of inactivation is a function of the instantaneous state of the system (e.g., temperature, fat content). Such state-dependent models may be ineffective when pathogens are subjected to sublethal heating (Jorgensen et al. 1996; Stephens and others 1994; Mackey and Derrick 1986), which can occur during slow-cooking processes, and which can cause cells to increase their thermotolerance (Bunning and others 1990; Mackey and Derrick 1986; Shah et al. 1991; Xavier and Ingham 1997a). If this occurs, then a state-dependent model might over-predict the process lethality, as bacterial inactivation rate does not depend solely on the state of the system, but also on the thermal path preceding the lethal condition.

Several prior studies have reported the effect of heating rate on inactivation rate (Mackey and Derrick 1987a; Quintavalla and Campanini 1991; Stephens et al. 1994). These studies reported that slow heating rates (on the order of < 1°C/min) induce a higher heat resistance in cells than did fast or instantaneous heating. This is consistent with the understanding that slow heating rates inherently expose the bacteria to extended periods in the temperature range previously described as inducing the heat shock response (i.e., approximately 40-50°C), thereby allowing sufficient time for that response to be expressed.

From this evidence, other studies did modify secondary models and incorporate heating rate as a variable to account for increasing thermotolerance due to heat adaptation (Corradini and

Peleg 2009; Valdramidis and others 2007; Stephens et al. 1994). However, the previously cited heat shock literature indicates that heat shock is a direct function of time spent in a critical temperature range, not of heating rate (Farber and Brown 1990; Pagan et al. 1997; Knabel et al. 1990; Diller 2006). In other words, the adaptive response of the bacteria is expressed over time when exposed to certain temperatures (i.e., a function of time and temperature), so that the cellular response is not a function specifically of the rate of temperature change. Therefore, heating rate as a variable in an inactivation model is a surrogate for the underlying cellular mechanisms of adaptation, and therefore may not be phenomenologically consistent with outcomes that can occur during certain treatments, such as those that include rapid heating rates but static holding periods at sublethal temperatures.

An alternative method to modeling heat inactivation with prior heat shock was developed by Vaidya et al. (2009). They added a "memory kernel" to their model, which allowed the model to account for "events at a temporal distance" that influenced the present state of a system. However, because a specific value was not assigned to the "temporal distance", the latter is only an arbitrary measure. This means that for any point in time, it is unknown how far the memory kernel goes back to account for heat effects. Also, using a constant "temporal distance" means the memory kernel makes no distinction between the sublethal and lethal regions. This is again inconsistent with heat shock literature, given that bacterial adaptation occurs only over a known temperature range (Pagan et al. 1997), and once the lethal temperatures are reached, adaptation essentially ceases, and only bacterial inactivation occurs.

Overall, the previously proposed models have potential for industry use, but they still present significant weaknesses. For instance, with the exception of Valdramidis et al. (2007), who reported accuracy and bias factors, sufficient statistical measures of model performance,

such as root mean squared errors (RMSEs), are rarely given to quantitatively test the predictive ability of the models. Such quantitative measures of the predictive ability and robustness of models are critical before they can be adapted to industrial applications.

A different approach was taken by Stasiewicz et al. (2008) (section 2.3.5). Their model incorporated an integral of thermal history in the sublethal region as a variable influencing subsequent inactivation rate. In comparing this path-dependent secondary model to a state-dependent secondary model, both were incorporated into a Weibull primary model applied to non-isothermal treatments with varying sublethal histories, the RMSE was lowered from 2.5 to 1.1 log CFU/g for the state- and path-dependent models, respectively (Stasiewicz et al. 2008). However, the experiments were constrained to only one type of meat (ground turkey thigh meat), and few data points accounted for very long exposures to sublethal temperatures that might correspond to commercial cooking schedules of slow-roasted products. Hence, it is important to test whether these results can be extended to longer exposures in the sublethal region, and to what degree substrate affects the model parameters. Therefore, the goals of this section were: (i) to extend the capabilities of the previously developed path-dependent model to account for longer sublethal heating times and three different meat species, and (ii) to validate the model against isothermal and non-isothermal independent data.

3.2 Materials and Methods

This study entailed non-isothermal heat treatment of *Salmonella*-inoculated meat samples (turkey, beef, and pork), including treatments designed to impart sublethal injury. The resulting data were used to estimate the parameters for a novel, path-dependent secondary inactivation

model that accounts for sublethal history. The model was then validated and compared to a traditional, state-dependent model.

3.2.1 Model development

The traditional, state-dependent inactivation model follows a Weibull distribution form (Peleg 2006):

$$\log S = \log \frac{N}{N_0} = -bt^n \qquad (1)$$

where S is the survivor ratio, N is the number of microorganisms at time t, and N_0 is the initial microorganism population. In this work, the expression “log reduction” will be used, just as is in the federal regulation; log reduction is equivalent to the negative of the survivor ratio (i.e., -log S) . The parameters b and n are estimated via non-linear regression, where n describes the shape of the survival curve. Although any suitable secondary model might be used to describe b as a function of temperature, Stasiewicz et al. (2008) previously reported, for data similar to the present study, that b can be described with an Arrhenius-type dependency:

$$b(T) = b_{ref} \cdot \exp\left[-\beta_1\left(\frac{1}{T(t)} - \frac{1}{T_{ref}}\right)\right] \qquad (5)$$

where β_1 describes the effect of temperature (K) on b. The state-dependent model in this study was equations (1) and (2). A previously reported, path-dependent inactivation model (Stasiewicz et al. 2008) takes the following modified form:

$$b(T,t) = b_{ref} \cdot \exp\left[-\beta_1\left(\frac{1}{T(t)} - \frac{1}{T_{ref}}\right) - \beta_2\tau(T,t)\right] \qquad (6)$$

and

$$\tau(T,t) = \int_{t_{T=HS_{upper}}}^{t_{T=HS_{lower}}} \langle T(t) - HS_{lower} \rangle dt \qquad (7)$$

where the sublethal history (τ) is quantified as the integral of the temperature vs. time curve in the heat shock region (i.e., from $T=HS_{lower}$ to $T=HS_{upper}$), where *Salmonella* can increase its thermal tolerance (Figure 1). In equation (3), β_2 scales the impact of this phenomenon so that increasing sublethal history (τ) causes a decrease in b. Based on prior research on heat shock response, Stasiewicz et al. (2008) set the heat shock region to be 38 to 52°C (HS_{upper} and HS_{lower}). The final, path-dependent model is obtained by combining equations 1, 3, and 4.

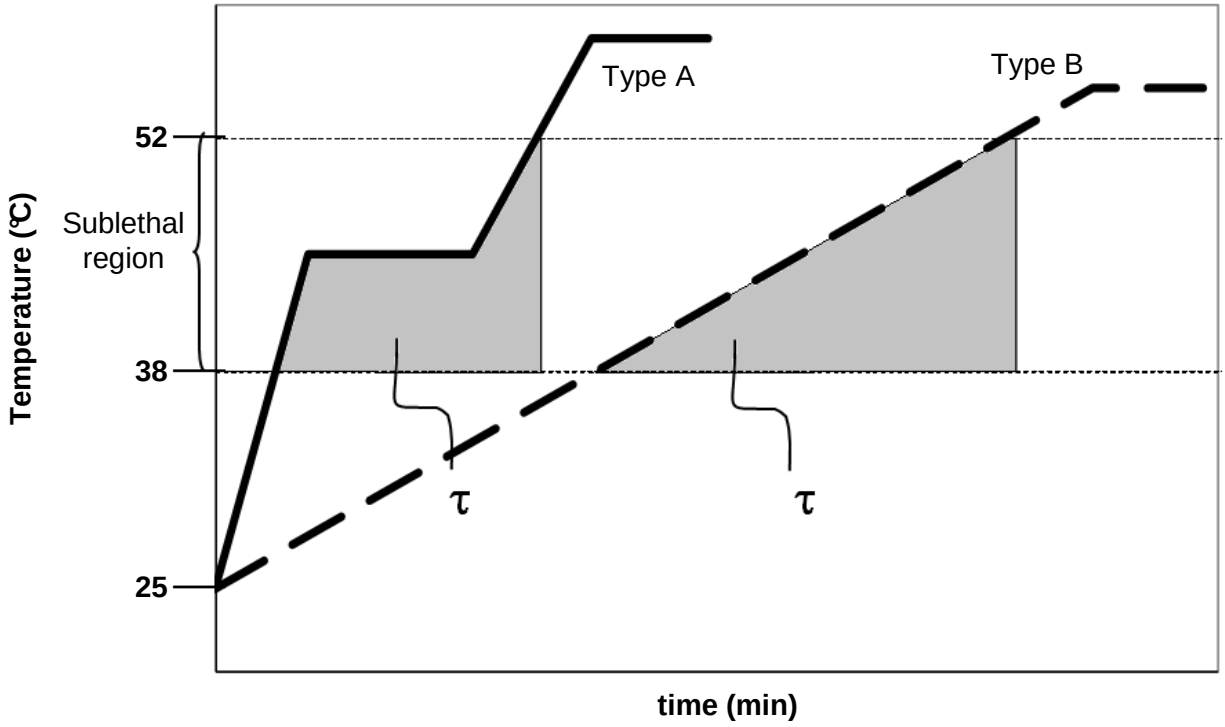

Figure 1. Representative heating profiles, where τ is the integral of the time-temperature profile within a prescribed heat shock region.

3.2.2 Inoculum

The inoculum was an 8-serovar *Salmonella* cocktail, previously obtained from Dr. V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA), which included: *S*. Thompson FSIS 120 (chicken isolate), *S*. Enteritidis H3527 and H3502 (clinical isolates phage types 13A and 4 respectively), *S*. Typhimurium DT 104 H3380 (human isolate), *S*. Hadar MF60404 (turkey isolate), *S*. Copenhagen 8457 (pork isolate), *S*. Montevideo FSIS 051 (beef isolate), and *S*. Heidelberg F5038BGI (human isolate). Before use, all serovars were kept separately at -80°C in vials containing tryptic soy broth (TSB; Difco Laboratories, Sparks, MD) and 20% glycerol. Cultures were started by transferring one loop of each frozen culture into separate tubes containing 9 ml of tryptic soy broth with 0.6% w/v yeast extract (TSBYE; Difco Laboratories, Sparks, MD) and incubating at 37°C. All serovars were separately grown for 24-36 h and transferred at least twice at ~24 h before use for inoculation.

3.2.3 Meat preparation

Whole-muscle skinless turkey breast, beef round, and pork loin were obtained from a local supplier as close to the time of harvest as possible, and transferred to Michigan State University's meat laboratory at <4.4°C. The meat was ground (Hobart, model 4146, Troy, OH) (twice through the 4.8 mm hole plate, then once through the 3.2 mm hole plate), vacuum packaged in double plastic bags, frozen and kept at -23°C. The packaged and frozen samples were irradiated to >10kGy (Food Technology Services, Incorporated, Mulberry, FL, 33860) to eliminate background microflora. The effectiveness of the irradiation was confirmed by thawing random samples, diluting them (1:5) in sterile 0.1% peptone water (Difco™, Becton, Dickinson and Company, Sparks, MD), and plating them on Petrifilm™ aerobic count plates (3M

Microbiology Products, St. Paul, MN). The irradiated samples were kept frozen until needed. Prior to inoculation, meat packets were thawed overnight at 4°C. Moisture and fat percentage measurements were determined on fresh samples using AOAC methods 950.46B and 960.39, respectively.

3.2.4 Inoculation

On the day of each experiment, a mixture containing 9 ml of each serovar in TSBYE was centrifuged (6000x*g* for 20 min at 4°C). The resulting pellet was then resuspended in 7.2 ml of peptone water to an inoculum population of ~10^{10} CFU/ml (confirmed by serially diluting in 0.1% peptone water and plating on Petrifilm^{TM} aerobic count plates). The inoculum (400 µl) was manually and aseptically mixed into 40 g of meat for 5 min, targeting a homogeneous population of ~10^{8} CFU/g in the sample. Individual 1 g samples were then pulled from the 40 g for the heat treatments.

3.2.5 Heat treatments

All treatments were carried out in a temperature-controlled programmable thermocycler (ENE Mate, Model FPROGO2G, ISC Bioexpress, Kaysville, UT), with a manufacturer-stated accuracy of ±0.1°C. For each test, the 1 g sample was divided into 0.2 g portions and inserted into five 0.2 ml thin-walled PCR microtubes with attached caps (Dot Scientific Incorporated, Burton, MI). For triplicate testing, fifteen microtubes were placed in the thermocycler and then equilibrated to 25°C before being subjected to one of the 53 different heating profiles (described below). Immediately after the heat treatment, all samples were cooled in ice and held at ~4°C for recovery and plating on the same day.

Each heating profile consisted of a randomly selected combination of a linear heating rate (1, 2, 3, 4, or 7 K/min), a variable-length sublethal holding period (at 40, 45, or 50°C), and a final lethal temperature (55, 58, 61, or 64°C). The sublethal holding period was determined depending on the randomly selected sublethal history (τ) target (15, 25, 34, 50, 100, 200, 370, or 500 K·min). The holding time at the lethal temperature was chosen to achieve a nominal target lethality of ~3 or 5 log reductions. Total treatment times were between 8.17 and 251.92 minutes, and the full sample set consisted of 159 data points for each species. There were two types of heating profiles (Figure 1): type A, which included the sublethal holding period and used only a heating rate of 7 K/min, and type B, which did not include a sublethal holding period, but did use all of the stated heating rates.

3.2.6 Recovery of samples

For each of the triplicate tests, five 0.2 g cooked subsamples were recovered, recombined into a 1 g sample, diluted (1:5), and serially diluted in 0.1% sterile peptone water for duplicate plating on Petrifilm™ aerobic count plates, which were incubated at 37°C for ~48 h before enumeration. A 5 g sample of uncooked, inoculated meat was diluted (1:5) and plated as a positive control against which the heated samples were compared to determine the process lethality (i.e., log reductions). In addition, a 5 g sample of non-inoculated meat was diluted (1:5) and plated as a negative control to verify meat sterility.

3.2.7 Model parameterization and validation

Before carrying out the state-dependent model parameter estimation on the non-isothermal data from this study, the Weibull model (equation (1)) and the corresponding simplified log-linear version (n=1 in equation (1)) were fitted to raw isothermal inactivation data

(individual inactivation curves) previously obtained for the same *Salmonella* cocktail and turkey, beef, and pork used in this study (Tuntivanich et al. 2008; Breslin 2009; Velasquez et al. 2010) (n_{obs}=90, 148, and 121 for each species, respectively, from a total of 13 different species-temperature combinations). Temperatures tested were 55, 58, 60, 62, 62.5, and 63°C. Model parameters were estimated via non-linear regression (Gauss-Newton with step halving, as used by JMP, Version 7. SAS Institute Inc., Cary, NC, 1989-2007) for the Weibull model and linear regression for the log-linear version. Regressions were performed for each temperature and for each species separately. Following fitting, two tests were conducted to evaluate which model better described the data: (i) Akaike's Information Criterion corrected for sample sizes (AIC_C), and (ii) a t-test. AIC_C compares models by creating a balance between goodness-of-fit and the number of parameters; then it determines the likelihood that one model is better at describing a set of data than the other (Motulsky and Christopoulos 2004). For example, model A might have a better goodness-of-fit (represented by the sum of squared errors, SSE) than a simpler model B, but when AIC_C is applied, model A is penalized for having more parameters than model B, and the result might show that the latter is more likely to be correct in describing the data. The t-test also can be used to test whether a certain parameter estimate is statistically different (α=0.05) from a fixed value (Bardsley and others 1995), in this case, for n=1 in equation (1). A t-value is calculated:

$$t = \frac{fixed\ \ value - parameter\ \ estimate}{estimated\ \ parameter\ \ standard\ \ error} \qquad (8)$$

and compared to the *t* distribution with n_{obs} minus n_{para} degrees of freedom, where n_{obs} is the total number of observations and n_{para} the number of parameters (Bardsley et al. 1995), to test the null hypothesis that n=1. Both AIC_c and the t-test calculations were completed in Excel (Microsoft Excel. Microsoft, 2003. Redmond, WA). Parameter estimates for each individual inactivation curve and their corresponding standard errors were obtained from the non-linear and linear regressions.

To obtain the state-dependent model parameters for all the pooled data, the same isothermal inactivation data sets were used (n_{obs}=90, 148, and 121, for turkey, beef, and pork, respectively). Global regressions on the respective data sets were done using completed by minimizing the sum of squared errors (SSE) using Excel's Solver function. Error was defined as the difference between the experimental log reduction and the state-dependent model predicted lethality (equations 1 and 2). Additionally, the non-isothermal calibration sets developed for this study (section 3.2.5) were used to calculate new parameters for the state-dependent model. Parameter estimation for these was done using MATLAB's *nlinfit* function (MATLAB R2011a, The MathWorks Inc., Natick, MA, 2011). Also using a global regression method, this function estimates the coefficients of a nonlinear function using the least squares estimation via the Gauss-Newton algorithm (with Levenberg-Marquardt modifications for global convergence). A sample code can be found in section 6.1. This analysis was carried out to determine whether poor predictions by the state-dependent model were due to sublethal heating rather than the use of an isothermally-calibrated model to predict non-isothermal microbial inactivation.

For the path-dependent model, all data from the 53 triplicated non-isothermal tests (n_{obs}=159 for each species) were used. To obtain the model parameters (b_{ref}, β_1, and β_2), 36 randomly selected tests were used (calibration set, n_{obs}=108 for each species). All parameters were obtained using MATLAB's *nlinfit* function, where the error was defined as the difference between the experimental and the path-dependent model prediction of log reductions (equations 1, 3, and 4). A sample code for this can be found in section 6.1.

Validation of the calibration results for each meat species was carried out against the remaining 17 non-isothermal tests (validation set, n_{obs}=51 for each species). In addition, the non-isothermal calibrations for both models were validated against the isothermal data to test whether: (i) the path-dependent model was reducible and applicable to the simpler isothermal case, and (ii) it was possible to obtain better inactivation predictions by changing from a state-dependent to a path-dependent model while using the same inactivation data.

3.3 Results and discussion

3.3.1 Meat sterility and composition

Tests determining irradiation effectiveness returned negative results (i.e., zero plate counts) for all irradiated samples and negative controls. For the isothermal tests, the product compositions were as follows: turkey breast was 72.5±0.2% water and 1.0±0.6% fat (Tuntivanich et al. 2008), beef round was 72.5±1.2% water and 2.7±1.3% fat (Breslin 2009), and pork loin was 73.6±2.7% water and 2.5±0.9% fat (Velasquez et al. 2010). For the non-isothermal tests, turkey breast was 74.0±0.9% water and 1.1±0.2% fat, beef round was 73.8±0.3% water and 2.3±0.6% fat, and pork loin was 68.5±0.9% water and 10.0±3.3% fat. Product composition

between isothermal and non-isothermal tests was noticeably different only for pork. However, based on prior results (Juneja et al. 2000a), for a fat difference of 2.5 to 10.0% and the time-temperature combinations used, lethality would be expected to vary at the most by 0.5 log CFU/g. Therefore, it was assumed for this study, that the estimated variation would not meaningfully affect the final conclusions.

3.3.2 Calibration: state-dependent model parameterization

AIC_c results showed that in 8 out of the 13 isothermal treatments (species-temperature combinations), the log-linear model (n=1 in equation 2) was more likely to be correct than the Weibull model (n≠1) in describing the isothermal data. Likeliness ranged from 52% to 100%, with the average being 74%. For the remaining 5 tests where the Weibull model was more likely to be correct, likeliness ranged from 62% to 99%, with the average being 86%. In average, for the 13 tests, the log-linear model was 51% more likely to be correct in describing the data. In addition, even with a relaxed significance level (α=0.1), the t-test did not reject the null hypothesis (H_0: n=1) in 10 out of the 13 tests. The results from both tests do not indicate that the use of a log-linear model will always give a better outcome, but did give enough evidence that it was marginally the better choice for most of the data used in this study. Although Stasiewicz et al. (2008) reported successful use of the Weibull model (n≠1) with the proposed path-dependent model (equations (1), (3), and (4)), the simpler log-linear version was used in the present study, given the results of the statistical tests reported above. In either case, the modification and testing of the path-dependent secondary model (equations (3) and (4)) was the primary objective of this study, so that confirmation of the performance of equations (3) and (4) with multiple primary models will further support the underlying construct of the secondary model form.

The calculated parameters and accuracy of the model, represented by RMSE and bias (mean residual), for the state-dependent model with both the isothermal and non-isothermal calibration sets are shown in Table 1. The reference temperature used was 60°C, as it is approximately the average of the lethal temperatures used in the treatments. The correlation coefficients, and standard and relative errors for the path-dependent model parameters (b_{ref}, β_1, β_2) can be found in section 6.2.

Table 1. Model parameters and results against calibration and validation sets.

Calibration								Validation against isothermal data		Validation against non-isothermal data (validation set, n_{obs}=51)	
			Parameters			Statistics		Statistics		Statistics	
Model	Data source	n_{obs}	b_{ref} (min^{-1})	β_1 (K)	β_2 (K^{-1} min^{-1})	RMSE	bias	RMSE	bias	RMSE	bias*
						(log CFU/g)					
State - dependent ♦	Isothermal turkey	90	0.97	49,315	NA	0.43	0.06	NA	NA	2.91	-1.50
	Isothermal beef	148	1.02	46,829	NA	0.90	-0.03	NA	NA	2.22	-1.25
	Isothermal pork	121	0.90	42,590	NA	0.99	-0.06	NA	NA	4.55	-2.58
	Non-isothermal turkey calibration set	108	0.57	48,491	NA	1.42	0.51	1.04	-0.77	1.75	0.80
	Non-isothermal beef calibration set	108	0.57	40,851	NA	1.53	0.56	1.45	-0.98	1.77	0.88
	Non-isothermal pork calibration set	108	0.45	52,382	NA	1.33	0.53	1.94	-1.45	1.49	0.82
Path-dependent †	Non-isothermal turkey calibration set	108	0.91	50,787	0.0017	0.66	0.07	0.46	-0.09	0.90	0.14
	Non-isothermal beef calibration set	108	0.94	44,710	0.0018	0.93	0.12	0.99	-0.19	0.81	0.24
	Non-isothermal pork calibration set	108	0.70	54,713	0.0016	0.87	0.18	1.52	-0.73	0.75	0.24

*bias: mean residual.

♦ state-dependent model was equations (1) and (2), with n=1 in equation (1).

†path-dependent model was equations (1), (3), and (4), with n=1 in equation (1).

3.3.3 Validation: application of the state-dependent model to non-isothermal data

When the isothermally-calibrated state-dependent model was applied to the non-isothermal validation sets, substantial over-prediction errors were observed (Figure 2, Figure 3, and Figure 4). The largest deviations were -10.1 log CFU/g, -7.5 log CFU/g, and -14.7 log CFU/g for turkey, beef, and pork respectively – all caused at the largest tested sublethal history (τ=500 K·min). Analysis of variance (ANOVA) revealed a statistically significant relationship between the sublethal history (τ) and the traditional state-dependent model error ($p<0.0001$ for all species), indicating that the state-dependent model error increased as τ increased. This agrees with previous work (Stasiewicz et al. 2008), and reaffirms the importance of a model that can account for sublethal injury in a variety of heating profiles. In addition, this concurs with heat shock literature (Farber and Brown 1990; Knabel et al. 1990; Pagan et al. 1997), in that a combination of time and temperature in the sublethal region, described by τ, causes an increase in bacterial thermotolerance. It can be seen (Table 1) that the RMSEs for the isothermally-calibrated, state-dependent model applied to the non-isothermal data are the largest of all model fittings, and that the corresponding biases are located in the fail-dangerous zone (i.e., bias<0 indicates over-prediction of lethality). These results show that the combined use of isothermal data and state-dependent models is ineffective for predicting microbial inactivation in cooking conditions where significant sublethal injury can occur.

The state-dependent model also was fitted to the non-isothermal calibration sets to verify that it was the model, rather than the isothermal calibration, that caused poor inactivation predictions. When applied to the non-isothermal validation sets, the RMSEs for this case (Table 1) were reduced from the isothermal calibration model predictions, but still reached values of

more than 1.49 log CFU/g. Overall, the biases were ~0.8 log CFU/g, which could be considered as being fail-safe. Even though application of such a model might mean a safe product, it would also mean overprocessing, which leads to lower product yield and therefore monetary losses. These findings confirm that the state-dependent model is unable to effectively describe inactivation profiles where sublethal heating takes place, even if non-isothermal data are used to estimate the model parameters.

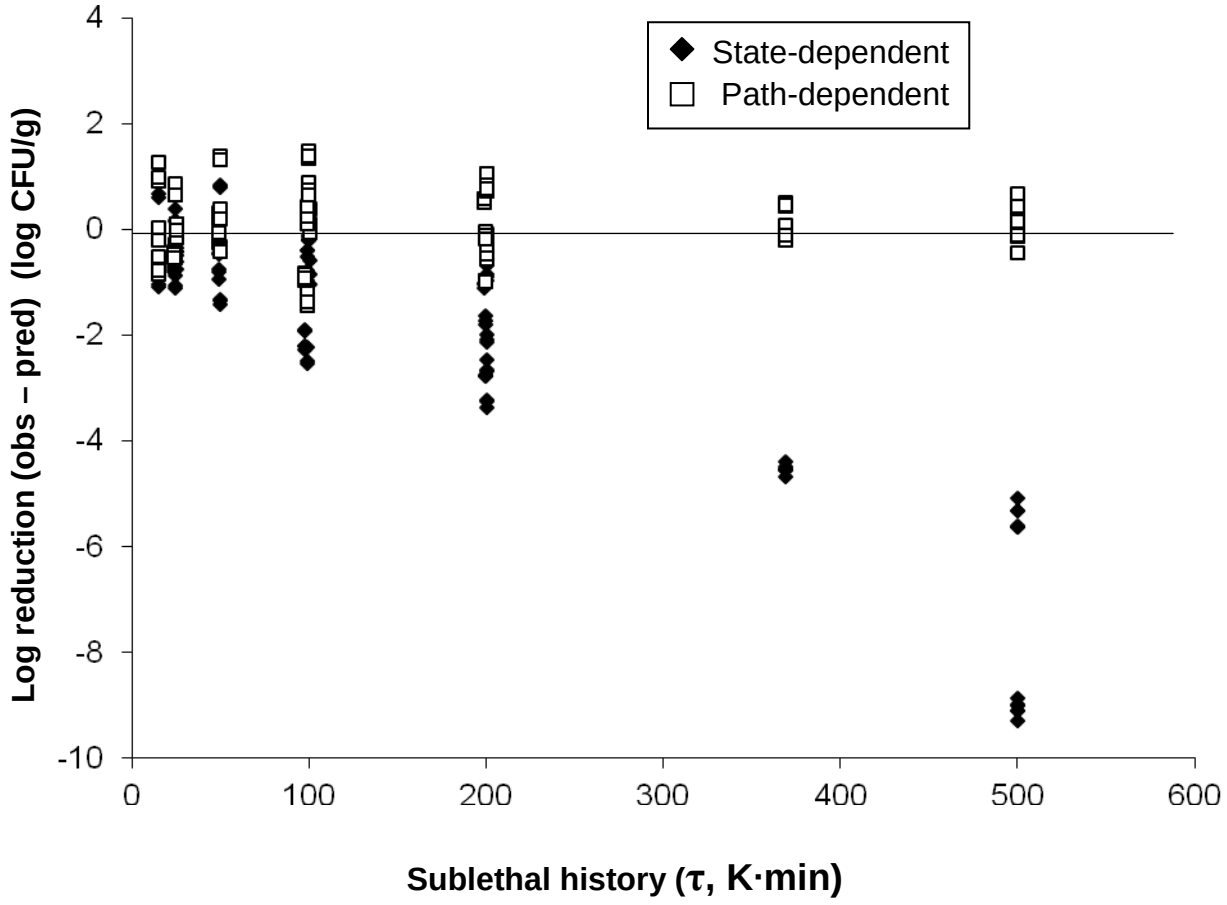

Figure 2. Log reduction errors (observed-predicted) for the state-dependent model and path-dependent model for the turkey calibration set.

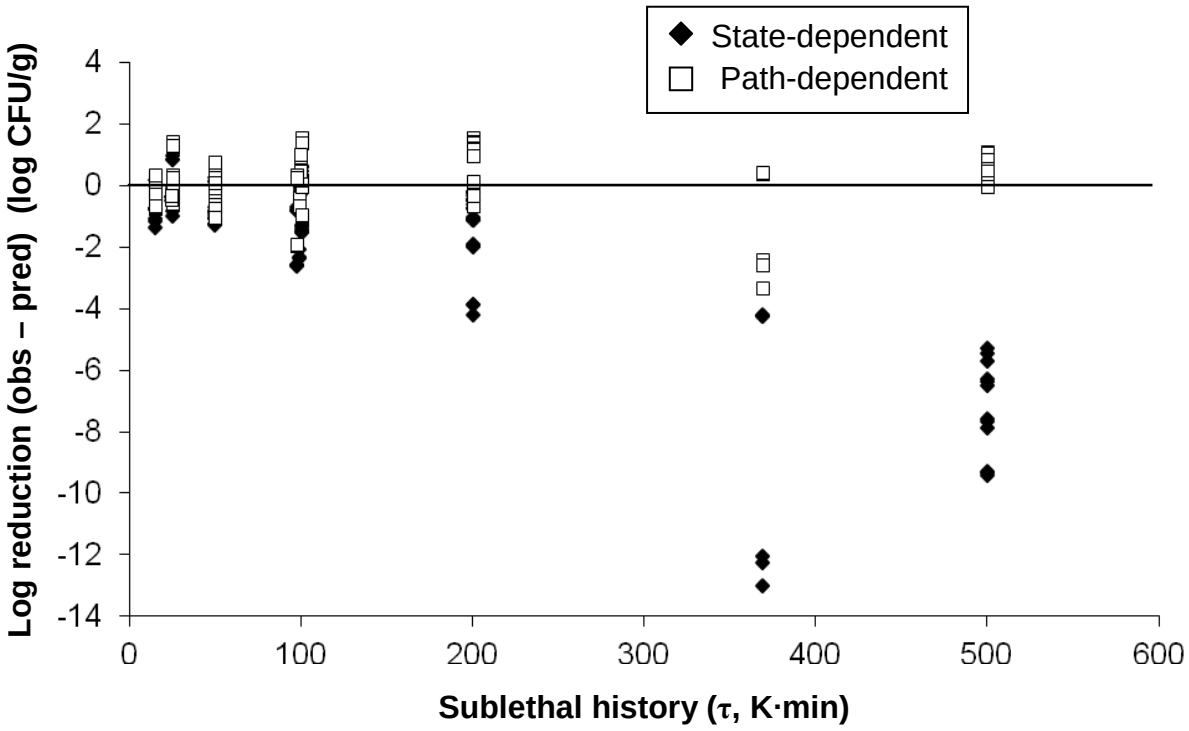

Figure 3. Log reduction errors (observed-predicted) for the state-dependent model and path-dependent model for the beef calibration set.

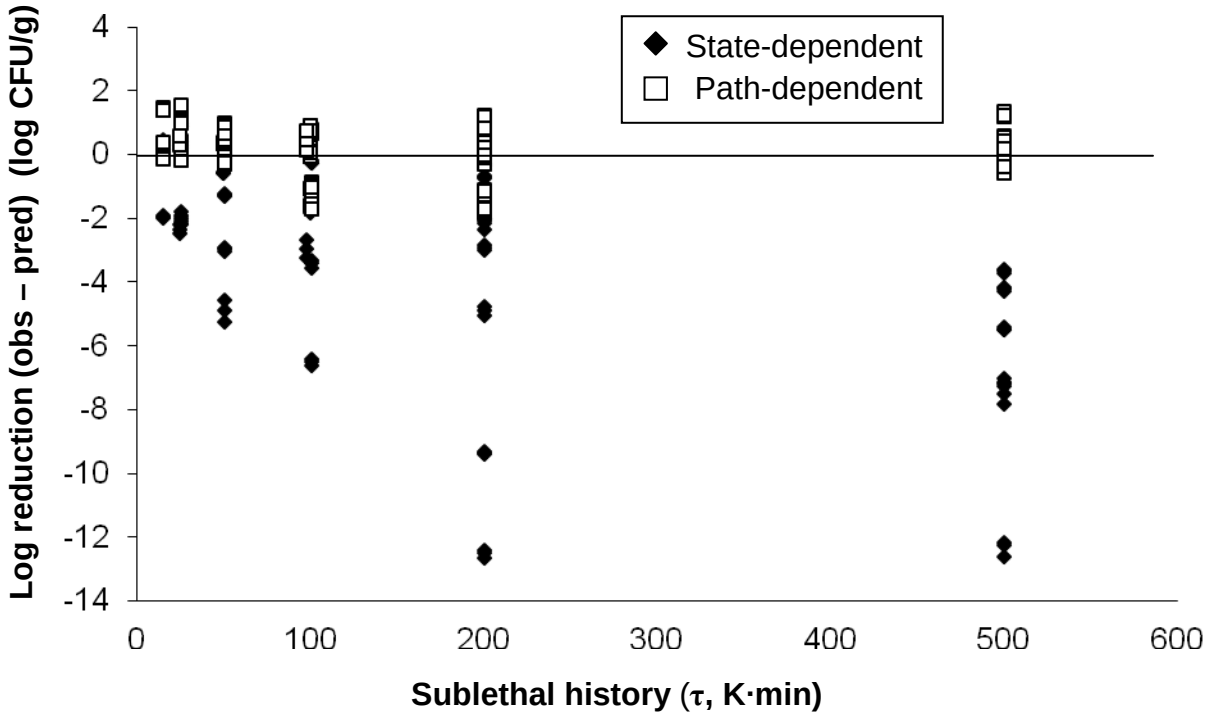

Figure 4. Log reduction errors (observed-predicted) for the state-dependent model and path-dependent model for the pork calibration set.

3.3.4 Calibration: path-dependent model parameterization

In comparison to the state-dependent model, the path-dependent model yielded better RMSEs (Table 1) in the case of beef and pork, and slightly worse values for turkey (0.43 vs. 0.66 log CFU/g), while bias was better for all species.

Evaluation of the residuals from the application of both the state- and path-dependent models to all calibration sets (Figure 2, Figure 3, and Figure 4) shows that the path-dependent model performed better than the state-dependent model, with maximum fail-dangerous errors of -3.3 and -14.7 log CFU/g, respectively. Prediction improvement by the path-dependent model is especially evident as sublethal history increases, showing the positive effect of accounting for this phenomenon in the thermal inactivation model.

3.3.5 Validation: application of the path-dependent model to isothermal and non-isothermal data

The use of the path-dependent model on the non-isothermal validation tests showed a substantial reduction in prediction error when compared to the state-dependent model (Table 1 and Figures 5-7). All RMSEs were below 0.9 log CFU/g, which translates into a 69%, 63%, and 82% reduction in RMSE from the state-dependent model for turkey, beef, and pork, respectively. This shows that the path-dependent model provides much improved accuracy, compared to the state-dependent model when describing data where sublethal heating has occurred. Additionally, all bias values are in the slight underprediction range (0.14-0.24 log CFU/g), a great improvement over the high overprediction biases from the state-dependent model (-1.5 to -2.6 CFU/g). Because the negative values are considered fail-dangerous, they show that a state-

dependent model cannot be relied upon to effectively predict microbial inactivation where non-isothermal conditions and significant sublethal heating has occurred.

When applied to the isothermal data, the state-dependent model also performed favorably (Table 1), with RMSEs comparable to those yielded by the isothermally-calibrated state-dependent model for turkey and beef, but slightly larger in the case of pork. The bias values show the same trend, and results are also on the slight overprediction range. These findings indicate that the path-dependent model is capable to also predict microbial inactivation when applied to isothermal data. In contrast, when the non-isothermally-calibrated state-dependent model was applied to the isothermal data, results were notably poorer (Table 1). This shows that even when the same calibration sets were used, the path-dependent model had a better predictive ability than the state-dependent model.

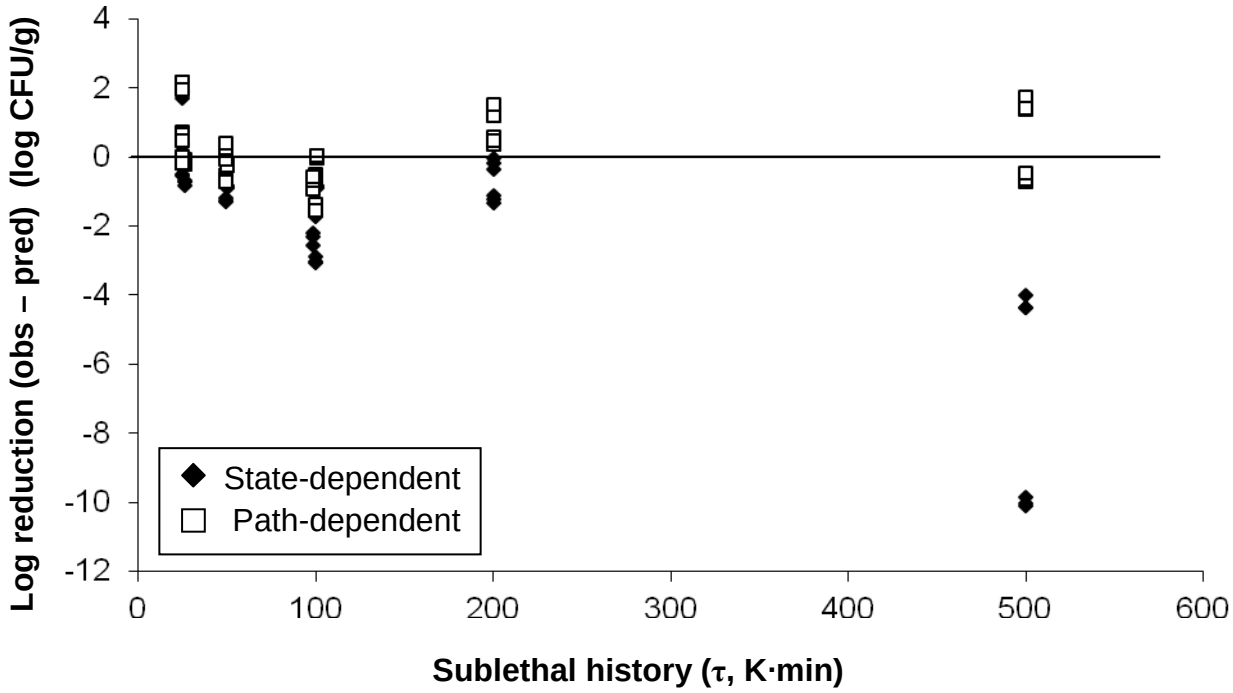

Figure 5. Log reduction errors (observed-predicted) for the state-dependent model and path-dependent model for the turkey validation set.

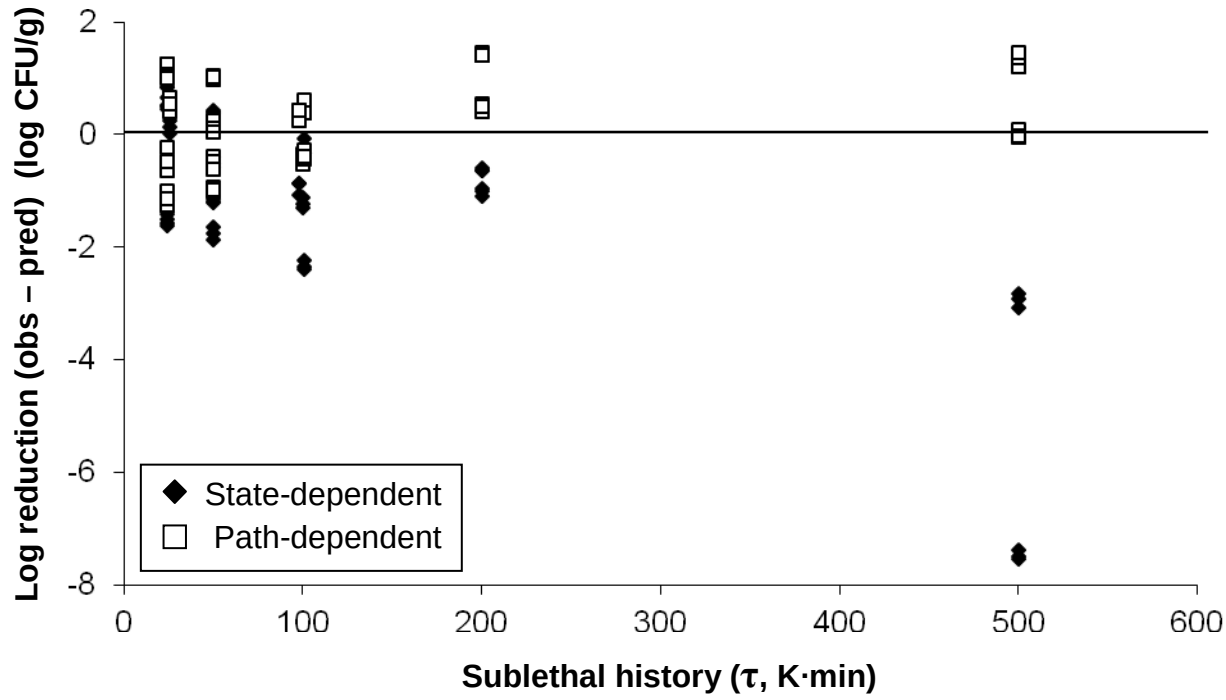

Figure 6. Log reduction errors (observed-predicted) for the state-dependent model and path-dependent model for the beef validation set.

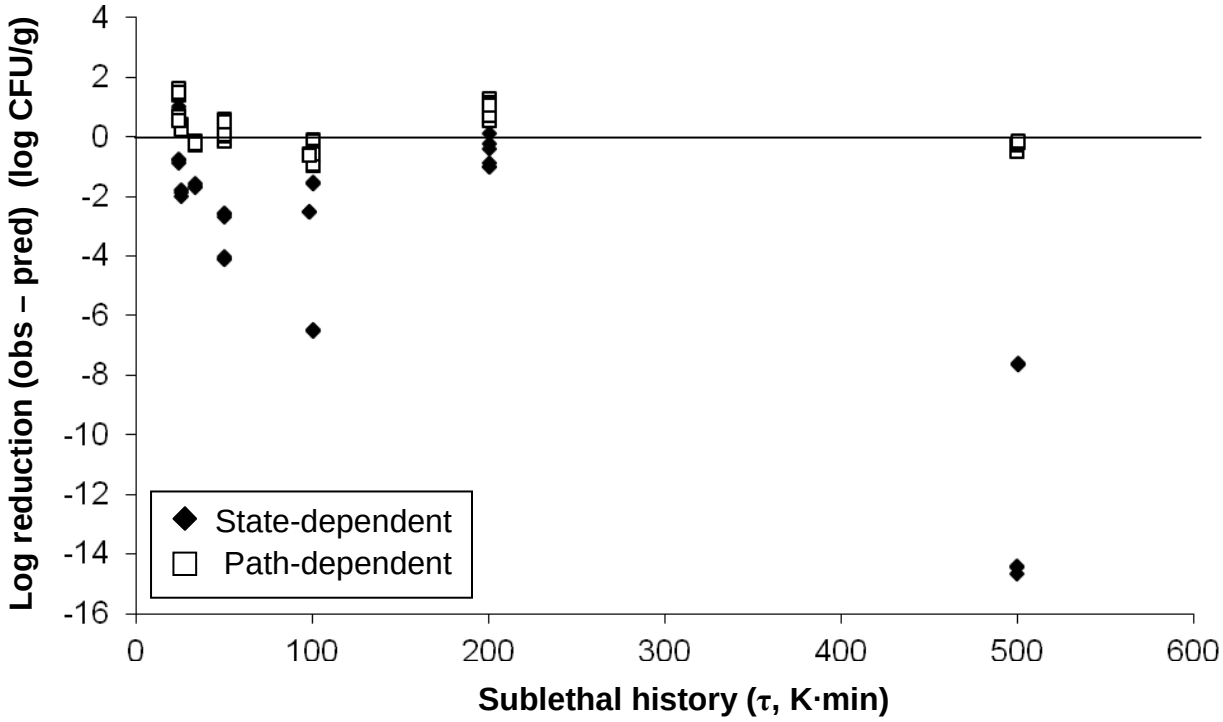

Figure 7. Log reduction errors (observed-predicted) for the state-dependent model and path-dependent model for the pork validation set.

3.4 Conclusions

This study revealed that the use of a model that considers sublethal thermal history in addition to the current state of the product can predict microbial lethality with significantly improved accuracy when applied to cases with or without sublethal heating, and across multiple meat products, and therefore can be useful to assure the safety of slow-cooked meat products.

All possible combinations between the use of isothermal and non-isothermal calibration and validation data sets, and the use of a state-dependent or a path-dependent model were analyzed for turkey, beef, and pork. Results demonstrated that isothermally-calibrated state-dependent models, as are typically reported and used for meat products, are ineffective when predicting lethality in processes where significant sublethal heating has occurred, and can produce fail-dangerous results that could jeopardize the safety of the products in question. On the other hand, the non-isothermally-calibrated, path-dependent model effectively described both isothermal and non-isothermal data sets. Slight fail-safe values resulted when validating against non-isothermal data, but slight fail-dangerous errors did result when the model was applied to isothermal data. These systematic errors could indicate that further studies are needed to improve the function that describes sublethal history (τ), so that it better reflects the complex cellular processes of stress adaptation.

4. MULTI-PRODUCT, MULTI-FACTOR MODEL

4.1 Introduction

As described in the literature review (section 2.4.1.3), there are modeling tools available to predict bacterial inactivation (i.e., ComBase, PMP, AMI Lethality Spreadsheet). However, these lack the elements necessary to comply with the lethality performance standards set by federal regulations, such as addressing *Salmonella* or considering product characteristics. On the other hand, secondary models are presented extensively in scientific literature (ICMSF 1996; FDA/CFSAN 2000), but each addresses a different factor affecting inactivation (in addition to temperature), and, with few exceptions, none has been validated against industry-relevant data. Therefore, the problem once again is delegated to processors to find data and/or models that would be applicable.

Ideally, a universal *Salmonella* thermal inactivation model applicable to various meat and poultry products would meet these requirements for the industry. Gathering inactivation data to understand pathogen inactivation behavior and develop models across product and processes has been attempted by few researchers, such as van Asselt and Zwietering (2006), Farakos and Zwietering (2011), and Halder and others (2010). Van Asselt gathered 4066 D-values for different pathogens at different temperatures in several food products and found that the parameter variability between sources was greater than that expected to be caused by product conditions (e.g., fat, pH). Farakos et al. collected D_P-values (equivalent to D_T-value for high hydrostatic pressure processes, HPP) across different temperatures, pressures, and microbial species in an attempt to develop global HPP inactivation models for each pathogen. They found that the obtained data were highly variable across these factors, especially temperature and

species, resulting in model parameter estimations with high standard deviations (e.g., log D_{Pref} = 0.27±0.25 log min for *Bacillus* spp.). Halder defined food groups based on the USDA national nutrient database food groups and set out to obtain growth and inactivation data for foodborne pathogens from ComBase and scientific literature, with the goal of developing "comprehensive food safety prediction software" (Halder et al. 2010) . The study avoided treating pathogen-specific effects as general, or being too specific on process and/or product characteristics. However, in the case of *Salmonella*, the inactivation model obtained was not sufficiently precise to validate industry processes in accordance to federal regulations (FSIS 2001). For example, one model is specified for red meat, but this food group can be understood to encompass beef, pork, lamb, and other meat species, not being specific enough to be used to validate a process; on the other hand, poultry is grouped with baby foods, soups and sauces, vegetables, and seafood, making the resulting model even less specific than in the red meat case.

Additionally, independent validation of a model is critically important if the model is to be used for industrial applications. Few studies have considered this step, and most of those validated against only laboratory-setting data, often with few quantitative measures. For example, although Halder stated the importance of model validation, only an example using one model from the study is shown, and no indication is given as to whether the other obtained models were validated (Halder et al. 2010). Wiegand et al. (2012) did validate an inactivation model against industry-scale data. Among other objectives, they evaluated the thermotolerance of *E. coli* O157:H7 in beef roasts under industry-relevant cooking conditions and compared those results to predictions by a model with parameters obtained under isothermal conditions. They reported high variability from the roasts lethality data, and found that the model predictions

greatly overestimated lethality, shedding light onto some of the few issues encountered when doing this kind of validation work. Breslin also conducted industry-relevant validations, by slow-cooking turkey, beef, or pork roasts inoculated with *Salmonella* (Breslin 2009). The study concluded that replication error significantly increases when scaling up from controlled laboratory experiments to pilot-scale cooks, which is an important consideration when applying lethality models to industry-scale processes. While insight from both projects is valuable in terms of scaled-up data, they do not provide quantitative means to account for the high variability possible in commercial applications.

Therefore, there is a need for a validated multi-product multi-factor *Salmonella* thermal inactivation model applicable for meat and poultry processors to use as means of complying with federal regulations. Therefore, the objectives of this study were to: (i) gather thermal inactivation data for *Salmonella* in poultry, beef, and pork products, (ii) compare multiple statistical methods to develop a multi-product multi-factor thermal inactivation model and its corresponding prediction intervals, and (iii) validate candidate models against industry-relevant, pilot-scale inactivation data.

4.2 Materials and Methods

4.2.1 Data compilation

To develop a multi-product multi-factor thermal inactivation model (i.e., a model suitable for predicting *Salmonella* thermal inactivation in various types of meat and poultry products and under differing conditions), all the relevant published raw data ideally should be compiled. Apart from laboratory data developed within our research group at Michigan State University, other data sets were sought in the electronic database ComBase (ComBase 2012) and in scientific

literature. To comply with the model goal described above, the data ultimately selected had to fulfill the following requirements:

1. Data had to describe *Salmonella* thermal inactivation: log N vs. time or log N/N_0 vs. time at constant or variable temperature. Plots of (a) log N vs. time, or (b) log N/N_0 vs. time with multiple sets indistinguishable from each other on one graph, were not used. This was because, for (a), it was impossible to determine which data points belonged to each set and thus obtain the standardized log N/N_0 value needed for parameter determination; and in the case of (b) it would not be possible to distinguish between data sets (see Figure 8), a factor needed for the mixed-effects statistical method (explained in detail in section 4.2.3.2). D- and z-values are the result of fitting raw data to the traditional log-linear model. Therefore, these values, without raw log N or log N/N_0 data, were not sufficient for the model development.
2. Data from *Salmonella* Senftenberg were not included, as this serovar is significantly more heat resistant than the rest of the serovars and is not linked to meat or poultry outbreaks (Goepfert et al. 1970; CDC 2011; Smith et al. 2001).
3. If experiments were done in replicates, the corresponding number of data points was expected (i.e., triplicate: 3 log N/N_0 points per time point); averaged values were already manipulated data, so they were not included in the raw data pool.
4. Muscle type (ground or whole), and species (e.g., turkey, beef, or pork) had to be stated.

5. Degree of grinding was not considered, as this variable does not affect *Salmonella* thermal inactivation (Mogollon et al. 2009).
6. Samples of any size/weight were considered. However, meat had to be inoculated raw and then cooked to simulate industrial processes.
7. Meat fat percentage (%) had to be reported.

A raw "data set" was considered to be a complete series of log N/N_0 vs. time observations at constant or variable temperature in one experimental trial. Each observation was then considered to be an individual raw data point (Figure 8).

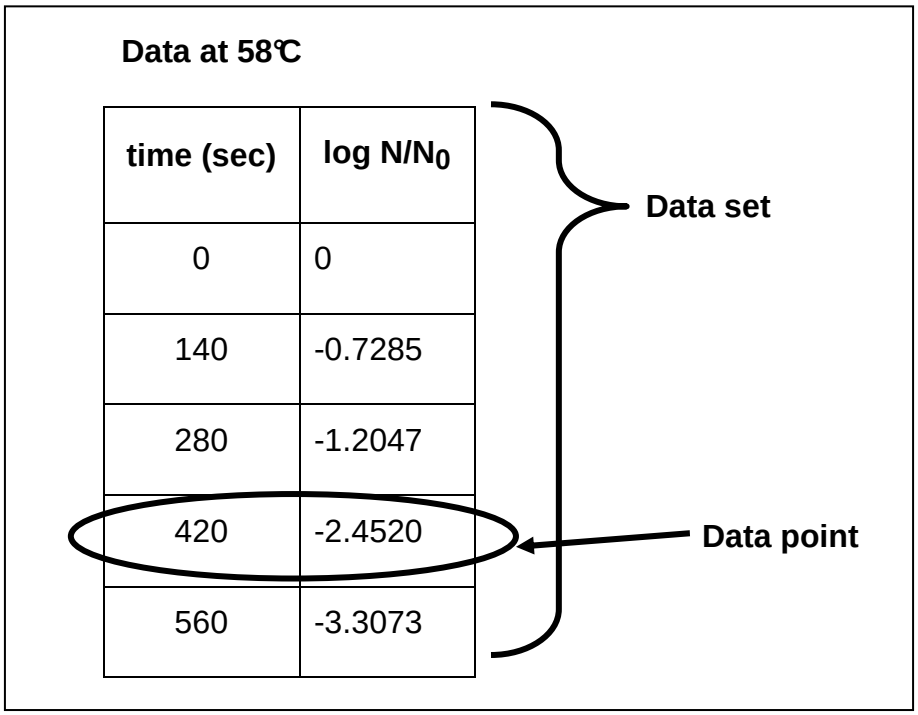

Figure 8. Definition of "data set" and "data point" for raw data.

The general approach was to include or exclude data sources following the above guidelines. The specific search methods in each case are described in detail in the following sections.

4.2.1.1 MSU laboratory data

The raw data collected from MSU and used for model development were all isothermal and came from the following experimental studies: (Breslin 2009), (Carlson et al. 2005), (Mogollon et al. 2009), (Orta-Ramirez et al. 2005), (Tuntivanich et al. 2008), (Velasquez et al. 2010), and (Wesche et al. 2005). For Carlson, only the data with the original product moisture was considered, as the model will not consider moisture content explicitly. In the case of Wesche, the data including pre-injured cells were ignored. For the rest, the isothermal data in both ground and whole muscle were considered.

4.2.1.2 Scientific journals

Relevant data from other research groups were sought electronically via searches of the Thomson Reuters' Web of Knowledge (Reuters 2011). Multiple combinations for the following search terms were used under the fields for Topic and Title:

- Salmonella
- Heat(ing)
- Thermal
- Inactivation
- Temperature
- Turkey
- Beef
- Pork
- Poultry
- Meat
- Ground
- Whole
- Whole muscle
- Fat
- Effect of…

- Resistance
- Processing
- Tolerance
- Survival
- Lethality
- Cooking
- Inoculated

Once all possible articles were listed, a complete copy of each paper was obtained either electronically or, for those not available online, through the MSU Library, to verify whether met criteria described in section 4.2.1. From this second evaluation, it was not possible to obtain raw data from some of the articles, so they were set aside, and their data sought in ComBase (see section 4.2.1.3). For the remainder, the available data sets were added to the pool to be used in this study.

4.2.1.3 ComBase Browser

As mentioned in section 2.4.1.3.3, ComBase is an online resource where food microbiology data are submitted from research groups on a voluntary basis. Although most of the data describe microbial growth, several sets for thermal inactivation where successfully obtained. The search parameters used to find relevant data were:

- Food type: Beef, Pork, Poultry
- Organisms: Salmonella spp
- Atmosphere: all selected
- Preparation: all selected
- Additives: all selected
- Other: all selected

- Temperature: 54°C to 120°C
- pH: 4.0 – 7.5 (default settings)
- Water activity: 0.71 – 1.0 (default settings)

As with the scientific journals, a second review was done on the initially collected data to ensure that the data complied with the criteria described in section 4.2.1. In addition, sources providing only D- and/or z-values were sought in the Web of Knowledge index and/or through other sources to see whether raw data could be obtained from them.

4.2.2 Model development

As described in section 3.2.1, the primary model can be described with a log-linear relationship:

$$\log S = \log \frac{N}{N_0} = -bt \qquad (9)$$

where S is the survival ratio, N is the number of microorganisms at time t, and N_0 is the initial microorganism population. In this work, the expression "log reduction" will be used, just as in the federal regulation; log reduction is equivalent to the negative of the survivor ratio (i.e., -log S). For this project, log-linear bacterial inactivation was assumed because, as described in section 3.3.2, AIC_c results indicated that this model was superior to the Weibull model ($n \neq 1$ in equation (2)) when describing the source data. Indeed, the results from section 3 show that the log-linear model performed well in describing both the isothermal and non-isothermal data (Table 1). Also, there is no known usage of non-log-linear inactivation models in the meat and

poultry industry or associated regulations. Therefore, to maximize the likelihood that the results of this study will be useful, it is reasonable to begin with the log-linear assumption. Additionally, the purpose of this work is to shed light onto the consequences of gathering data from different sources, not to address specific effects of tailing and similar non-log-linear phenomena that are addressed with non-log-linear models. Overall, this study does not suggest that the log-linear model is always the best choice, but only that it is the best for the data and goals of this study.

Parameter b, the rate of inactivation, can be described by a variety of secondary models, including an Arrhenius-type secondary model dependency for temperature (Stasiewicz et al. 2008), which is the most important factor for thermal inactivation (Jay et al. 2005):

$$b(T) = b_{ref} \cdot \exp\left[-\beta_1\left(\frac{1}{T(t)} - \frac{1}{T_{ref}}\right)\right] \qquad (5)$$

Although other models could also be suitable, the Arrhenius model has been shown to work in other studies. For example, a section of the study by McQuestin et al. (2009) assessed the goodness-of-fit between the Arrhenius temperature dependency and "other empirical equations" when fitted to inactivation data of *Escherichia* coli in fermented meat; the study concluded that the Arrhenius version was more adequate for describing the data (RMSE of 1.01 vs. 1.19 log CFU/g for the empirical model). Xu et al. fitted Bigelow and Arrhenius models to *Bacillus anthracis* thermal inactivation data, and found that the Arrhenius version described the data better (R^2>0.99 in all strains, vs. R^2~0.86-0.94 for the Bigelow model) (Xu and others 2006). Stasiewicz et al. (2008), among other objectives, used *Salmonella* inactivation data to fit Arrhenius, log-logistic, and empirical models; results showed that the Arrhenius version gave the best fit to the model parameters (RMSE of 0.16 log CFU/g vs. 0.45 and 0.23 log CFU/g for log-

logistic and empirical models, respectively). Based on these results and preliminary analyses of the data included in the study, the Arrhenius temperature dependency was used.

From section 2.3, other factors that can be considered to include in the secondary model include fat, muscle type, moisture, sublethal injury, and media. From these, moisture, cold shock injury, and starvation injury were excluded from the model. In the case of moisture content, fat percentage in the product was sufficient to account for these two factors, as they are inversely proportional to each other (section 2.3). Cold and starvation shock were excluded because, apart from the lack of data to obtain their describing parameters, it is not feasible, with current technology, to quantify them during processes. For media, meat products from turkey, beef, and pork were chosen due to their dominance in the available data pool. Therefore, the final version for the secondary model was established as:

$$b(T,\tau,F,M,K,B,P) = b_{ref} \cdot \exp\left[-\beta_1\left(\frac{1}{T(t)}-\frac{1}{T_{ref}}\right)-\beta_2\tau-\beta_3F-\beta_4M-\beta_5Y-\beta_6B-\beta_7P\right] \quad (10)$$

The meaning of each parameter and variable is described in Table 2. Except for the case of temperature, a simple relationship was chosen for all parameters, as their mathematical association to lethality has not yet been determined. Additionally, this allowed the model to remain simple, which was beneficial when individually determining the effects of each factor.

Table 2. Description of parameters and variables for secondary model.

Parameter or Variable	Description
b	b, inactivation rate (min), dependent on T, τ, F, M, Y, B, and P (see definitions below)
b_{ref}	Inactivation rate (min^{-1}) at a reference temperature T_{ref}
T	Product temperature (K), dependent on process time (t)
T_{ref}	Reference temperature (K)
τ	Thermal sublethal (injury) history (K·min). Explained in detail in section 3.2.1, (Stasiewicz et al. 2008)
F	Meat product fat content (%)
M	Muscle structure of the meat product. M = 1 for ground meat, M = 0 for whole muscle
Y	Variable takes value of 1 if meat product is poultry. Otherwise, it is 0
B	Variable takes value of 1 if meat product is beef. Otherwise, it is 0
P	Variable takes value of 1 if meat product is pork. Otherwise, it is 0
β_1	Temperature parameter (K). Describes the effect of temperature on b
β_2	Sublethal history parameter $(K \cdot min)^{-1}$. Describes the effect of τ on b
β_3	Fat content parameter ($\%^{-1}$). Describes the effect of F on b
β_4	Muscle type parameter (unitless). Describes the effect of M on b
β_5	Species parameter (unitless). Describes the effect of Y on b
β_6	Species parameter (unitless). Describes the effect of B on b
β_7	Species parameter (unitless). Describes the effect of P on b

4.2.3 Non-linear parameter estimation

Two methods for model calibration were used on the different versions derived from equation (10): the ordinary least squares method, and the mixed-effects method. Each one is detailed in the following sections. For initial estimates, the parameter values obtained from

section 3 were used. In addition, model parameters were recalculated by varying these initial estimates by at least 500%, and were compared to the original calibration values to test for parameter sensitivity to initial estimates.

To estimate the sublethal history parameter (β_2), the collected isothermal data were not useful because of the parameter's nature (equation (4)). Therefore, the parameter values used in the models were based on those found from the studies in section 3 (Table 1).

The statistical measures of performance obtained from each model fitting were the RMSE (a measure of the goodness-of-fit), the bias (mean residuals), and the AIC_c (Akaike's information criterion corrected for finite sample sizes, as described in section 3.2.7).

4.2.3.1 Ordinary least squares (OLS) method

4.2.3.1.1 Theory

The ordinary least squares method (OLS) tests different parameter values to minimize the sum of squares of the errors. For these tests, the residuals were defined as the difference between the experimental log reductions and the log reductions predicted by the model:

Error = log reduction experimental – log reduction predicted

4.2.3.1.2 Parameter estimation

As mentioned in section 4.2.3, the values used for the sublethal injury parameter (β_2) were those estimated from section 3. In addition, for the OLS method, the temperature effect was accounted for using only the MSU data. This was because:

- The inactivation rates resulting from the MSU data and the information from other sources were highly different; for example, for ground pork at 60°C, D-value was 6.21 min from a study by Juneja and others (2000b), while it was 0.99 min from a MSU study (Velasquez et al. 2010). Using them both together with the OLS method would cause the prediction intervals to be particularly large, which would not be useful for our purposes.
- Data differences between sources could not be explained from simple analysis of the data and acquisition methods, so it was not possible to scale all the data to a common baseline.
- Most of the data obtained for model calibration and all the data for pilot-scale validation came from MSU, so by choosing the MSU data to parameterize the temperature effect, better consistency across data sets would be kept.

This also meant that it would not be possible to estimate the fat parameter (β_3), as the MSU data did not contain studies analyzing the effect of fat on thermal inactivation. Therefore, the parameter used was obtained with model regression from data that did study this effect (Juneja et al. 2001; Juneja and Eblen 2000; Juneja and others 2000b). First, an optimum T_{ref} was calculated (procedure described below) with these data, and then model parameters were estimated using MATLAB's *nlinfit* function (described in section 3.2.7). The β_3 used in the final OLS models was based on the parameter obtained from this procedure.

For parameter estimation, the first step was to find an optimum reference temperature (T_{ref}) to minimize errors in future calculations (Datta 1993). The process chosen minimizes the correlation coefficient between the inactivation rate parameter (b_{ref}) and the temperature

dependency parameter (β_1) (Dolan et al. 2007). To do this, the parameters related to T_{ref} (b_{ref} and β_1, equation(2)) were estimated several times while varying T_{ref}. For each case, the correlation coefficient between b_{ref} and β_1 was plotted against T_{ref}. The optimum T_{ref} is the one at which the correlation coefficient is a minimum (in this case, $r \sim 1x10^{-4}$).

Parameter estimation was done using MATLAB's *nlinfit* function A sample code can be found in section 6.3.1. For parameterization, different versions of the "full" model (equation (10)) were independently calibrated. This is because, even though all factors described by the model are relevant, the model with the most parameters is not necessarily the best (Zwietering and den Besten 2011; Motulsky and Christopoulos 2004). Following a naming convention, the model took the initials of the factors it included (i.e., T F (w) model includes temperature and fat effects, and uses the whole-muscle data for obtaining parameters). The models tested are shown in Table 3. Note that the fat and sublethal history parameters (β_2 and β_3) were obtained separately as mentioned at the beginning of this section.

Table 3. Versions of the "full" model parameterized with OLS method.

Model name♦	Factors included	Factors excluded	Data set(s) used for parameterization*	Parameters in model	No. of models
T (g)	Temperature	Fat Muscle type Sublethal history Species	G turkey	b_{ref}, β_1	3
			G beef		
			G pork		
T F (g)	Temperature Fat	Muscle type Sublethal history Secies	G turkey	b_{ref}, β_1, β_3	3
			G beef		
			G pork		
T τ (g)	Temperature Sublethal history	Fat Muscle type Species	G turkey	b_{ref}, β_1, β_2	3
			G beef		
			G pork		
T F τ (g)	Temperature Fat Sublethal history	Muscle type Species	G turkey	b_{ref}, β_1, β_2, β_3	3
			G beef		
			G pork		
T(w)	Temperature	Fat Muscle type Sublethal history Species	W turkey	b_{ref}, β_1	3
			W beef		
			W pork		
T F (w)	Temperature Fat	Muscle type Sublethal history Species	W turkey	b_{ref}, β_1, β_3	3
			W beef		
			W pork		
T τ (w)	Temperature Sublethal history	Fat Muscle type Species	W turkey	b_{ref}, β_1, β_2	3
			W beef		
			W pork		
T F τ (w)	Temperature Fat Sublethal history	Muscle type Species	W turkey	b_{ref}, β_1, β_2, β_3	3
			W beef		
			W pork		

*G: ground muscle, W: whole muscle

♦(g): model calibrated with ground-muscle data, (w): model calibrated with whole-muscle data

4.2.3.1.3 Prediction intervals

A 95% prediction interval (PI) is the region around a predicted value where a new individual observation is expected to fall with a confidence of 95%. In contrast, a 95% confidence interval (CI) is the region around a predicted value where the mean of new observations is expected to fall with a confidence of 95% (Motulsky and Christopoulos 2004). Although CIs are more commonly used in model predictions, the application of our model in food safety requires the use of PIs, because food safety goods should be based on the safety of individual servings, not on the mean.

When using the OLS method, there are two ways to calculate a close approximation of the asymmetric PIs. The first is to use the *nlpredci* function in MATLAB, which gives the asymptotic PIs. Although these are good approximate PIs, their width can be further approximated to the asymmetric value by further parameter estimation with the ellipse method via QR decomposition (Bates and Watts 1988), bootstrapping (Mishra and others 2011), or Monte Carlo (van Boekel 1996) simulations. These will be referred to as the PI methods from this point forward. As our objective is to estimate PIs useful to industry (i.e., those that are both reliable and small estimates), the PI methods were preferred over the *nlpredci* function in MATLAB.

The end result of the PI methods is a region made of n_{sim} different combinations of possible parameter values with a 1-α confidence (when $\alpha = 0.05$, confidence = 95%), where n_{sim} is the number of simulations executed. Carrying out these methods with two parameters gives a contouring area; three parameters would mean the creation of a three-dimensional space; using four parameters would add another dimension, and so on. In level of complexity, the ellipse

method is the simplest, followed by bootstrapping, and finally by the Monte Carlo simulations. For each case of parameter estimation (Table 3), the three methods were carried out in MATLAB, and compared against each other to determine the best approach for calculating PIs.

Although it is possible to use any number of parameters, studies where the PI methods are applied on models with more than two parameters are extremely scarce, as the methodologies for the use of more than two dimensions are not well developed (Dolan 2012). For that reason, a different empirical approach had to be taken for the model cases with 3 or more parameters. The two parameters chosen as main factors in the PI methods were b_{ref} and β_1. This is because the reference inactivation rate (b_{ref}) and temperature (β_1) are the most determinant parameters for thermal inactivation. The PIs calculated with these two parameters will be known as the main PIs from this point forward. To calculate the PI when the remaining parameters (β_2, β_3, etc) were used, it was determined that the main PI would be used and then it would be modified by adding the remaining parameter confidence intervals to account for their variability. This empirical approach would inevitably make the PIs wider, but the other option would have been to develop three- and four- dimensional regions – work that is much beyond the scope of this project.

Once the best PI method was chosen, to obtain the PI for each validation data set, a prediction was calculated with each of the n_{sim} parameter combinations. This yielded n_{sim} predictions, which were arranged in increasing (or decreasing) order. The 2.5% and 97.5% values represented the upper and lower PI for the data set in question.

4.2.3.2 Non-linear mixed-effects (NLME) method

4.2.3.2.1 Theory

All statistical models are composed of variables, which change value depending on the conditions the model is using to estimate (e.g., temperature (T) in equation (6)), and fixed effects, which are the model parameters – values related to an entire population or with repeatable levels of experimental factors (e.g., b_{ref} in equation (6)) (Pinheiro 2000). In addition, some models include a random effect, which are values linked with "individual experimental units drawn at random from a population" (Pinheiro 2000). In the case of this project, an "experimental unit" would be a data set (Figure 8). A mixed-effects model is a model that incorporates both fixed and random effects, such as that shown by the simple linear equation (11). Here, y_i is the model prediction for the i^{th} "experimental unit", x is the predictor, m and b are the fixed effects (model parameters), and the random effect for the i^{th} "experimental unit" is symbolized by ξ_i.

$$y_i = mx + b + \xi_i \tag{11}$$

A simple graphical way to represent a mixed-effects model is shown in Figure 9. Here, we have two different representations of a linear model (y=mx+b). The first shows a "random" distribution in the y-axis intercept b, while the second shows the same for the lines' slopes m. Therefore, in mixed-effects modeling, a random effect would be associated with the intercept parameter b in the first case, while it would be associated with the slope parameter m in the second case. When parameterizing any of the model versions derived from equation (10), the

fitting software would evaluate the variability caused by the "experimental units" on each of the model parameters (bref, b1, b2, etc), and determine which one would carry a random effect.

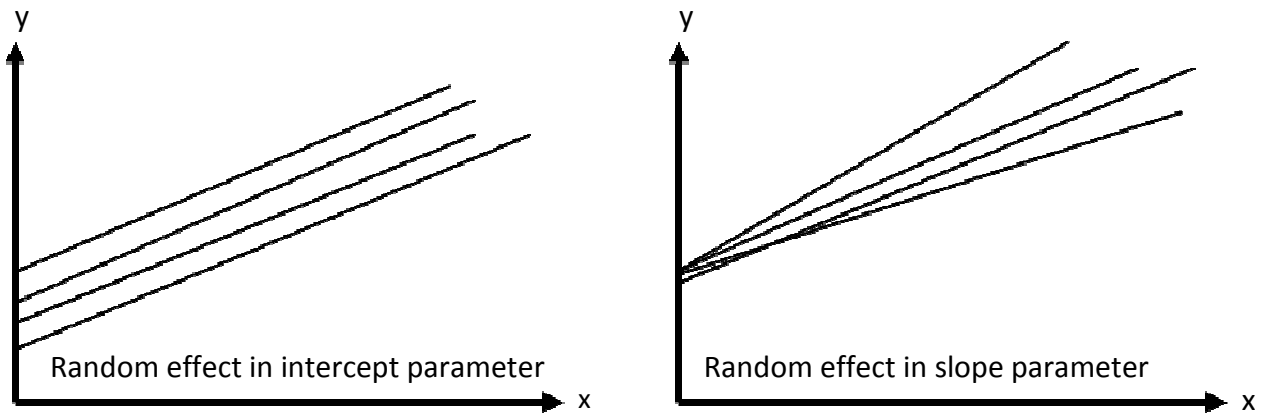

Figure 9. Graphical representations of mixed-effects models.

For the purpose of developing a multi-product multi-factor model with *Salmonella* inactivation data from different sources, the use of a mixed-effects model for repeated measures is extremely helpful. This is because these types of models are designed to handle data "generated by observing a number of individuals repeatedly under differing experimental conditions where the individuals are assumed to constitute a random sample from a population of interest" (Lindstrom and Bates 1990). Basically, if we assume the "population of interest" to be all the data describing *Salmonella* thermal inactivation in meat and poultry products, and a "random sample of individuals" treated under "differing experimental conditions" to be the data obtained from different laboratory settings, then the mixed-effects model would be able to characterize all the data sets accounting for the variability generated by different research groups.

4.2.3.2.2 Parameter estimation

Parameter estimation was done using the non-linear mixed-effects (*nlme*) function from the R statistical package (R: A Language and Environment for Statistical Computing, Vienna, Austria, 2011). This function fits a nonlinear mixed-effects model using the formulation and computational methods described in Lindstrom and Bates (Lindstrom and Bates 1990). A sample code for this method can be found in section 6.3.2.

To determine which parameters carried a random effect, several versions of the model were fitted, associating a random effect with one parameter each time. The statistical measures to determine the most appropriate model were AIC_C, RMSE, and variance of the random effect. For AIC_C and RMSE, the model yielding the lowest values of both would be preferred. For the variance of the random effect, a large value is desired, because its approximation to zero would mean there is no unexplained variability between data sets, thus no random effect associated (Pinheiro 2000). After the final model version was selected, the rest of the data were analyzed.

As with the least squares method (section 4.2.3.1.2), different versions for the "full" model (equation (7)) were parameterized. The same naming convention was followed; the model took the initials of the factors it described (e.g., T F (g) model describes temperature and fat calibrated with ground-muscle data). The models tested are described in Table 4.

Table 4. Versions of the "full" model parameterized with mixed-effects method (continued next page).

Model name♦	Factors included	Factors excluded	Data set(s) used for parameterization*	Parameters in model	No. of models
T (g)	Temperature	Fat Muscle type Sublethal history Species	G turkey G beef G pork	b_{ref}, β_1	3
T (w)	Temperature	Fat Muscle type Sublethal history Species	W turkey W beef W pork	b_{ref}, β_1	3
T F (g)	Temperature Fat	Muscle type Sublethal history Species	G turkey G beef G pork	b_{ref}, β_1, β_3	3
T F (w)	Temperature Fat	Muscle type Sublethal history Species	W turkey W beef W pork	b_{ref}, β_1, β_3	3
T F τ (g)	Temperature Fat Sublethal history	Muscle type Species	G turkey G beef G pork	b_{ref}, β_1, β_2, β_3	3
T F τ (w)	Temperature Fat Sublethal history	Muscle type Species	W turkey W beef W pork	b_{ref}, β_1, β_2, β_3	3
T F S (g)	Temperature Fat Species	Muscle type Sublethal history	G turkey, beef, pork	b_{ref}, β_1, β_3, β_5, β_6, β_7	1
T F S (w)	Temperature Fat Species	Muscle type Sublethal history	W turkey, beef, pork	b_{ref}, β_1, β_3, β_5, β_6, β_7	1

*G: ground muscle, W: whole muscle

♦(g): model calibrated with ground-muscle data, (w): model calibrated with whole-muscle data. Where not present, model was calibrated with both ground- and whole-muscle data.

Table 4 (cont'd). Versions of the "full" model parameterized with mixed-effects method (continued next page).

Model name♦	Factors considered	Factors left out	Data set(s) used for parameterization*	Parameters in model	No. of models
T F S τ (g)	Temperature Fat Species Sublethal history	Muscle type	G turkey, beef, pork	b_{ref}, β_1, β_2, β_3, β_5, β_6, β_7	1
T F S τ (w)	Temperature Fat Species Sublethal history	Muscle type	W turkey, beef, pork	b_{ref}, β_1, β_2, β_3, β_5, β_6, β_7	1
T F M	Temperature Fat Muscle type	Sublethal history Species	G + W turkey G + W beef G + W pork	b_{ref}, β_1, β_3, β_4	3
T F M τ	Temperature Fat Muscle type Sublethal history	Species	G + W turkey G + W beef G + W pork	b_{ref}, β_1, β_2, β_3, β_4	3
T F M S	Temperature Fat Muscle type Species	Sublethal history	G + W Turkey, beef, pork	b_{ref}, β_1, β_3, β_4, β_5, β_6, β_7	1
T F M τ S	Temperature Fat Muscle type Sublethal history Species	None	G + W Turkey, beef, pork	b_{ref}, β_1, β_2, β_3, β_4, β_5, β_6, β_7	1

*G: ground muscle, W: whole muscle

♦(g): model calibrated with ground-muscle data, (w): model calibrated with whole-muscle data. Where not present, model was calibrated with both ground- and whole-muscle data.

4.2.3.2.3 Prediction intervals

The importance of PIs for our application is described in section 4.2.3.1.3. For the *nlme* method, the approach taken was that described by Gelman (Gelman and Hill 2007) (pp.272-275) with the addition of Monte Carlo simulations. These calculations were also carried out in R. A sample code for this method can be found in section 6.3.2. The code imported a validation data set, and computed PIs doing n_{sim} Monte Carlo simulations taking into account the random effect and residual variances. As described in section 4.2.3.1.3, the simulations were carried out n_{sim} times, yielding n_{sim} predictions that were arranged in increasing (or decreasing) order to obtain the 2.5% and 97.5% percentiles, representing the upper and lower PI values for the corresponding data set.

4.2.4 Model validation against pilot-scale data

Validation of a model against independent data is of utmost importance to determine model performance. In addition, it provides insight into model suitability in cases where it is tested outside its application range (Halder et al. 2010). Although manuscripts describing thermal lethality abound in scientific journals, very few of them validate the resulting models. From these few studies, most are validated against other laboratory-based data (Mattick and others 2001; Peleg and others 2007), and the rationale for selecting a specific data set is rarely reported (Corradini and Peleg 2009; Vaidya and Corvalan 2009; Aragao and others 2007; Peleg et al. 2007). In a few other instances, a validation is carried out, but few statistical parameters to describe goodness-of-fit or quantify model performance are reported (Vaidya and Corvalan 2009; Porto-Fett and others 2008; Sallami and others 2006). While all these works provide

valuable information on their research topics, their results are not directly applicable for validating industrial processes. If a thermal inactivation model is to be useful for lethality predictions in independent tests, it is essential that the model be suitably validated. For our application, it is imperative that the models be tested against data produced under pilot-scale, industry-like conditions. For these reasons, each model was validated against the pilot-scale sets of data described in the following sections, from both rapid (i.e., impingement) and slow convection processes. For each validation data set, the following statistical values were calculated: RMSE, bias (mean residual), maximum error (fail-safe), minimum error (fail-dangerous), and percentage (%) of data points that fell within the PIs and above the fail-dangerous PI.

4.2.4.1 Steaks/fillets and patties in impingement oven

These tests involved the cooking of *Salmonella*-inoculated (~10^7 – 10^8 CFU/g) whole-muscle cuts, and ground and formed patties in a pilot-scale, moist-air impingement oven, as reported in detail by Hildebrandt (2012a). Overall, the samples (chicken breast fillets, beef steaks from boneless round roasts, pork chops from boneless loin, and ground and formed patties of turkey, beef, and pork) were ~120 g and ~11-12 mm thick, with fat contents of 0.33 to 10%. There were 6 different cooking treatments, which combined conditions relevant to commercial applications: oven air temperature (149 or 204°C), humidity (20 or 50% moisture by volume), and target lethality (4 or 6 log reduction, in order to have reliable survivor counts). The full factorial combination of the treatments (in triplicate for steaks/fillets, in duplicate for patties) yielded a total of 144 data points, with total cooking times of 4 to 11 min. All raw data obtained

from these experiments complied with the criteria described in section 4.2.1, and were comprised of log reduction results for the product cores.

4.2.4.2 Whole-muscle roasts

These experimental data, from Breslin (2009), resulted from pilot-scale, slow cooking of *Salmonella*-inoculated (~$10^{6.5}$ CFU/g at the core) turkey breast, beef round, and pork loin roasts in a commercial, moist-air convection oven. Roast sample size was ~680 g. There were 7 different cooking schedules representing industry processes, which combined the following parameters: cook (in-bag or out-of-bag), time (total cook time 86-253 min), oven temperature (constant at 93.3°C or ramp-up), and humidity (20, 50, or 78% RH). All raw data obtained from these experiments complied with the parameters described in section 4.2.1. However, only the data in which cooks were done to a specified lethality were taken into account, ignoring those which reached 71.1°C, because those generally yielded no survivors.

4.2.4.3 Hot dogs

For these experiments, commercially formulated beef and turkey emulsions (batter) were acquired from a local processor, inoculated with *Salmonella* (~10^{8} CFU/g), vacuum-stuffed, and cooked in a pilot-scale convection oven using a cook schedule for low-fat hot dogs similar to that used in industry. Samples were ~60 g (~15.5 cm long, 2 cm diameter) and had a thermocouple inserted for temperature logging. The cooking cycle increased temperature (60 to 82 °C) and humidity (38 to 79%RH) over ~145 min. Cooking was stopped by quenching the samples in liquid nitrogen when the data-logger signaled a predicted lethality of 4 or 6 log. All raw data

obtained from these experiments complied with the parameters described in section 4.2.1. A more detailed description of the tests can be found in section 0.

4.3 Results and discussion

4.3.1 Data compilation results

Table 5 shows a description of the data obtained from the MSU studies. Although the experiments did not include the effect of fat percentage (%) on *Salmonella* inactivation, the data provided valuable information with respect to muscle type.

Table 5. MSU data characteristics by source.

Reference	Species and muscle type	Data points*	Data sets*	Test temperatures	Fat %
Breslin (2009)	Ground beef	156	21	55, 58, 60, 62, 63°C	2.7%
	Whole beef	164	25		
Carlson et al. (2005)	Ground turkey	22	4	60°C	1.8%
Mogollon et al. (2009)	Ground beef	67	7	60°C	4.5%
	Whole beef	22	2		
Orta-Ramirez et al. (2005)	Ground beef	80	10	55, 60, 62.5°C	5.6%
	Whole beef	27	6		
Tuntivanich et al. (2008)	Ground turkey	90	9	55, 60, 62.5°C	1%
	Whole turkey	67	9		
Velasquez et al. (2010)	Ground pork	121	16	55, 58, 60, 62, 63°C	2.5%
	Whole pork	120	15		
Wesche et al. (2005)	Ground turkey	48	6	60°C	1.5%

*See Figure 8 for illustration and definition of data point and data set (section 4.2.1).

Regarding data from other research groups, a total of 36 journal articles from 12 different journals were considered possible sources for inactivation data. After applying the criteria set in section 4.3.1, 4 journal articles were selected for our database, with all of them directed to ComBase for raw-data search.

From the 43,153 total data sets available in ComBase at the time of the initial search, 419 matched the characteristics from section 4.2.1.3. From these, 106 data sets were selected according to the criteria previously described for the study (section 4.2.1). The raw data missing from scientific articles (section 4.2.1.2) were available to download from ComBase, allowing the completion of the data pool.

Because these data were ultimately pooled with the combined searches from previous literature and ComBase, it is fitting to present the results as one (Table 6). While muscle type was not addressed in these studies, the data sets provide beneficial information regarding fat percentage (%) in the meat products.

Table 6. Scientific journals and ComBase Browser data characteristics by source.

Reference	Species and muscle type	Data points	Data sets	Test temperatures	Fat %
Juneja et al. (2001)	Ground turkey	244	32	58, 60, 62.5, 65°C	1, 7, 10, 12%
Juneja (2003)	Ground beef	44	6	55, 57.5, 60°C	25%
Juneja and Eblen (2000)	Ground beef	100	12	58, 60, 62.5, 65°C	7, 12, 18, 24%
Juneja et al. (2000b)	Ground beef	202	24	58°C	7, 12, 18, 24%
	Ground pork	268	32	58, 60, 62.5, 65°C	4, 10, 24, 28%

Table 7 shows the characteristics for all the data compiled. Beef comprised most of the collected data, particularly the ground product, while the least common data were for whole-muscle turkey, with only 9 data sets with 67 data points. Both the MSU data and those from other sources included the temperature effect on *Salmonella* inactivation with a useful range of temperatures. Generally, the MSU data focused on assessing the effect of muscle type, while those from other sources included varying fat content. This shows that, depending on the application, merging results from different studies could be beneficial in trying to explain different phenomena that have not been studied together. In this case, the merged data showed great variability across source studies, due to different sample preparation, processing, and recovery methods. Therefore it was decided that both methods of parameter estimation would be used to obtain the multi-product multi-factor model: the standard non-linear estimation (OLS) (section 4.2.3.1), and the mixed-effects method (section 4.2.3.2).

Table 7. Final compiled data characteristics and sources (continued next page).

Data characteristics	MSU data	Scientific journals and ComBase Browser	All sources
All data			
Data points	995	908	1903
Data sets	134	106	240
Temperature range	55 - 63°C	55 - 65°C	55 - 65°C
Fat % range	1 – 5.6%	1 – 28%	1 – 28%
Ground Turkey			
Data points	160	244	448
Data sets	19	32	59
Temperature range	55 – 62.5°C	58 - 65°C	55 - 65°C
Fat % range	1 – 1.8%	1 – 12%	1 – 12%

Table 7 (cont'd). Final compiled data characteristics and sources.

Data characteristics	MSU data	Scientific journals and ComBase Browser	All sources
Whole Turkey			
Data points	67	-	67
Data sets	9	-	9
Temperature range	55 – 62.5°C	-	55 – 62.5°C
Fat % range	1%	-	1%
Ground Beef			
Data points	303	352	655
Data sets	38	42	80
Temperature range	55 - 63°C	55 - 65°C	55 - 65°C
Fat % range	2.7 – 5.6%	7 – 25%	2.7 – 25%
Whole Beef			
Data points	224	-	224
Data sets	29	-	29
Temperature range	55 - 63°C	-	55 - 63°C
Fat % range	2.7 – 5.6%	-	2.7 – 5.6%
Ground Pork			
Data points	121	268	389
Data sets	16	32	48
Temperature range	55 - 63°C	58 – 65°C	55 - 65°C
Fat % range	2.5%	4 – 28%	2.5 – 28%
Whole Pork			
Data points	120	-	120
Data sets	15	-	15
Temperature range	55 - 63°C	-	55 - 63°C
Fat % range	2.5%	-	2.5%

4.3.2 OLS model parameters, statistics, and PIs

The reference temperatures obtained for each data set are shown in Table 8. As expected, the reference temperature falls approximately in the middle of the lethal temperature range data in the data pool. The obtained parameters and corresponding statistics for the OLS model are shown in Table 9. Note that the RMSE and bias for the models does not change by the addition of the estimated β_2, the sublethal injury parameter from section 3, as the tests are carried out in temperature ranges above that of the sublethal injury region. On the other hand, β_2 should prove useful when validating the pilot-scale data, especially the longer-cook roasts. The standard and relative errors for the model parameters of all model versions, including the fits made to obtain β_3, can be found in section 6.5.1. The correlation coefficients can be found in section 6.6.1.

Table 8. Reference temperatures by data set.

Data	Reference temperature (T_{ref}, °C)*
Ground turkey	59.5943
Ground beef	59.7356
Ground pork	59.0460
Whole turkey	58.9800
Whole beef	59.4870
Whole pork	59.2068

*Temperature in models is in K.

Table 9. Parameters and statistics for OLS models (calibrated with only MSU data).

Data source for calibration	Model	Parameters				Statistics	
		b_{ref}	β_1	β_2*	β_3**	RMSE	Bias
		min^{-1}	K	K^{-1}	fat % $^{-1}$	log (CFU/g)	
Ground turkey n_{obs} = 160	T (g)	1.11	52,269	-	-	1.21	-0.04
	T F (g)	1.11	52,269	-	0.0300	1.24	-0.13
	T τ (g)	1.11	52,269	0.0017	-	1.21	-0.04
	T F τ (g)	1.11	52,269	0.0017	0.0300	1.24	-0.13
Whole turkey n_{obs} = 67	T (w)	0.37	48,589	-	-	0.42	0.003
	T F (w)	0.37	48,589	-	0.0300	0.43	-0.03
	T τ (w)	0.37	48,589	0.0017	-	0.42	0.003
	T F τ (w)	0.37	48,589	0.0017	0.0300	0.43	-0.13
Ground beef n_{obs} = 303	T (g)	0.83	44,242	-	-	0.77	-0.02
	T F (g)	0.83	44,242	-	0.0227	0.80	-0.18
	T τ (g)	0.83	44,242	0.0018	-	0.77	-0.02
	T F τ (g)	0.83	44,242	0.0018	0.0277	0.80	-0.18
Whole beef n_{obs} = 224	T (w)	0.44	44,799	-	-	0.87	-0.03
	T F (w)	0.44	44,799	-	0.0227	0.87	-0.16
	T τ (w)	0.44	44,799	0.0018	-	0.87	-0.03
	T F τ (w)	0.44	44,799	0.0018	0.0277	0.87	-0.16
Ground pork n_{obs}= 121	T (g)	0.63	41,750	-	-	0.99	-0.05
	T F (g)	0.63	41,750	-	0.0137	0.99	-0.13
	T τ (g)	0.63	41,750	0.0016	-	0.99	-0.05
	T F τ (g)	0.63	41,750	0.0016	0.0137	0.99	-0.13
Whole pork n_{obs} =120	T (w)	0.45	47,164	-	-	1.03	-0.04
	T F (w)	0.45	47,164	-	0.0137	1.03	-0.12
	T τ (w)	0.45	47,164	0.0016	-	1.03	-0.04
	T F τ (w)	0.45	47,164	0.0016	0.0137	1.03	-0.12

*Parameters obtained from sublethal injury studies, Chapter 3, Table 1.
**Obtained from calibrating only data from Table 6.

Regarding the PIs, the contours produced by all methods (ellipse, bootstrap, Monte Carlo) were essentially the same in all cases (ground turkey in Figure 10, the rest in section 6.7.1), and so the PIs generated were also extremely similar, with <0.05 log N/N_0 difference between them (ground turkey in Figure 11 and Figure 12), the rest in section 6.7.2). Therefore, to avoid unneeded complexity, it was decided to use the results from the ellipse method to determine the final PIs for each model.

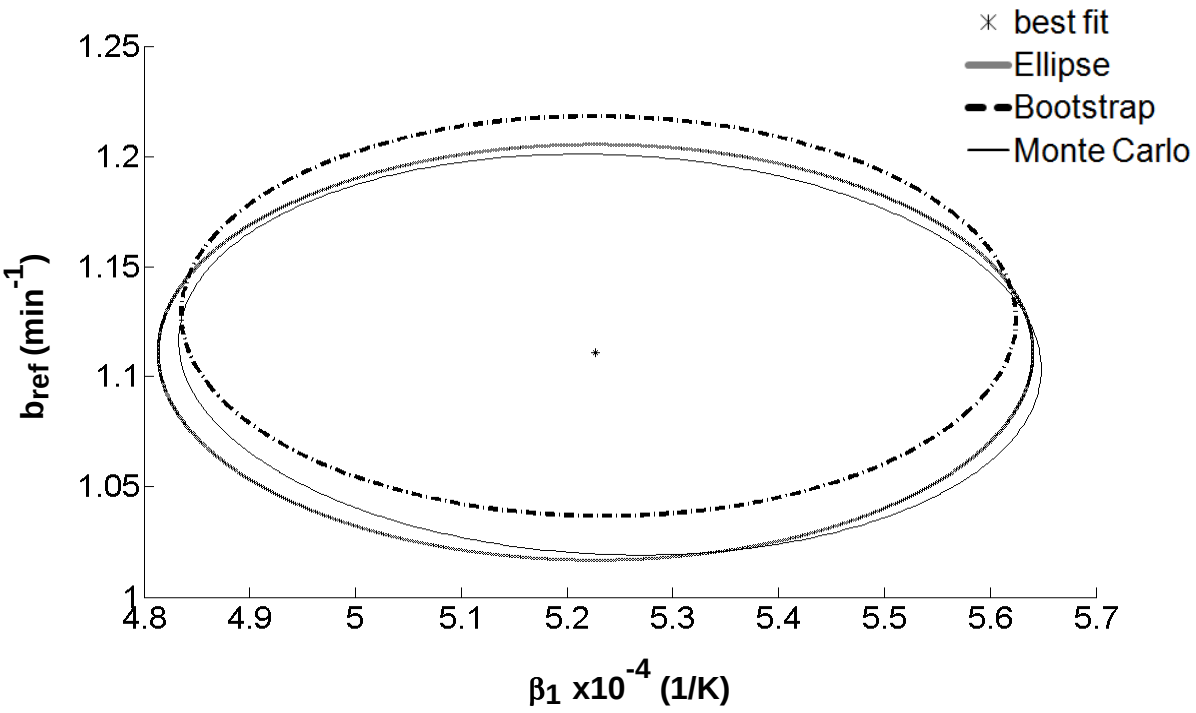

Figure 10. PI methods parameter contours for ground turkey calibration set.

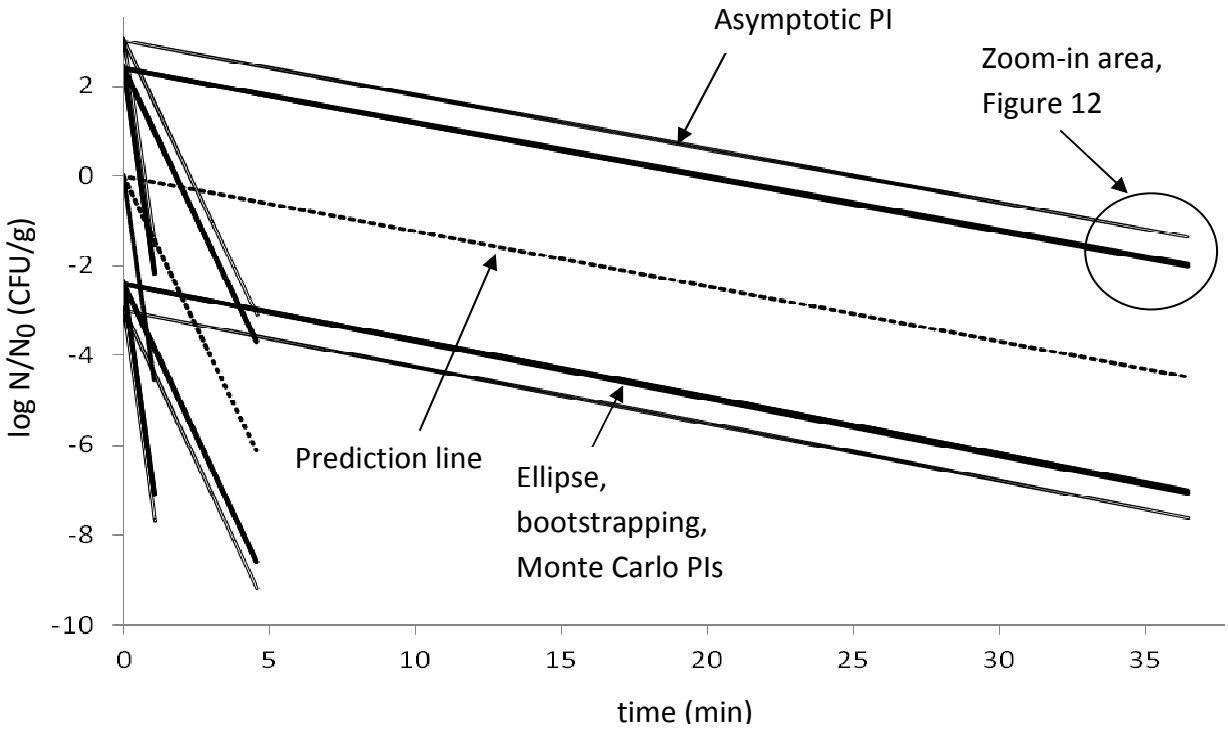

Figure 11. PIs with all methods for ground turkey calibration data set.

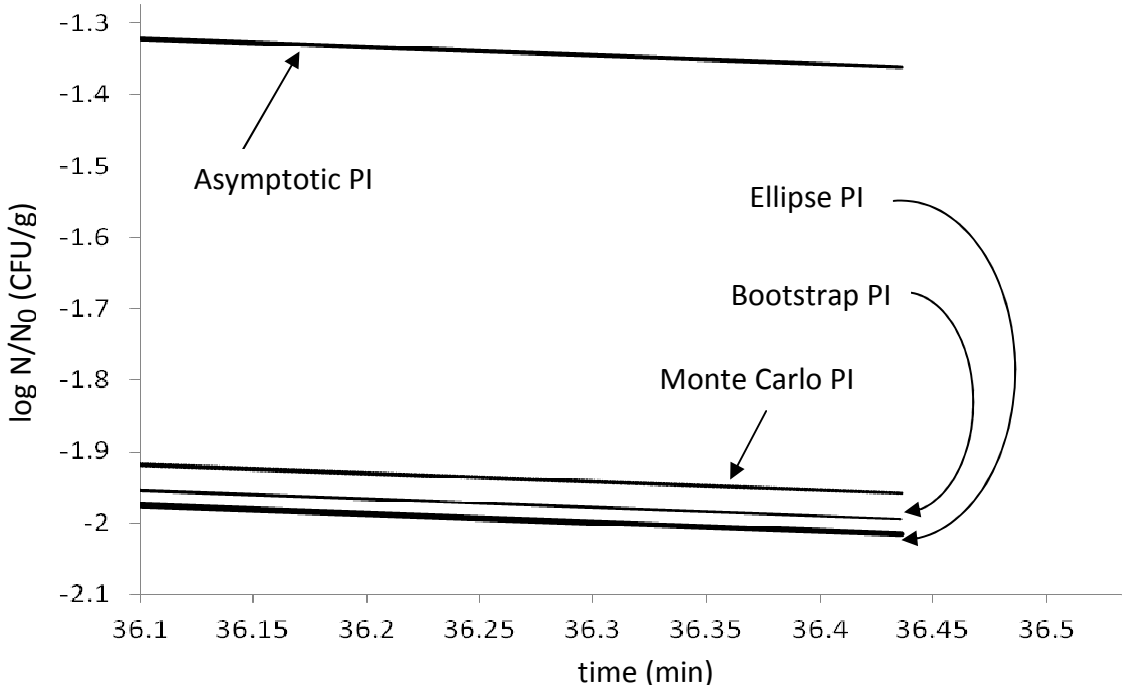

Figure 12. Zoom-in section from Figure 11.

4.3.3 Mixed-effects model parameters and statistics

Table 10 shows the parameters and statistics for the different model versions obtained with the mixed-effects method. In the cases where the sublethal injury parameter (β_2) was added, the RMSE did not change, as mentioned in section 4.3.2, and the AIC_c could not be computed. This is because the number of observations (n_{obs}) changes, but the RMSE does not take this into account; therefore, the AIC_c that would be obtained does not accurately represent the model in question. Standard and relative errors for the all the parameters in the different model versions can be found in section 6.5.2, while the correlation coefficients are in section 6.6.2, and the random effects for all data sets (groups) in the same model versions are in section 6.8.

Table 10. Parameters and statistics for mixed-effects model (continued next page).

Model	Data source for calibration	n_{obs}	n_{groups}	Parameters								Statistics	
				b_{ref}	β_1	β_2	β_3	β_4	β_5	β_6	β_7	RMSE♦	AICc
				min^{-1}	K	$K \cdot min^{-1}$	fat $\%^{-1}$	•	•	•	•	log (CFU/g)	•
T (g)	G turkey	404	51	0.67	50,750	-	-	-	-	-	-	0.38	392
	G beef	649	80	0.56	44,710	-	-	-	-	-	-	0.70	714
	G pork	389	48	0.43	53,950	-	-	-	-	-	-	0.56	377
T (w)	W turkey	67	9	0.59	50,750	-	-	-	-	-	-	0.43	20
	W beef	224	29	0.54	44,710	-	-	-	-	-	-	0.62	145
	W pork	120	15	0.65	53,950	-	-	-	-	-	-	0.77	115
T F (g)	G turkey	404	51	1.58	36,470	-	0.21	-	-	-	-	0.38	294
	G beef	649	80	0.95	36,320	-	0.06	-	-	-	-	0.70	640
	G pork	389	48	0.74	35,940	-	0.06	-	-	-	-	0.55	313
T F (w)	W turkey	67	9	1.36	44,710	-	0.90	-	-	-	-	0.45	23
	W beef	224	29	0.80	44,710	-	0.13	-	-	-	-	0.67	142
	W pork	120	15	1.05	53,950	-	0.19	-	-	-	-	0.77	117

♦RMSE values for models containing τ are repeated from the same models without τ (see text).

Table 10 (cont'd). Parameters and statistics for mixed-effects model (continued next page).

Model	Data source for calibration	n_{obs}*	n_{groups}*	Parameters								Statistics	
				b_{ref}	β_1	β_2**	β_3	β_4	β_5	β_6	β_7	RMSE	AICc
				min^{-1}	K	$K \cdot min^{-1}$	fat $\%^{-1}$	•	•	•	•	log (CFU/g)	•
T F τ (g)	G turkey	404+108	51+36	1.58	36,470	0.0018	0.21	-	-	-	-	0.38	NA
	G beef	649+108	80+36	0.95	36,320	0.0018	0.06	-	-	-	-	0.70	NA
	G pork	389+108	48+36	0.74	35,940	0.0016	0.06	-	-	-	-	0.55	NA
T F τ (w)	W turkey	67+108	9+36	1.36	44,710	0.0018	0.90	-	-	-	-	0.45	NA
	W beef	224+108	29+36	0.80	44,710	0.0018	0.13	-	-	-	-	0.67	NA
	W pork	120+108	15+36	1.05	53,950	0.0016	0.19	-	-	-	-	0.77	NA
T F S (g)	G turkey, beef, pork	1442	179	1.53	49,800	-	0.07	-	0.83	0.78	1.09	0.58	1409
T F S (w)	W turkey, beef, pork	358	53	5.20	45,690	-	0.13	-	2.15	1.88	1.79	0.70	267
T F S τ (g)	G turkey, beef, pork	1442 + 324 ×	179+108 ×	1.53	49,800	0.0017†	0.07	-	0.83	0.78	1.09	0.58	NA
T F S τ (w)	W turkey, beef, pork	358 + 324×	53+108×	5.20	45,690	0.0017†	0.13	-	2.15	1.88	1.79	0.70	NA

*108 data points correspond to the data used to obtain β_2 in section 3. These correspond in turn to 36 data groups.

** Parameters obtained from sublethal injury studies, Chapter 3, Table 1.

† Average from the 3 species; individual values obtained from Table 1.

×324 data points correspond to the data used to obtain β_2 in section 3 (for the 3 species). These correspond in turn to 108 data groups.

NA: not applicable for models containing τ, as n_{obs} is modified (see text).

Table 10 (cont'd). Parameters and statistics for mixed-effects model.

Model	Data source for calibration	n_{obs}*	n_{groups}*	Parameters								Statistics	
				b_{ref}	β_1	β_2**	β_3	β_4	β_5	β_6	β_7	RMSE	AICc
				min^{-1}	K	$K \cdot min^{-1}$	fat $\%^{-1}$	•	•	•	•	log (CFU/g)	•
T F M	G + W turkey	471	60	0.52	36,860	-	0.21	-1.14	-	-	-	0.39	321
	G + W beef	873	109	0.61	37,490	-	0.06	-0.45	-	-	-	0.68	791
	G + W pork	509	63	0.67	40,300	-	0.07	-0.14	-	-	-	0.61	428
T F M τ	G + W turkey	471+108	60+36	0.52	36,860	0.0018	0.21	-1.14	-	-	-	0.39	NA
	G + W beef	873+108	109+36	0.61	37,490	0.0018	0.06	-0.45	-	-	-	0.68	NA
	G + W pork	509+108	63 + 36	0.67	40,300	0.0016	0.07	-0.14	-	-	-	0.61	NA
T F M S	G + W turkey, beef, pork	1853	232	1.09	49,410	-	0.06	-0.51	0.65	0.65	0.86	0.59	1612
T F M S τ	G + W turkey, beef, pork	1853 + 324×	232 + 108×	1.09	49,410	0.0017†	0.06	-0.51	0.65	0.65	0.86	0.59	NA

*108 data points correspond to the data used to obtain β_2 in section 3. These correspond in turn to 36 data groups.

** Parameters obtained from sublethal injury studies, Chapter 3, Table 1.

† Average from the 3 species; individual values obtained from Table 1.

× 324 data points correspond to the data used to obtain β_2 in section 3 (for the 3 species). These correspond in turn to 108 data groups.

NA: not applicable for models containing τ, as n_{obs} is modified (see text).

4.3.4 Validation against pilot-scale data

Validation for all developed models against the independent pilot-scale data is shown in the following sections. The comparisons to consider when determining model performance are those within the same types of products, i.e., ground-muscle calibrated models against ground-muscle data and not against whole-muscle data. However, current limitations in practice might entail use of ground-muscle calibrated models to validate whole-muscle products (because of the lack of adequately-calibrated models). These comparisons were also carried out to showcase possible outcomes. When validating turkey-calibrated models against the impingement oven whole-muscle samples, the cooked poultry product was chicken instead of turkey. In all cases, the maximum (+, most fail-safe) and minimum (-, most fail-dangerous) errors, and plots showing model predictions for representative data sets can be found in section 6.9.

Given the various statistical measures of model performance, it was considered for this study that an ideal (although impossible) model would predict lethality with RMSE and bias of 0.0 log CFU/g, i.e., a perfect fit, PI width would be near zero, and percentage of data points inside it would be 100% (Figure 13). However, due to the inherent variability and experimental error in real-world tests, a best practically possible and industry-useful model would present a low prediction RMSE (say ~1.0 log CFU/g), positive bias to avoid fail-dangerous errors, moderately narrow 95% PIs (say ~±1.5 log CFU/g), and 100% of the predicted data points captured by the 95% PI (Figure 14). When a model like this is not obtained, one yielding conservative predictions could also be highly functional (Figure 15). Also, a model with all data points above the fail-dangerous PI band could still be useful, depending on the other statistical parameters. For example, with a model capturing 100% of the data points above the fail-

dangerous PI band, but with a wide PI, negative bias and/or high RMSE, the model user would need to determine whether the width of the PI is useful for a particular process or not (Figure 16 and Figure 17). In this case, bias and RMSE would be secondary parameters to consider, as there can be a highly negative bias or a highly scattered data set (high RMSE); however, with all points above the fail-dangerous PI band and an acceptable PI width, process safety can still be assured. On the other hand, a model of no use would not necessarily be the complete opposite of the ideal model; it suffices to have most of the data points below the fail-dangerous PI band for the model to be unacceptable, regardless of PI width (Figure 18).

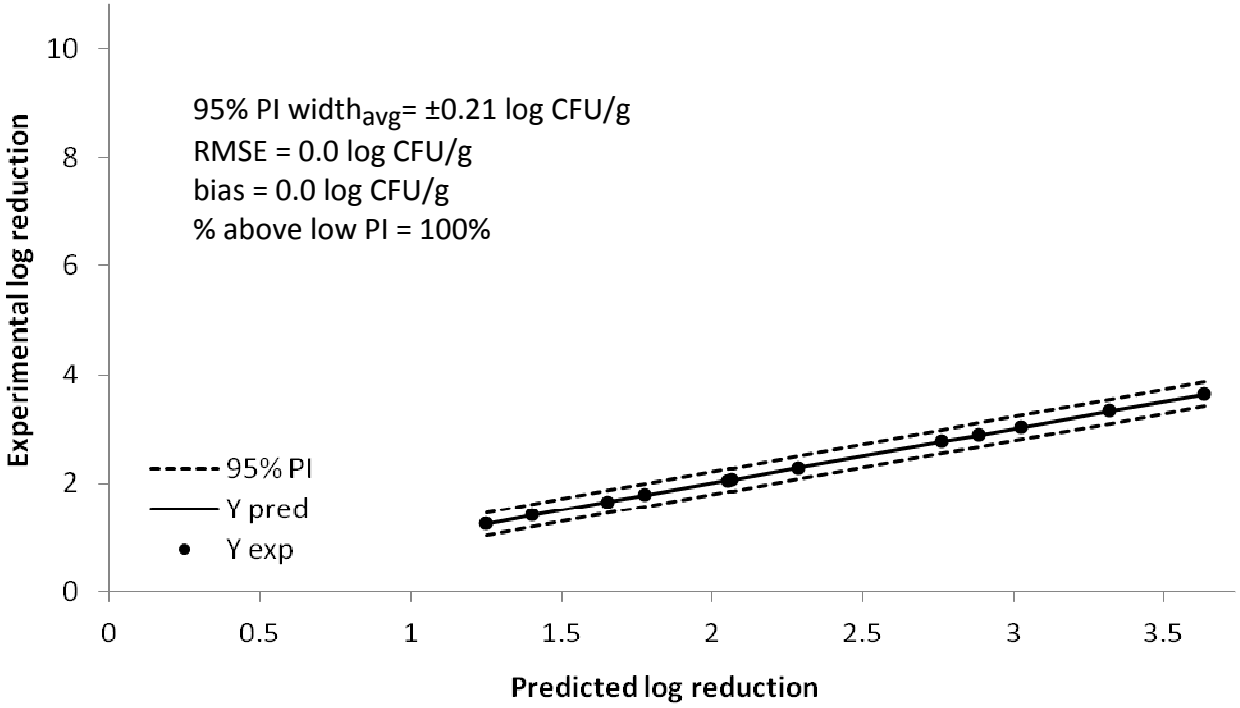

Figure 13. Example performance of an ideal model.

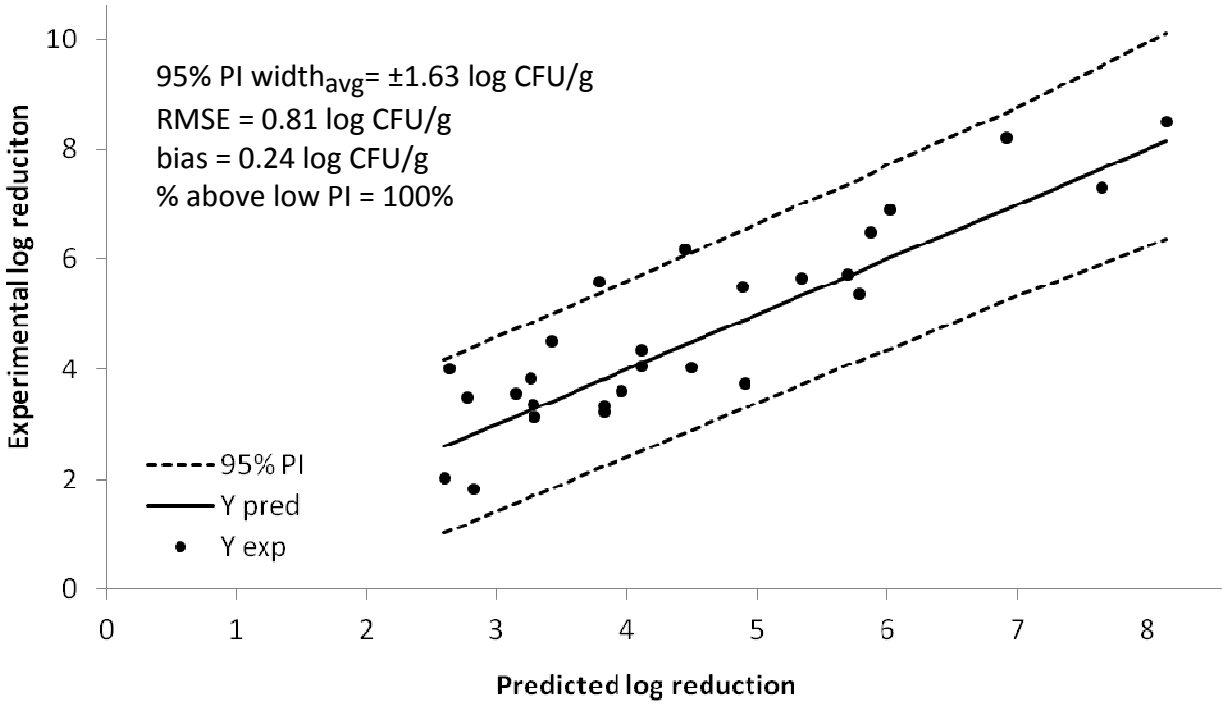

Figure 14. Example performance of a practically possible ideal model.

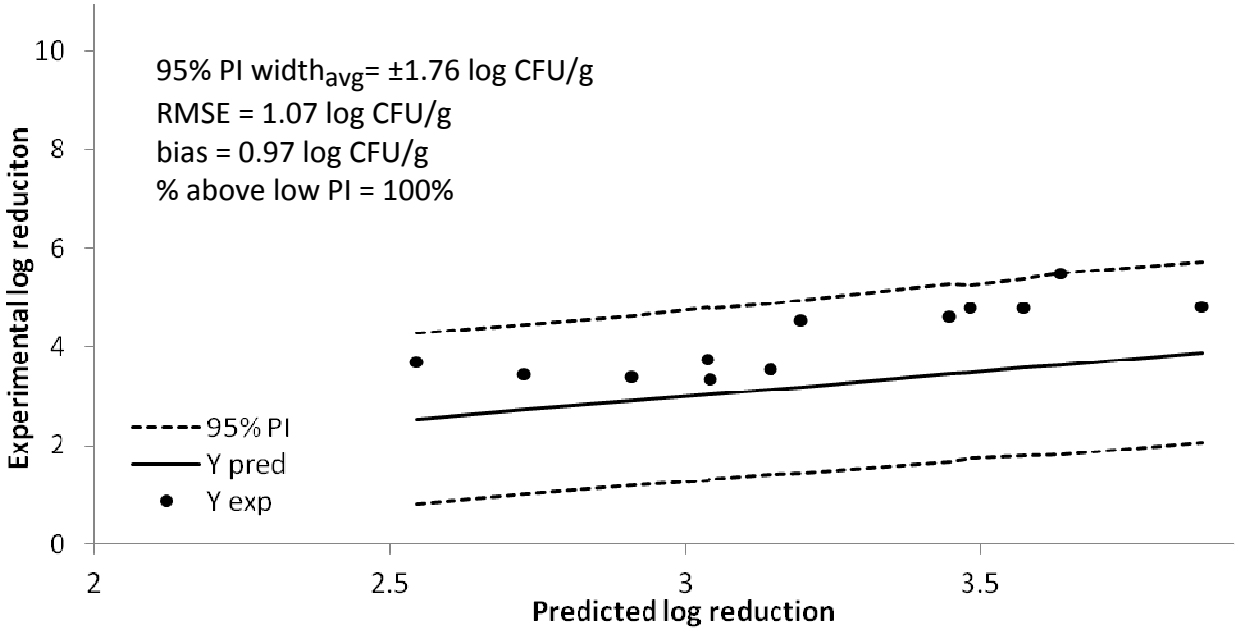

Figure 15. Example performance of a conservative, but possibly useful model.

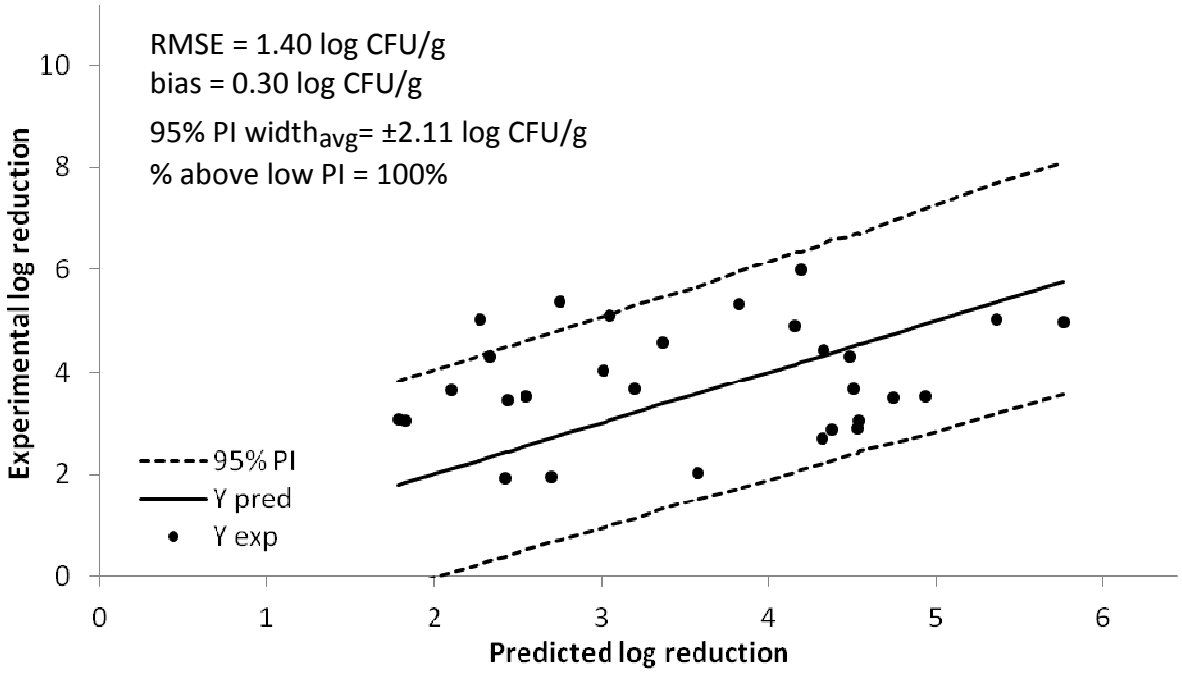

Figure 16. Example performance of a potentially useful model; all data points fall above the fail-dangerous PI, but PI width would need to be evaluated by the user to determine usefulness, especially as several data points are quite close to the lower PI band.

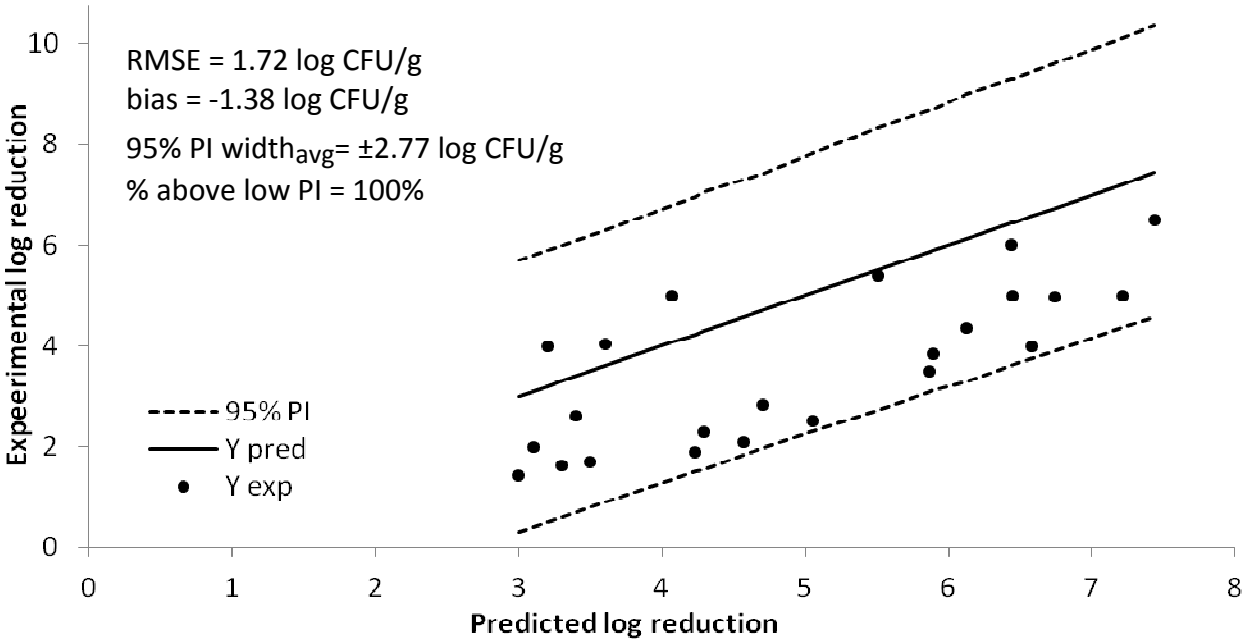

Figure 17. Example performance of a possibly useful model; all data points fall above the fail-dangerous PI, but bias is highly negative, and PI width would need to be evaluated by the user to determine usefulness.

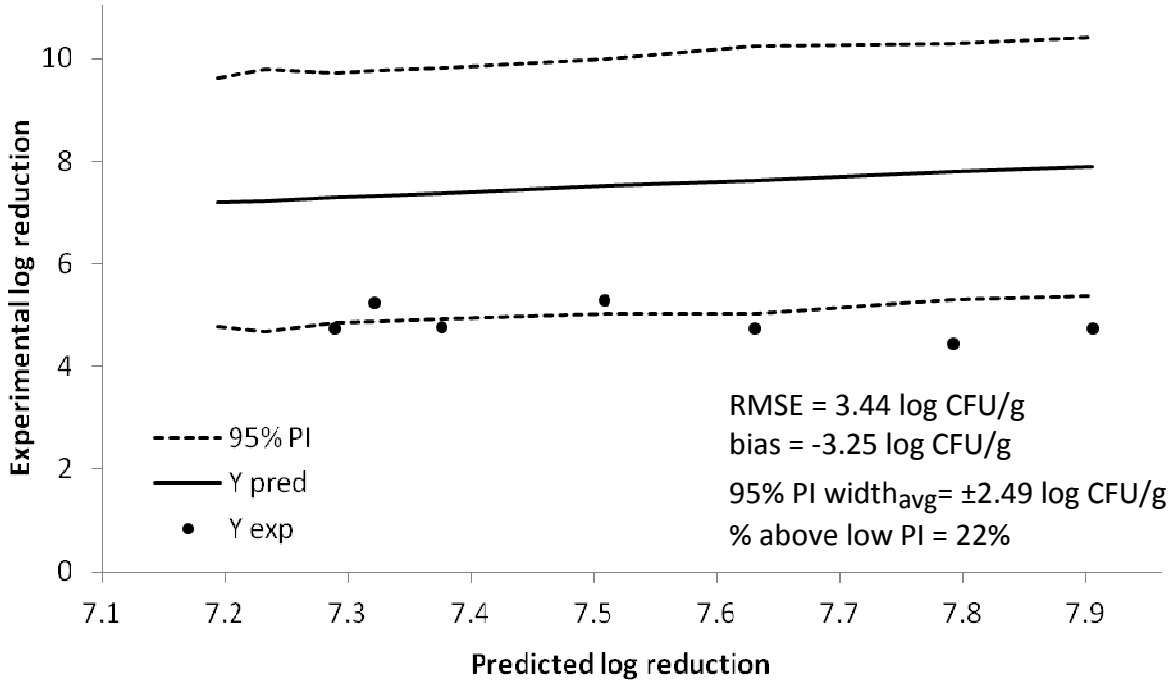

Figure 18. Example performance of a useless model. In addition to not capturing all data points above the fail-dangerous PI band, notice the width of the PI.

In the next sections, model performance on the pilot-scale data is analyzed based on the criteria described above.

4.3.4.1 Steaks/fillets and patties

Tables 11-13 show the OLS models' performance when validated against the impingement-cooked products (whole-muscle and ground-and-formed poultry, beef, and pork). Overall, as expected, the whole-muscle-calibrated models fared better than their ground-muscle-calibrated counterparts when predicting lethality in whole-muscle products. This can be attributed to the fact that the whole-muscle model parameters reflect the significantly greater thermal resistance of *Salmonella* in whole-muscle than in ground products (Tuntivanich et al. 2008; Orta-Ramirez et al. 2005; Velasquez et al. 2010). In both the ground-muscle calibrated and the whole-muscle calibrated versions, and against most of the validation data sets, the addition of

the fat and sublethal injury parameters (β_3 and β_2, respectively) improved the models' performance; decreased RMSE, "pulled" all points to the fail-safe side of prediction, which improved the bias and the percentage of observations that fell above the lower PI. In some cases, for example, the ground beef samples, the RMSE did not improve when β_2 and/or β_3 were added, but the percentage of points falling above the lower PI did, which is more important for the food safety application of this project. In other cases, for example the whole chicken samples, this percentage did not change, but the bias was improved, meaning that the predictions were less fail-dangerous. Finally, in all cases, the addition of the fat parameter (β_3) was fairly more beneficial to the model prediction than the addition of the sublethal injury parameter (β_2). This is especially noticeable in the ground pork samples (10% fat) where RMSE improved by 0.4 log CFU/g and bias by 0.6 log CFU/g with the addition of the fat content term (Figure 19 and Figure 20). From these observations, it was concluded that the consideration of fat percentage and sublethal injury by the models was beneficial to their predictions of the independent validation results.

Table 11. OLS models validated against chicken fillets and turkey patties (impingement cooked).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Ground turkey + Whole chicken n_{obs} = 44	T (g)	27	27	2.61	4.51	-3.94
	T F (g)	27	27	2.60	4.36	-3.80
	T τ (g)	27	27	2.60	4.41	-3.84
	T F τ (g)	27	27	2.60	4.26	-3.70
	T (g) + T (w)	52	34	1.83	3.59	-1.99
	T F (g) + T F (w)	52	34	1.81	3.44	-1.86
	T τ (g) + T τ (w)	52	34	1.82	3.52	-1.91
	T F τ (g) + T F τ (w)	55	34	1.81	3.37	-1.79
Ground turkey n_{obs} = 23 fat = 1.05% τ_{avg} = 8.77 K·min	T (g)	26	26	2.62	4.79	-4.17
	T F (g)	26	26	2.59	4.58	-3.96
	T τ (g)	26	26	2.61	4.69	-4.07
	T F τ (g)	26	26	2.58	4.48	-3.86
Whole chicken n_{obs} = 21 fat = 0.33% τ_{avg} = 7.92 K·min	T (g)	29	29	2.60	4.17	-3.68
	T F (g)	29	29	2.60	4.11	-3.62
	T τ (g)	29	29	2.59	4.08	-3.59
	T F τ (g)	29	29	2.60	4.01	-3.52
	T (w)	81	43	0.96	1.34	0.41
	T F (w)	81	43	0.95	1.34	0.44
	T τ (w)	81	43	0.95	1.35	0.45
	T F τ (w)	86	43	0.95	1.35	0.47

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 12. OLS models validated against beef steaks and patties (impingement cooked).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Ground + Whole beef n_{obs} = 44	T (g)	70	66	1.56	1.88	-0.93
	T F (g)	77	70	1.55	1.72	-0.69
	T τ (g)	75	70	1.56	1.84	-0.86
	T F τ (g)	80	73	1.55	1.69	-0.62
	T (g) + T (w)	91	80	1.65	1.35	0.20
	T F (g) + T F (w)	95	75	1.64	1.38	0.38
	T τ (g) + T τ (w)	95	82	1.65	1.36	0.25
	T F τ (g) + T F τ (w)	95	73	1.64	1.39	0.43
Ground beef n_{obs} = 19 fat = 2.32% τ_{avg} = 9.28 K·min	T (g)	84	74	1.54	1.45	-0.07
	T F (g)	95	79	1.54	1.44	0.12
	T τ (g)	95	84	1.54	1.45	-0.01
	T F τ (g)	95	79	1.54	1.45	0.18
Whole beef n_{obs} = 25 fat = 2.68% τ_{avg} = 8.18 K·min	T (g)	60	60	1.57	2.14	-1.58
	T F (g)	64	64	1.56	1.91	-1.30
	T τ (g)	60	60	1.57	2.09	-1.51
	T F τ (g)	68	68	1.56	1.86	-1.23
	T (w)	96	84	1.73	1.27	0.41
	T F (w)	96	72	1.73	1.33	0.58
	T τ (w)	96	80	1.73	1.29	0.45
	T F τ (w)	96	68	1.73	1.35	0.62

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 13. OLS models validated against pork steaks and patties (impingement cooked).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Ground + Whole pork n_{obs} = 56	T (g)	77	75	2.06	1.97	-1.30
	T F (g)	79	75	2.02	1.79	-0.88
	T τ (g)	75	73	2.05	1.98	-1.13
	T F τ (g)	79	75	2.02	1.76	-0.82
	T (g) + T (w)	86	82	2.09	1.89	-0.76
	T F (g) + T F (w)	91	84	2.06	1.66	-0.43
	T τ (g) + T τ (w)	88	84	2.08	1.86	-0.71
	T F τ (g) + T F τ (w)	91	84	2.06	1.65	-0.40
Ground pork n_{obs} = 27 fat = 10% τ_{avg} = 9.34 K·min	T (g)	78	78	2.05	2.06	-1.53
	T F (g)	89	85	2.00	1.64	-0.98
	T τ (g)	81	81	2.05	2.01	-1.47
	T F τ (g)	89	85	2.00	1.61	-0.93
Whole pork n_{obs} = 29 fat = 1.53% τ_{avg} = 8.21 K·min	T (g)	83	79	2.12	1.95	-0.82
	T F (g)	69	66	2.04	1.92	-0.78
	T τ (g)	69	66	2.05	1.94	-0.82
	T F τ (g)	69	66	2.04	1.89	-0.72
	T (w)	93	86	2.12	1.71	-0.04
	T F (w)	93	83	2.11	1.69	0.08
	T τ (w)	93	86	2.11	1.71	0.00
	T F τ (w)	93	83	2.11	1.69	0.08

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

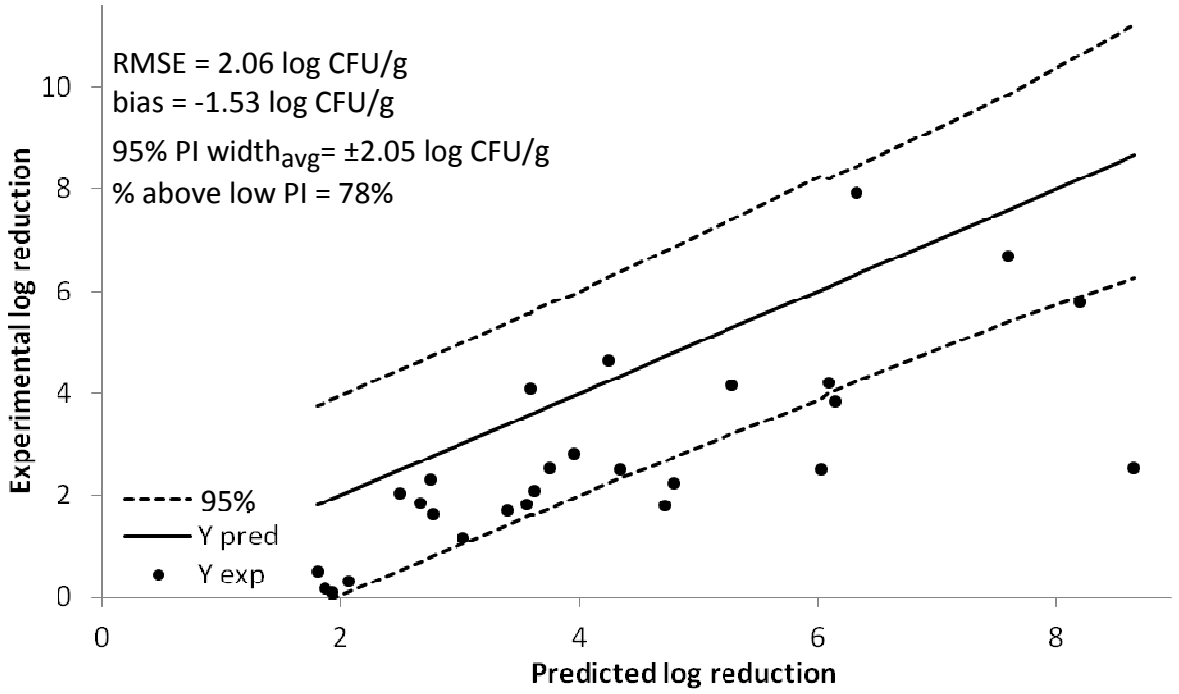

Figure 19. OLS T (g) model validated against ground pork data. Compare with Figure 20.

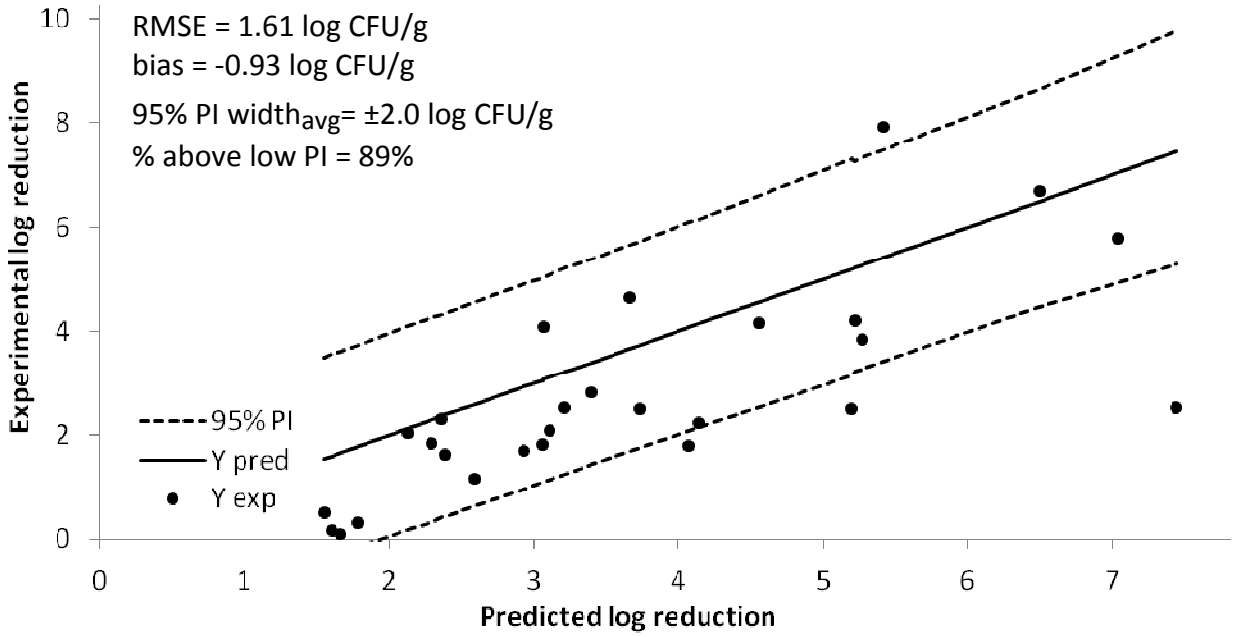

Figure 20. OLS T F τ (g) model validated against ground pork data. Notice that by considering fat content (F) and sublethal history (τ), model performance is improved.

In the poultry products, the PIs for the whole-muscle validated models also performed better than their ground-muscle counterparts, in terms of data points above the fail-dangerous PI band (~80% vs. ~25%) (Figure 21 and Figure 22). However, for beef and pork, both model versions showed high percentages for this measure (>80%) in their corresponding validation data sets. Additionally, the prediction interval "widths" were different across species and whole- or ground-muscle validated models. For example, for turkey, the ground-muscle-validated models PIs were more than twice as wide than their whole-muscle-validated counterparts (~2.60 vs ~0.95 log CFU/g). In the case of beef, PI width for the ground-muscle-validated models was only ~0.4 log CFU/g narrower than their whole-muscle-validated partners'; and finally for pork, they were essentially the same. While these interval widths evidently have an effect on the percentage of data points falling above the fail-dangerous PI band, they are mostly a consequence of the source data used to calibrate the models. Also, for our application, the choice of a model can be balanced between percentage of data points above the fail-dangerous PI band and the PI width.

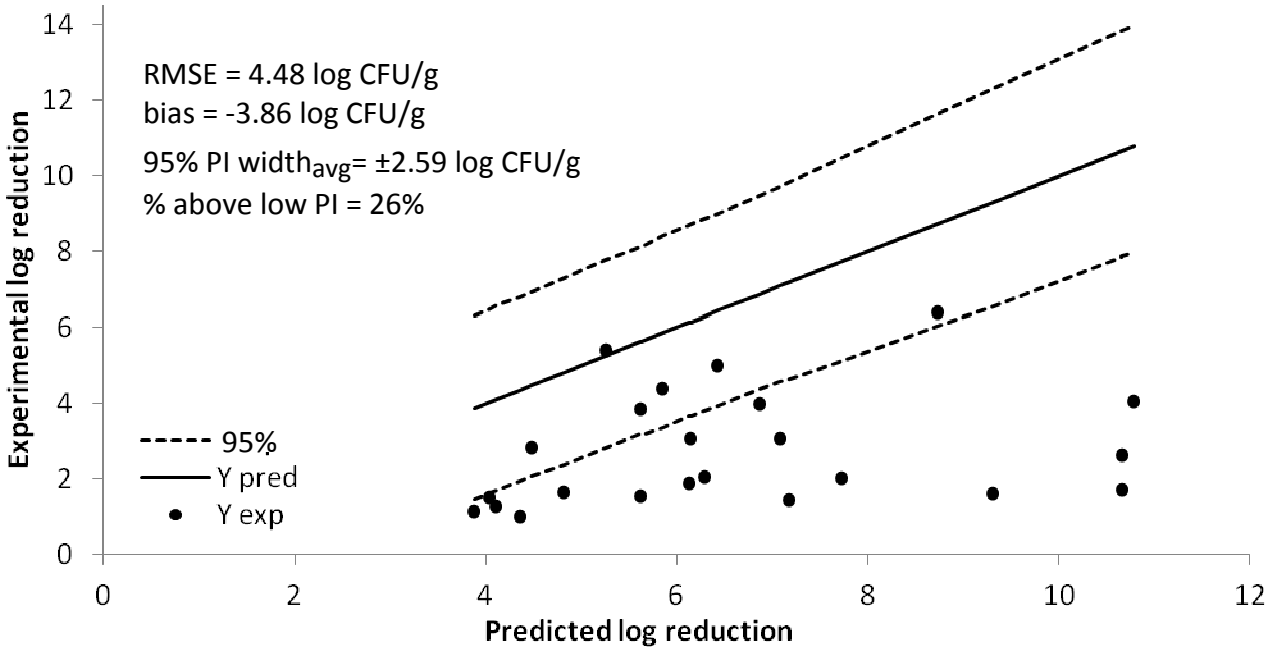

Figure 21. OLS T F τ (g) model validated against ground turkey data. Compare with Figure 22.

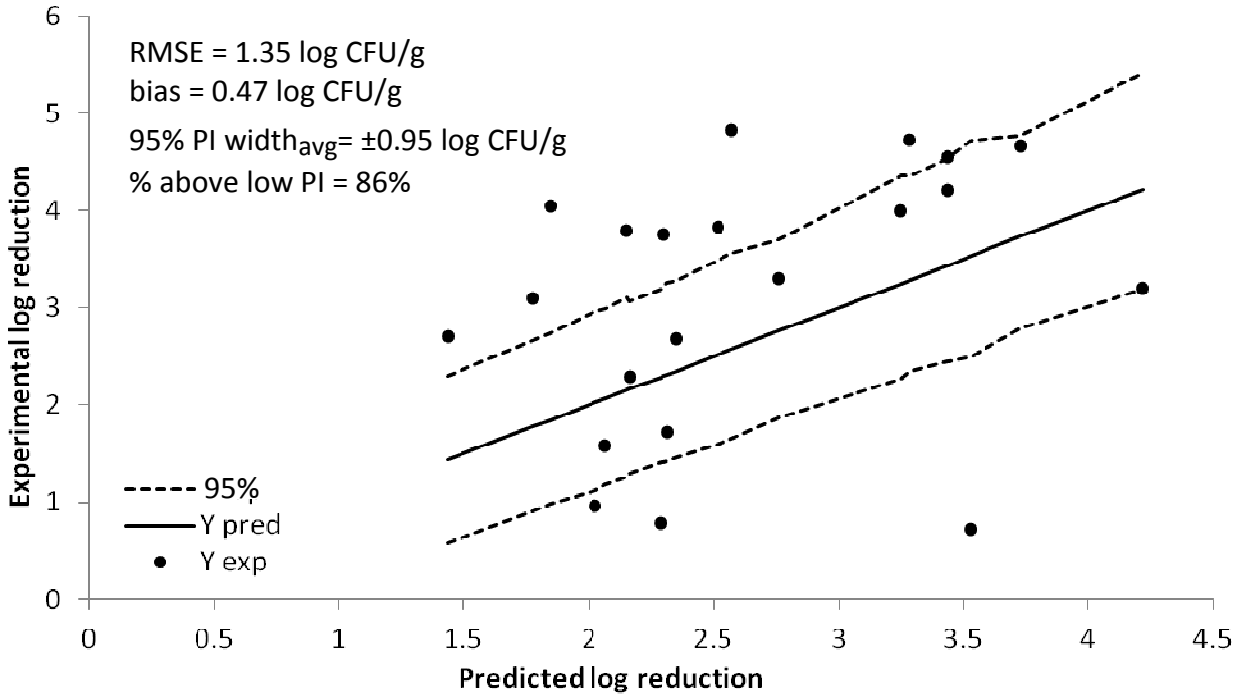

Figure 22. OLS T F τ (w) model validated against whole-muscle chicken breast data.

Table 14 shows model predictions when all the impingement oven data were pooled together. As with the individual species and muscle samples, the addition of the fat and sublethal injury parameters (β_2 and β_3, represented by F and τ in model names) improved model performance. However, substantial differences in the performance parameters were not evident, meaning, as expected, that temperature is the most influential factor in determining process lethality. On the other hand, the fat and sublethal injury factors may influence model predictions significantly more in products where they appear to be more prominent, such as sublethal history for slow-cooking roasts, and fat content for products such as hot dogs (as reported in the next section).

Table 14. OLS models validated against ALL impingement oven data.

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL impingement oven data	T (g) + T (w)	77	67	1.87	2.42	-0.84
	T F (g) + T F (w)	81	66	1.85	2.30	-0.62
	T τ (g) + T τ (w)	79	68	1.87	2.38	-0.78
n_{obs} = 144	T F τ (g) + T F τ (w)	81	65	1.86	2.26	-0.57

*In all cases, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data. In addition, models used were species specific, that is, turkey models predicted for turkey data, and so on.

Tables 15-17 show the mixed-effects models performance when validated against whole-muscle products and ground patties in the impingement oven. These are discussed in detail in the following paragraphs.

For the poultry products (Table 15), the models fared better when predicting lethality in the whole chicken breasts. For example, while the PIs for the more complex models encompassed high percentages (~80-100%) of data points for both ground- and whole-muscle products, the simpler models produced much lower numbers (~40%) against the ground turkey patties than against the whole-muscle chicken samples (~70%). The exception to this would be the low percentage (38%) obtained with the T F and T F τ models in the whole chicken breasts (Figure 23); however, notice here that PI width is only 0.85 log CFU/g. On the other hand, the RMSE and PI widths were slightly larger for the ground turkey patties, while the bias was also more prominent towards the fail-dangerous side. Overall, when both poultry data sets were pooled together (n_{obs}=44), the most complex model (T F M τ S) performed, in a conservative manner, the best.

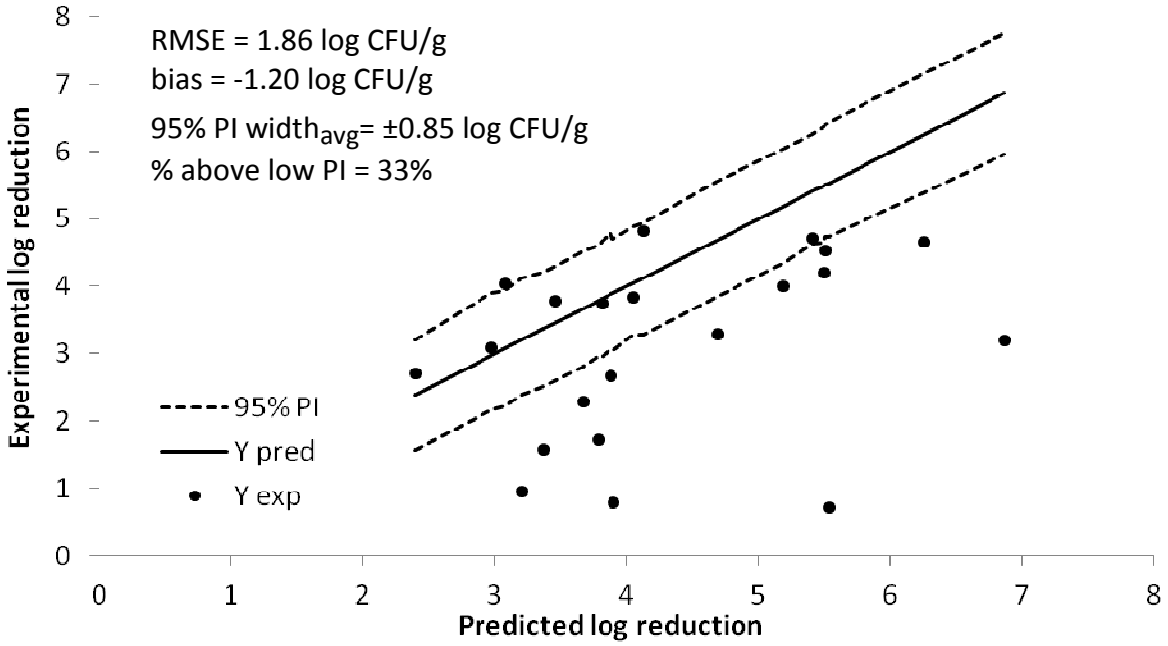

Figure 23. Mixed-effects T F (w) model validated against whole-muscle chicken fillets.

Table 15. Mixed-effects models validated against chicken steaks and turkey patties (impingement cooked) (continued next page).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Ground Turkey + Whole Chicken n_{obs} = 44	T (g)	100	100	3.66	1.54	-0.38
	T F (g)	66	66	2.90	2.65	-2.14
	T F τ (g)	66	66	2.89	2.58	-2.06
	T F S (g)	100	100	4.75	1.46	-0.16
	T F τ S (g)	100	100	4.67	1.45	-0.12
	T (g) + T (w)	91	73	2.33	1.53	-0.20
	T F (g) + T F (w)	50	48	1.85	2.27	-1.64
	T F τ (g) + T F τ (w)	50	48	1.86	2.21	-1.57
	T F S (g) + T F S (w)	98	91	3.24	1.48	0.09
	T F τ S (g) + T F τ S (w)	98	91	3.21	1.47	0.13
	T F M	73	45	2.09	2.28	-0.54
	T F M τ	73	45	2.04	2.25	-0.49
	T F M S	100	98	3.15	1.90	-0.58
	T F M τ S	100	98	3.14	1.88	-0.53
Ground turkey n_{obs} = 23 fat = 1.05% τ_{avg} = 8.77 K·min	T (g)	100	100	3.70	1.70	-0.59
	T F (g)	61	61	2.76	2.58	-2.03
	T F τ (g)	61	61	2.78	2.52	-1.96
	T F S (g)	100	100	4.69	1.57	-0.28
	T F τ S (g)	100	100	4.62	1.56	-0.24
	T F M	48	48	2.76	2.69	-2.16
	T F M τ	48	48	2.68	2.62	-2.08
	T F M S	100	100	3.69	2.29	-1.52
	T F M τ S	100	100	3.72	2.24	-1.46

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 15 (cont'd). Mixed-effects models validated against chicken steaks and turkey patties (impingement cooked).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Whole chicken n_{obs} = 21 fat = 0.33% τ_{avg} = 7.92 K·min	T (g)	100	100	3.30	1.34	-0.16
	T F (g)	71	71	3.05	2.72	-2.25
	T F τ (g)	71	71	3.02	2.65	-2.18
	T F S (g)	100	100	4.82	1.33	-0.04
	T F τ S (g)	100	100	4.73	1.32	0.01
	T (w)	81	43	0.83	1.31	0.23
	T F (w)	38	33	0.85	1.86	-1.20
	T F τ (w)	38	33	0.85	1.82	-1.14
	T F S (w)	95	81	1.66	1.36	0.50
	T F τ S (w)	95	81	1.66	1.38	0.53
	T F M	100	43	1.35	1.73	1.22
	T F M τ	100	43	1.34	1.75	1.25
	T F M S	100	95	2.56	1.36	0.45
	T F M τ S	100	95	2.51	1.37	0.48

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

For the beef samples (Table 16), all models performed relatively well, with high percentages of data points (>95%) above the lower fail-dangerous PI band (Figure 24), RMSEs ranging from ~1.2-1.9 log CFU/g, and almost all bias values on the fail-safe side. Some models against the whole beef samples produced negative bias values, but no larger than -0.34 log CFU/g. Overall, the PIs were slightly, but consistently across models, wider than in the poultry samples.

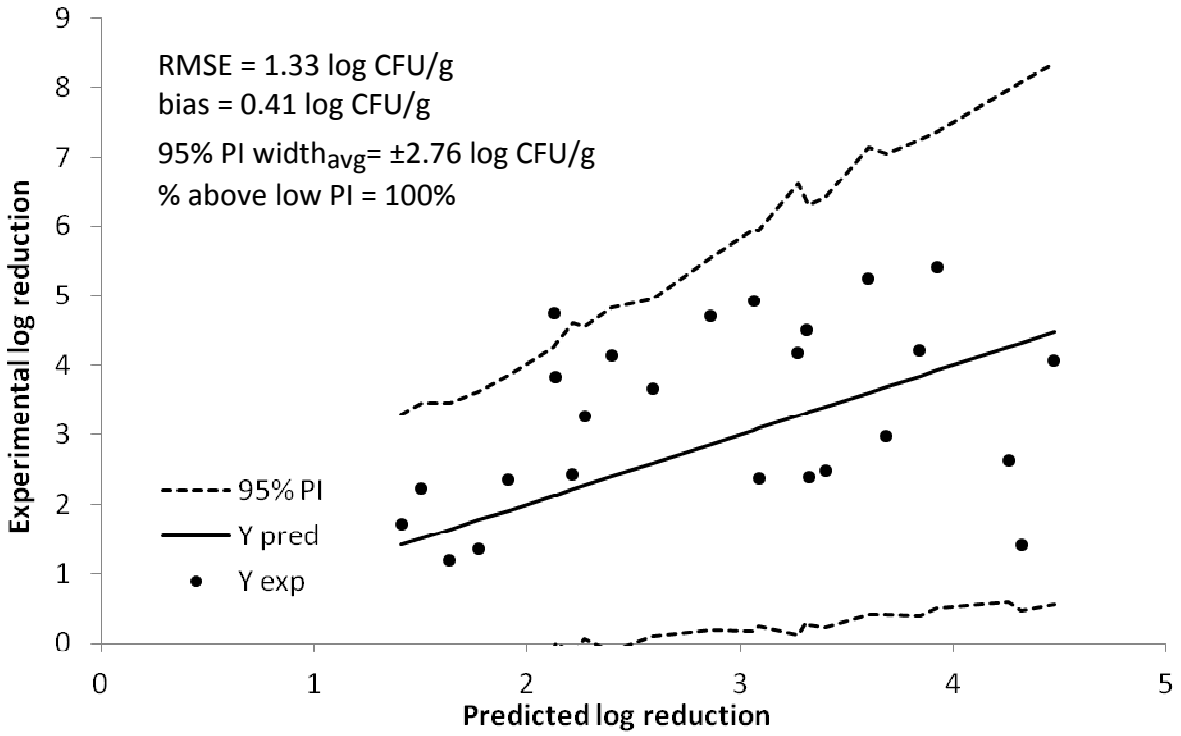

Figure 24. Mixed-effects T F M τ S model validated against whole-muscle beef steaks.

Table 16. Mixed-effects models validated against beef steaks and patties (impingement cooked) (continued next page).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Ground + Whole beef n_{obs} = 44	T (g)	100	93	2.56	1.58	0.76
	T F (g)	100	95	2.83	1.33	0.14
	T F τ (g)	100	95	2.80	1.34	0.19
	T F S (g)	100	100	4.96	1.55	0.20
	T F τ S (g)	100	100	4.83	1.56	0.25
	T (g) + T (w)	98	82	2.11	1.59	0.81
	T F (g) + T F (w)	98	86	2.01	1.37	0.43
	T F τ (g) + T F τ (w)	98	82	2.00	1.38	0.48
	T F S (g) + T F S (w)	98	95	2.78	1.47	0.50
	T F τ S (g) + T F τ S (w)	98	95	2.72	1.48	0.55
	T F M	100	86	2.26	1.48	0.78
	T F M τ	100	84	2.23	1.51	0.85
	T F M S	100	95	2.90	1.41	0.22
	T F M τ S	100	98	2.90	1.41	0.27
Ground beef n_{obs} = 19 fat = 2.32% τ_{avg} = 9.28 K·min	T (g)	100	84	2.75	1.93	1.34
	T F (g)	100	89	2.59	1.51	0.63
	T F τ (g)	100	89	2.56	1.53	0.70
	T F S (g)	100	100	4.14	1.71	0.92
	T F τ S (g)	100	100	4.00	1.73	0.96
	T F M	100	89	2.42	1.48	0.56
	T F M τ	100	89	2.38	1.50	0.61
	T F M S	100	95	3.13	1.51	0.03
	T F M τ S	100	100	3.08	1.51	0.09

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 16 (cont'd). Mixed-effects models validated against beef steaks and patties (impingement cooked).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Whole beef n_{obs} = 25 fat = 2.68% τ_{avg} = 8.18 K·min	T (g)	100	100	3.46	1.25	0.32
	T F (g)	100	95	3.01	1.18	-0.26
	T F τ (g)	100	100	2.98	1.17	-0.20
	T F S (g)	100	100	5.59	1.42	-0.34
	T F τ S (g)	100	100	5.46	1.40	-0.29
	T (w)	96	80	1.62	1.27	0.41
	T F (w)	96	84	1.57	1.24	0.26
	T F τ (w)	96	76	1.58	1.25	0.31
	T F S (w)	96	92	1.75	1.25	0.19
	T F τ S (w)	96	92	1.74	1.26	0.23
	T F M	100	84	2.14	1.47	0.95
	T F M τ	100	80	2.11	1.51	1.03
	T F M S	100	96	2.72	1.32	0.37
	T F M τ S	100	96	2.76	1.33	0.41

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

For the pork products (Table 17), the models gave slightly better predictions for the ground-muscle patties in terms of the percentage of data points above the fail-dangerous PI band. In addition, the PI widths for the ground-muscle calibrated models (g) were slightly narrower than those of the whole-muscle calibrated models (w). RMSE was consistent (~1.4-1.9 log CFU/g) throughout all the data set, except for a few exceptions (~2.3-2.6 and ~3.1 log CFU/g). On the other hand, bias values were more scattered, ranging from -2.2 to 1.2 log CFU/g. However, when both ground- and whole-muscle samples were pooled together (n_{obs}=56), RMSE, bias, and PI width were consistent across models once more.

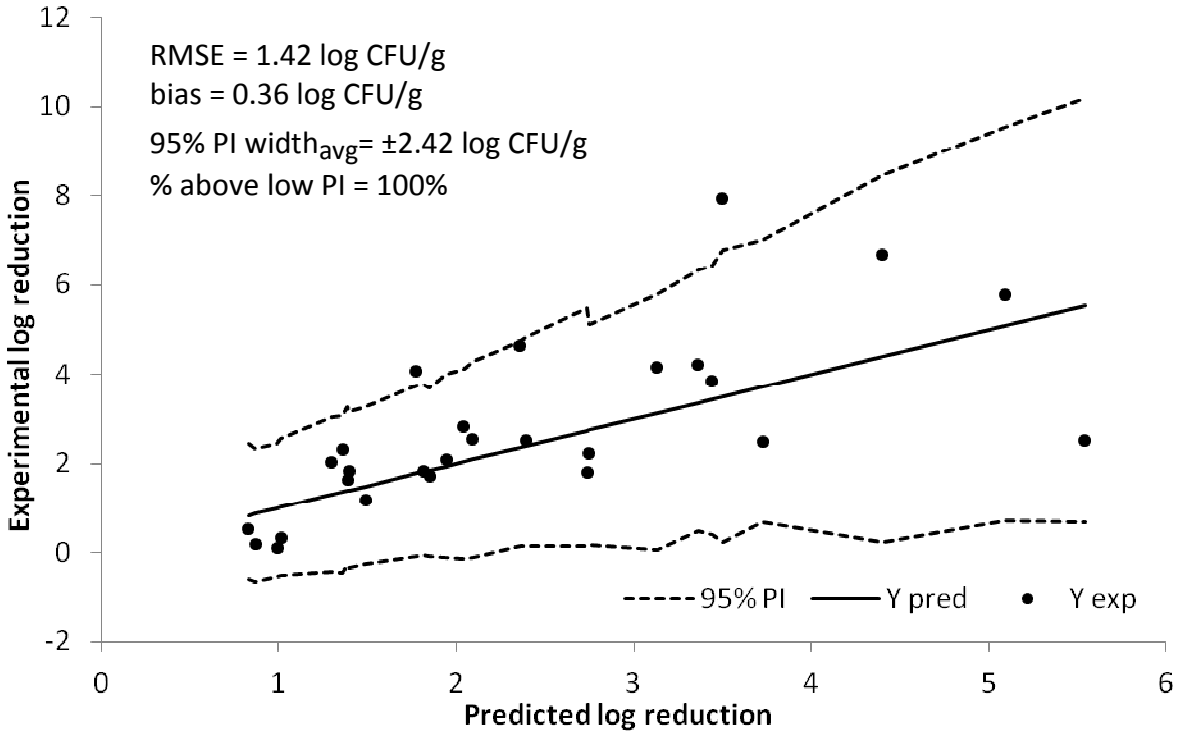

Figure 25. Mixed-effects T F M τ S model validated against ground-muscle pork patties.

Table 17. Mixed-effects models validated against pork steaks and patties (impingement cooked) (continued next page).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Ground + Whole pork n_{obs} = 56	T (g)	93	89	3.54	1.55	0.12
	T F (g)	82	100	1.87	1.60	0.80
	T F τ (g)	100	82	1.87	1.62	0.82
	T F S (g)	100	93	3.54	1.82	0.93
	T F τ S (g)	100	93	3.66	1.71	0.84
	T (g) + T (w)	82	80	2.79	1.98	-0.72
	T F (g) + T F (w)	89	75	2.36	2.60	-0.60
	T F τ (g) + T F τ (w)	88	73	2.34	2.57	-0.56
	T F S (g) + T F S (w)	86	50	1.27	1.98	0.57
	T F τ S (g) + T F τ S (w)	89	80	2.30	1.86	0.46
	T F M	100	88	1.98	1.54	0.70
	T F M τ	100	84	1.98	1.56	0.73
	T F M S	100	82	2.49	1.66	0.67
	T F M τ S	100	86	2.48	1.53	0.60
Ground pork n_{obs} = 27 fat = 10% τ_{avg} = 9.34 K·min	T (g)	85	85	2.87	1.47	-0.13
	T F (g)	100	74	1.59	1.83	1.11
	T F τ (g)	100	74	1.59	1.85	1.14
	T F S (g)	100	89	2.43	1.85	1.16
	T F τ S (g)	100	89	2.72	1.87	1.18
	T F M	100	85	1.73	1.67	0.92
	T F M τ	100	81	1.75	1.70	0.95
	T F M S	100	89	2.43	1.40	0.32
	T F M τ S	100	93	2.42	1.42	0.36

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 17 (cont'd). Mixed-effects models validated against pork steaks and patties (impingement cooked).

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Whole pork n_{obs} = 29 fat = 1.53 % τ_{avg} = 8.21 K·min	T (g)	100	93	4.17	1.61	0.35
	T F (g)	100	90	2.12	1.35	0.50
	T F τ (g)	100	90	2.13	1.36	0.53
	T F S (g)	100	97	4.57	1.80	0.71
	T F τ S (g)	100	97	4.54	1.56	0.52
	T (w)	79	76	2.71	2.35	-1.26
	T F (w)	79	76	3.08	3.15	-2.20
	T F τ (w)	76	72	3.03	3.10	-2.13
	T F S (w)	86	76	1.92	1.87	-0.25
	T F τ S (w)	79	72	1.91	1.86	-0.21
	T F M	100	90	2.20	1.40	0.49
	T F M τ	100	86	2.19	1.41	0.52
	T F M S	100	76	2.55	1.87	1.00
	T F M τ S	100	79	2.54	1.64	0.83

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

The fact that the mixed-effects models performed better with the beef and pork samples (especially in terms of the percentage of data points on the safe side) is a measure of the suitability of the calibration data for the chosen models. Just as with the OLS models, the addition of the sublethal injury parameter (β_2, represented by τ in model names), improved overall, even if slightly, in some manner the models- be it by reducing RMSE, bias, PI width, or a combination of the three. On the other hand, the combination of ground- and whole-muscle data to obtain models with the M (muscle) parameter (Table 4) had mixed outcomes; it improved

predictions for the poultry samples, but had both positive and negative effects for the beef and pork products. In addition, the separation of data only by muscle type (that is, lumping all ground turkey, beef, and pork data in one calibration group, and their whole-muscle counterparts in another (Table 4) to yield the S (species) parameter, had this same effect. On the other hand, within the TFS models, those calibrated with ground-muscle data (T F S (g) and T F τ S (g)) had wider PIs (~±2.50 vs. ~±1.0 log CFU/g), but consequently were able to capture higher percentages of data points above the fail-dangerous PI band (~±100% vs. ~±90%). Although these percentage differences could be considered small, on food safety applications it is desirable to err on the safe side. In terms of RMSE, the ground-calibrated models fared slightly better (~0.2 log CFU/g) for the beef and pork samples, while the bias values were evenly fail-safe and fail-dangerous across all samples.

Table 18 shows the mixed-effects models performing against all impingement oven data. Percentage of data points above the fail-dangerous PI band was overall satisfactory, with the two most complicated models (T F M S and T F M τ S) capturing 100% of them, and all capturing at least 79%. RMSEs were also acceptable, given the expected variability in this kind of data, and bias values were mostly positive, except for the first three model versions. On the other hand, PI width was large -bigger than what could possibly be useful for industrial applications, especially in the case of the last two models.

When comparing the OLS models with the mixed-effects versions against all the impingement oven data pooled together (Table 14 and Table 18), it is evident each has its positive features and drawbacks. For example, while the OLS models placed, at most, 81% of the data points above the fail-dangerous PI band, the mixed-effects models were able to capture

>80% of them in the same region. However, this could mostly be due to the PI widths (~2.0-2.8 log CFU/g for mixed-effects models vs. ~1.86 log CFU/g for the OLS versions). On the other hand, RMSE and bias were more favorable to the mixed-effects models, with a maximum RMSE = 2.18 log CFU/g for the mixed-effects models and minimum RMSE=2.26 log CFU/g for OLS versions; and in the case of bias, all were fail-dangerous for the OLS models, while 67% of them were fail-safe for the mixed-effects versions.

Table 18. Mixed-effects models validated against ALL impingement oven data.

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL impingement oven data n_{obs} =144	T (g) + T (w)	90	78	2.44	1.73	-0.09
	T F (g) + T F (w)	80	70	2.10	2.18	-0.60
	T F τ (g) + T F τ (w)	79	68	2.09	2.16	-0.55
	T F S (g) + T F S (w)	93	76	2.34	1.69	0.40
	T F τ S (g) + T F τ S (w)	94	88	2.70	1.64	0.39
	T F M	92	74	2.10	1.78	0.34
	T F M τ	92	72	2.07	1.78	0.39
	T F M S	100	91	2.82	1.67	0.15
	T F M τ S	100	93	2.81	1.61	0.16

*In all cases, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data. In addition, models used were species specific, that is, turkey models predicted for turkey data, and so on.

4.3.4.2 Whole-muscle roasts

Table 19 shows the OLS models predictions against the pilot-scale roasts. Because these are the biggest pilot-scale samples, high variability and potential fail-dangerous predictions were expected (Breslin 2009). For the turkey roasts, the ground-calibrated models did not predict satisfactorily; PIs were wide, RMSEs very big, and bias values extremely high on the fail-dangerous side, as would be expected (Figure 26). On the other hand, even though the percentage of data points above the fail-dangerous PI band remained the same at 0%, when the whole-muscle validated models were applied to these whole-muscle roasts, the prediction statistics were better: narrower PIs, lower RMSEs, and less dangerous bias values (Figure 27). This shows the importance of using models calibrated with products that match the characteristics of those to be involved in model predictions. In the case of beef and pork, the same trends were produced with whole-muscle calibrated models predicting better than their ground-muscle counterparts. However, the pork models performed significantly better, in terms of reaching 100% of data points above the fail-dangerous PI band compared to 62% in the case of beef. This could be attributed to the PI widths of pork being larger than those of beef (~2.0 CFU/g vs ~1.75 CFU/g). On the other hand, the RMSEs for beef were significantly smaller than those of pork (~1.0 CFU/g vs. ~3.0 CFU/g), and bias values were also more prominent on the fail-safe side for the same products (~-1.6 CFU/g vs. ~0.2 CFU/g for beef and pork, respectively).

Table 19. OLS models validated against roasts.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Turkey roasts n_{obs} = 9 fat = 0.27% τ_{range} = 200 – 500 K·min	T (g)	0	0	2.82	12.41	-12.36
	T F (g)	0	0	2.80	12.27	-12.23
	T τ (g)	0	0	2.68	10.95	-10.89
	T F τ (g)	0	0	2.66	10.83	-10.77
	T (w)	0	0	1.11	3.44	-3.25
	T F (w)	0	0	1.10	3.39	-3.19
	T τ (w)	0	0	1.02	2.81	-2.58
	T F τ (w)	0	0	1.01	2.76	-2.53
Beef roasts n_{obs} = 13 fat = 2.68% τ_{range} = 100 – 480 K·min	T (g)	0	0	1.63	7.57	-6.56
	T F (g)	0	0	1.59	6.94	-5.92
	T τ (g)	0	0	1.58	6.94	-5.90
	T F τ (g)	8	8	1.55	6.34	-5.30
	T (w)	46	46	1.75	2.72	-1.91
	T F (w)	62	62	1.74	2.98	-1.71
	T τ (w)	62	62	1.74	3.00	-1.69
	T F τ (w)	62	62	1.72	2.73	-1.35
Pork roasts n_{obs} = 20 fat =1.53% τ_{range} = 100 – 600 K·min	T (g)	60	60	2.02	2.37	-1.98
	T F (g)	65	65	2.01	2.26	-1.86
	T τ (g)	65	65	2.00	1.96	-1.63
	T F τ (g)	70	70	1.99	1.86	-1.52
	T (w)	100	100	2.06	0.94	0.10
	T F (w)	100	100	2.06	0.96	0.22
	T τ (w)	100	100	2.05	0.90	0.31
	T F τ (w)	100	100	2.04	0.93	0.42

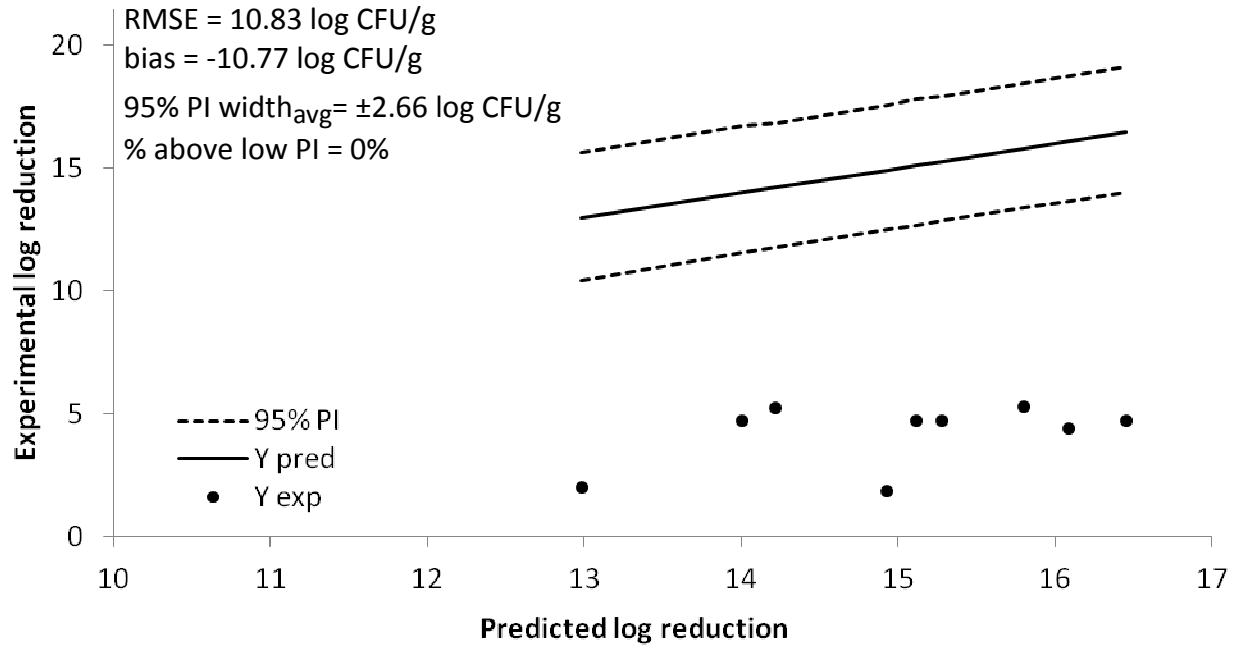

Figure 26. OLS T F τ (g) model validated with turkey roast data. Compare with Figure 27.

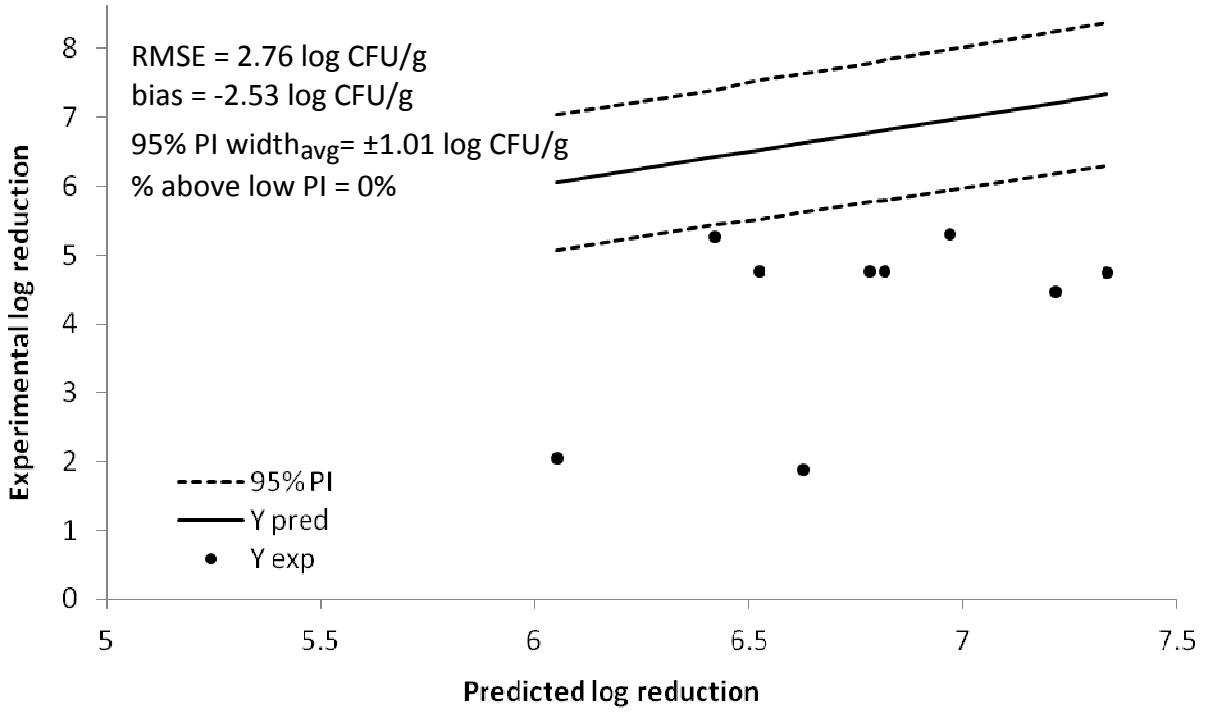

Figure 27. OLS T F τ (w) model validated against turkey roast data. Notice the significantly better performance than its (g) counterpart (Figure 26).

Table 20 shows model predictions when the roast data for all three species were pooled together. As with the species-specific roasts and the impingement oven data, the addition of the fat and sublethal injury parameters (β_3 and β_2, respectively) enhanced model performance, but not substantially. On the other hand, while β_3 had a greater effect on the impingement oven data predictions, β_2 here has a larger impact, reducing PI width, RMSE, and bias values more significantly than β_3. This is due to the effect the high sublethal history values of these data (ranging from 100 to 600 K·min) have on the model predictions (compared to ~8-9 K·min for the impingement oven data).

Table 20. OLS models validation against ALL roast data.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL roast data n_{obs} = 42	T (w)	62	62	1.76	2.29	-1.24
	T F (w)	67	67	1.75	2.38	-1.11
	T τ (w)	67	67	1.73	2.20	-0.93
	T F τ (w)	67	67	1.72	2.09	-0.76

Table 21 shows the mixed-effects models when validated against the roast products. Performance is once more negatively affected when using ground-muscle calibrated models to predict lethality on whole-muscle data, as expected. Just as with the impingement oven samples, turkey roasts were once more the most affected by this, with highly dangerous bias (> -5.0 log CFU/g), extremely wide PIs (> ±8.0 log CFU/g), large RMSEs (<5.50 log CFU/g), and low percentage of data points above the fail-dangerous PI band. It can be argued that the T F τ S (g) model on the turkey roasts encompassed 100% of the data points, but looking at the PI width

(±8.05 log CFU/g), this becomes obvious and not useful for our application. For the beef and pork roasts, the ground-calibrated modes fared better in terms of the percentage values (>90% above low PI), but most PI widths were still outside the scope of usefulness, as explained in section 4.2.4 (>±5.5 log CFU/g for beef, and >2.2 log CFU/g for pork, one of the few exceptions in Figure 28). Even though performance on pork was satisfactory in the case of the T F τ (g) model, the whole-muscle validated counterparts provided better predictions. Overall, the whole-muscle validated models, those including sublethal history (τ), or those accounting for muscle type (M) had better predictions across the products, with the more complicated model (T F M τ S) performing the best conservatively.

Table 21. Mixed-effects models validated against roasts (continued next page).

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Turkey roasts n_{obs} = 9 fat = 0.27% τ_{range} = 200 – 500 K·min	T (g)	100	100	9.00	4.34	-4.19
	T F (g)	0	0	12.20	18.65	-18.54
	T F τ (g)	11	0	7.70	9.77	-9.57
	T F S (g)	100	100	10.64	11.53	-11.38
	T F τ S (g)	100	100	7.83	1.59	-0.92
	T (w)	0	0	0.83	3.27	-3.00
	T F (w)	0	0	0.85	18.65	-18.54
	T F τ (w)	0	0	0.85	4.83	-4.54
	T F S (w)	33	33	2.79	3.77	-3.58
	T F τ S (w)	89	89	2.10	1.37	-0.61
	T F M	78	78	3.87	3.42	-3.15
	T F M τ	89	89	2.52	1.18	-0.25
	T F M S	100	100	5.92	5.44	-5.25
	T F M τ S	100	100	3.82	1.25	-0.18

Table 21 (cont'd). Mixed-effects models validated against roasts.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Beef roasts n_{obs} = 13 fat = 2.68% τ_{range} = 100 – 480 K·min	T (g)	100	92	6.55	3.48	-2.30
	T F (g)	85	85	7.44	6.58	-5.80
	T F τ (g)	92	92	5.34	4.06	-3.00
	T F S (g)	100	100	10.08	3.92	-2.61
	T F τ S (g)	100	92	7.44	2.68	-0.68
	T (w)	57	50	2.35	3.18	-2.10
	T F (w)	54	54	2.24	3.58	-2.42
	T F τ (w)	77	54	1.89	2.42	-0.52
	T F S (w)	62	62	2.49	3.58	-2.38
	T F τ S (w)	96	92	1.74	1.26	0.23
	T F M	92	92	4.32	3.17	-2.19
	T F M τ	100	92	3.21	2.10	-0.32
	T F M S	100	100	4.70	2.85	-1.35
	T F M τ S	100	85	3.53	2.26	0.22
Pork roasts n_{obs} = 20 fat = 1.53% τ_{range} = 100 – 600 K·min	T (g)	100	100	3.14	1.71	1.50
	T F (g)	90	90	3.12	1.91	-1.17
	T F τ (g)	100	90	2.39	0.82	0.19
	T F S (g)	100	100	4.04	1.46	1.15
	T F τ S (g)	100	100	3.18	1.97	1.82
	T (w)	100	100	2.25	0.86	0.33
	T F (w)	100	100	2.50	0.92	-0.35
	T F τ (w)	100	90	2.12	0.97	0.69
	T F S (w)	90	90	1.95	1.12	-0.50
	T F τ S (w)	100	95	1.75	0.91	0.63
	T F M	100	100	2.70	1.23	-0.28
	T F M τ	100	100	2.19	1.11	0.82
	T F M S	100	85	2.31	1.66	1.38
	T F M τ S	100	45	1.97	2.15	1.99

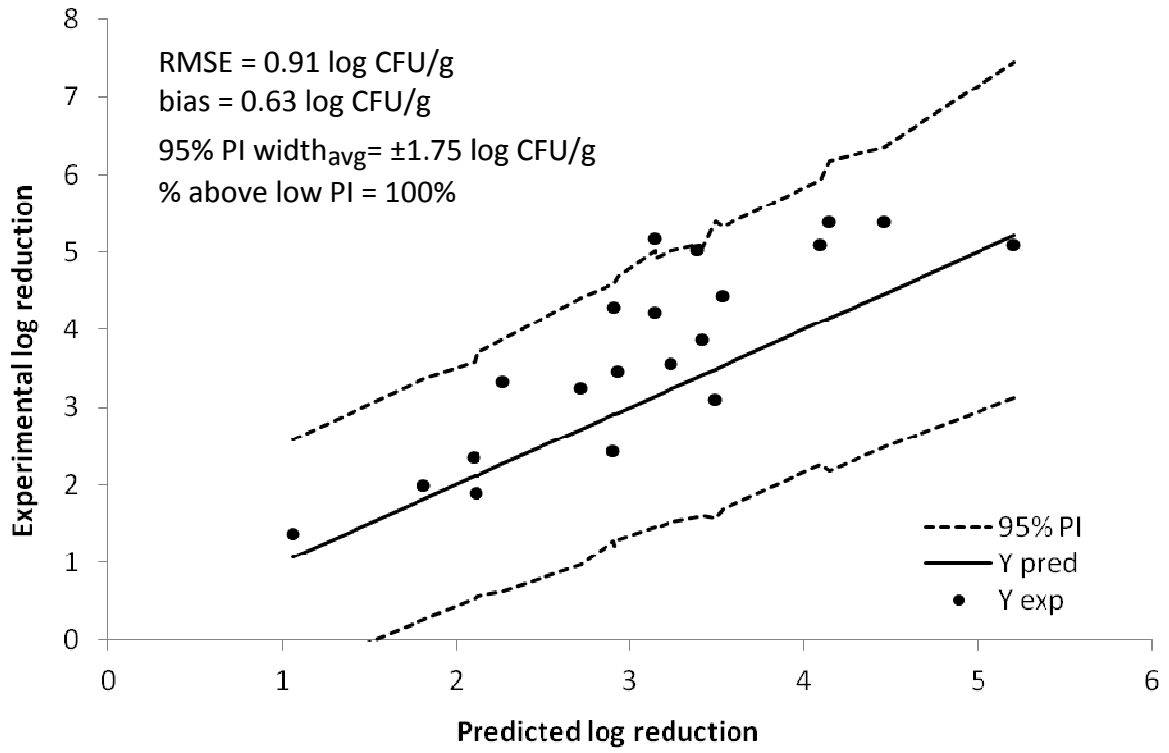

Figure 28. Mixed-effects T F τ S (w) model validated against pork roasts. Notice the favorable fitting statistics.

Table 22 shows the whole-muscle-validated and those accounting for muscle type (M) mixed-effects models against all roast data put together. The most complicated models (lower down the table) fared better in terms of the percentage of data points captured above the fail-dangerous PI band, mostly because of the low percentage values from the simpler models' predictions in the turkey roasts (Table 21). On the other hand, the simpler models had narrower PIs, but also higher RMSE and more fail-dangerous bias values.

Table 22. Mixed-effects models validated against ALL roast data.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL roast data n_{obs} = 42	T (w)	67	64	1.98	4.00	-2.28
	T F (w)	64	64	2.07	8.88	-4.89
	T F τ (w)	71	60	1.83	2.67	-1.04
	T F S (w)	69	69	2.30	2.76	-1.74
	T F τ S (w)	90	83	1.92	1.63	0.02
	T F M	93	93	3.45	2.52	-1.49
	T F M τ	98	95	2.58	1.50	0.24
	T F M S	100	93	3.82	3.19	-0.89
	T F M τ S	100	69	2.85	2.03	0.98

Comparing the OLS models (Table 20) vs. the mixed-effects models (Table 22) performances in the all the roast data pooled together, once more the OLS models reach a plateau in capturing data points above the fail-dangerous PI band (~67% maximum), while model complexity in the mixed-effects versions allows the percentage to reach 100%. On the other hand, PI widths on the OLS models are more industry-useful than those yielded by the mixed-effects models (~1.75 log CFU/g vs. 1.83-3.82 log CFU/g). Finally RMSE and bias values were inconsistent across all models, but overall presenting more high numbers compared to the impingement oven data.

4.3.4.3 Hot dogs

Table 23 shows the OLS models predicting lethality for the hot dog data. With these few validations and data sets, it is easier to see the positive effect fat and sublethal history have when included in the models. In the case of the turkey hot dogs, no improvement in the percentage of

data points above the fail-dangerous PI band was observed with the separate addition of the two parameters in question. However, RMSE and bias did improve. Nevertheless, when applied together, the percent captured increased from 33% to 58%, RMSE decreased from 3.31 to 2.19 log CFU/g and bias decreased from -2.60 to -1.40 log CFU/g. On the other hand, for the beef hot dogs, fat content and sublethal injury had significant effects when considered individually by the model; each increased the percentage of data points above the lower PI band from 33% to 92%. Additionally, when acting together, the PI encompassed 100% of the data points. It can be noted that the fat parameter performed particularly well in these ~15% fat beef products, decreasing the RMSE and bias more than the sublethal injury parameter did.

Table 23. OLS models validated against hot dogs.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Turkey hot dogs n_{obs} = 12 fat = 4.28% τ_{range} = 50 – 125 K·min	T (g)	33	33	2.47	3.31	-2.60
	T F (g)	33	33	2.43	2.74	-2.02
	T τ (g)	33	33	2.43	2.63	-1.90
	T F τ (g)	58	50	2.41	2.19	-1.40
Beef hot dogs n_{obs} = 12 fat =15.42% τ_{range} = 100 – 275 K·min	T (g)	33	33	1.55	2.03	-1.90
	T F (g)	92	92	1.52	0.81	-0.69
	T τ (g)	92	92	1.52	0.97	-0.82
	T F τ (g)	100	100	1.52	0.40	0.07

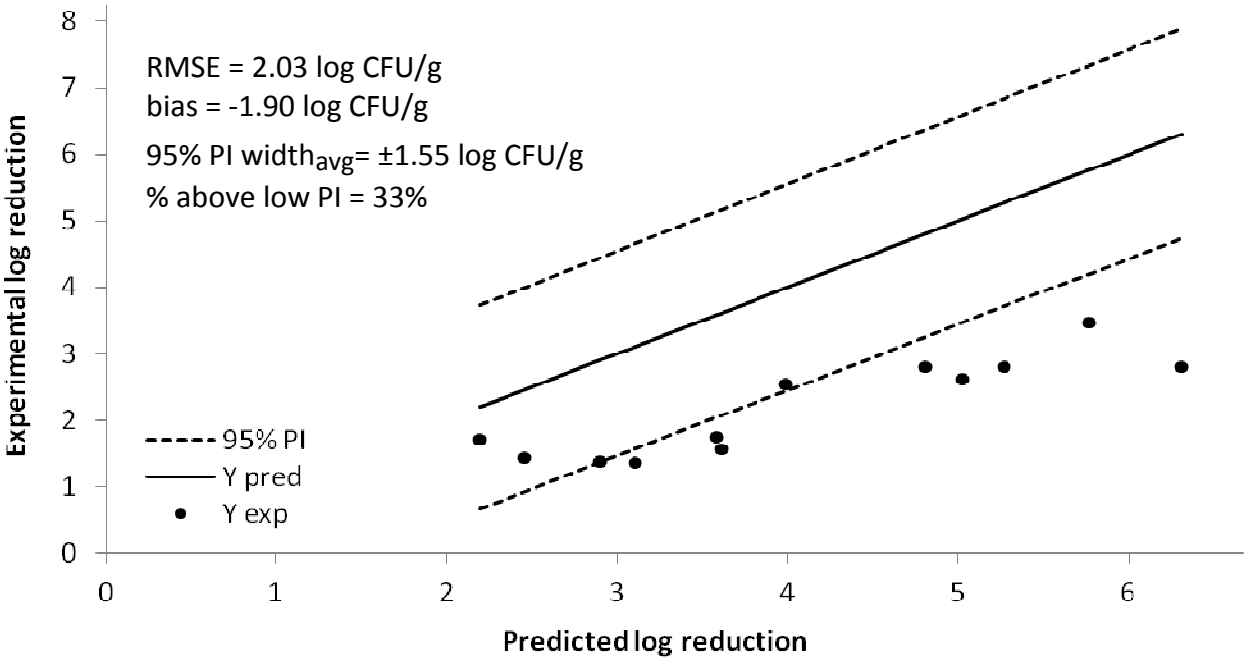

Figure 29. OLS T (g) model validated against beef hot dogs. Compare with Figure 30.

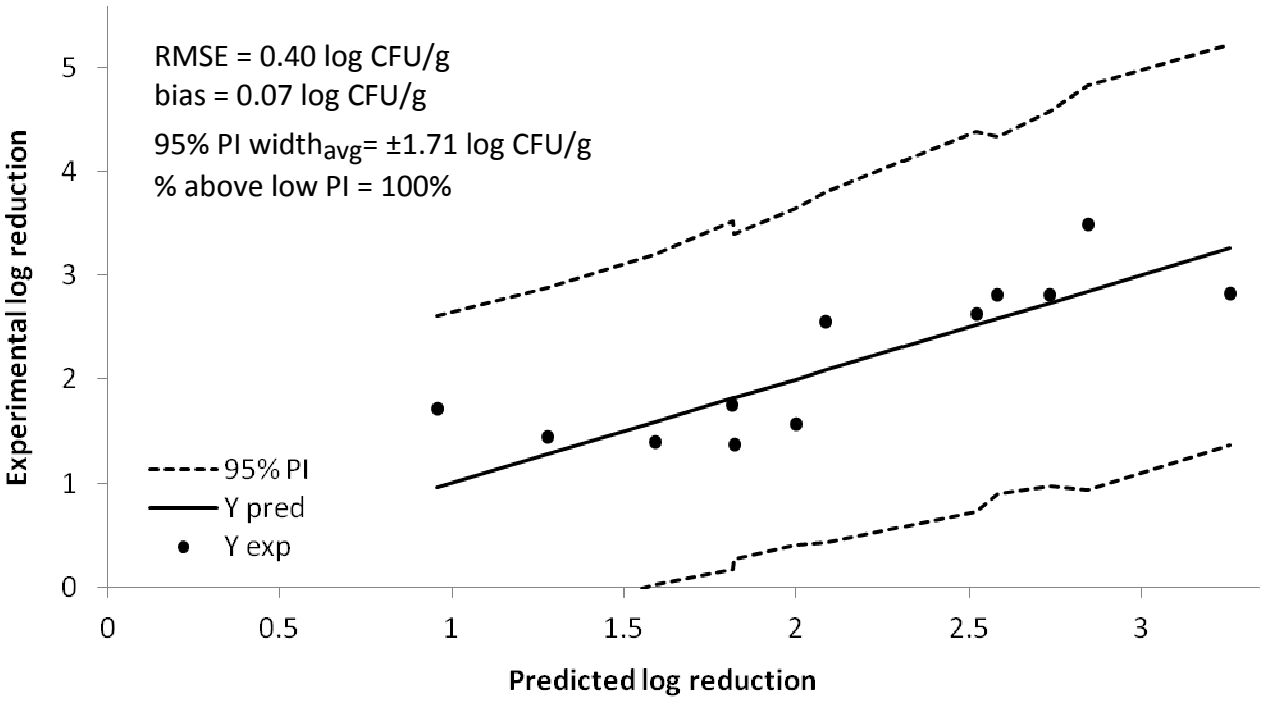

Figure 30. OLS T F τ (g) model validated against beef hot dog data. Notice the significantly better performance than the simpler T (g) model (Figure 29).

Table 24 shows the OLS models validated against both the turkey and beef hot dogs as a single data set. Again, the trends shown in the individual data sets are evident; fat and sublethal injury considerations meaningfully improved model predictions.

Table 24. OLS models validated against ALL hot dog data.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL hot dog data n_{obs} = 24	T (g)	33	33	2.01	2.75	-2.25
	T F (g)	63	63	1.97	2.02	-1.35
	T τ (g)	63	63	2.06	1.98	-1.36
	T F τ (g)	75	75	2.03	1.69	-1.11

Table 25 shows the different versions of the mixed-effects models validated against the hot dog data. Again, the models performed less satisfactorily on the turkey than on the beef samples, with wider PIs, and higher RMSEs and fail-dangerous bias values. However, the most complicated model (T F M τ S) fared sufficiently well for both cases (i.e., Figure 31), with 100% of the data points above the lower PI band, even though the interval was relatively wide for the turkey samples (±2.77 log CFU/g).

Table 25. Mixed-effects models validated against hot dogs.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
Turkey hot dogs n_{obs} = 12 fat =4.28% τ_{range} = 50 – 125 K·min	T (g)	100	83	2.89	1.40	-0.29
	T F (g)	67	58	2.29	2.18	-1.48
	T F τ (g)	67	50	2.03	3.73	-0.94
	T F S (g)	100	83	3.25	1.34	0.29
	T F τ S (g)	100	83	2.83	2.41	0.57
	T F M	67	50	2.19	2.82	-1.50
	T F M τ	67	50	1.93	1.82	-0.96
	T F M S	100	83	2.67	3.49	-0.59
	T F M τ S	100	83	2.40	2.51	-0.18
Beef hot dogs n_{obs} = 12 fat = 15.42% τ_{range} = 100 – 275 K·min	T (g)	100	100	2.99	0.43	-0.25
	T F (g)	100	100	2.18	0.33	0.07
	T F τ (g)	100	100	1.91	1.07	0.59
	T F S (g)	100	100	1.98	1.36	1.23
	T F τ S (g)	100	100	2.59	1.57	1.48
	T F M	100	100	1.99	0.34	0.10
	T F M τ	100	100	1.82	0.78	0.65
	T F M S	100	100	1.75	1.38	0.82
	T F M τ S	100	92	1.60	1.65	1.18

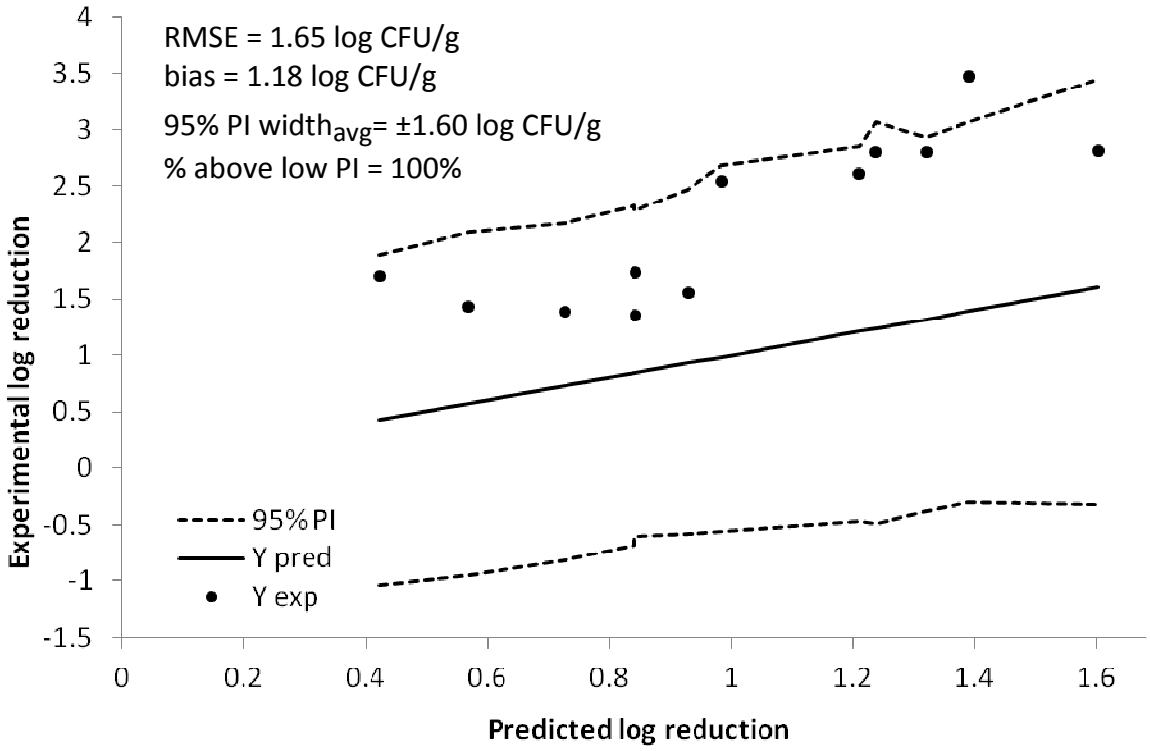

Figure 31. Mixed-effects T F M S τ model validated against beef hot dogs.

Table 26 shows the performance of the mixed-effects models when the turkey and beef hot dog data were pooled together. Here, it is more evident that the T F M τ S model gave the best predictions among the model versions; 100% of the data points fell above the fail-dangerous PI band, RMSE was 1.34 log CFU/g, bias was 0.50 log CFU/g, and the PI width was of ±2.0 log CFU/g, which is a fairly acceptable value, compared to the other validated products. It can be argued that models T F S (g) and T F τ S (g) also covered 100% of the data points in the safe region. However, even though their RMSE and bias values were similar, their PI widths were bigger (±2.62 log CFU/g and ±2.71 log CFU/g).

Table 26. Mixed-effects models validated against ALL hot dog data.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (± value)	RMSE	bias
				log (CFU/g)		
ALL hot dog data n_{obs} = 24	T (g)	100	4	2.94	1.04	-0.27
	T F (g)	83	4	2.23	1.56	-0.71
	T F τ (g)	83	4	1.97	1.38	-0.17
	T F S (g)	100	4	2.62	1.30	0.76
	T F τ S (g)	100	4	2.71	1.48	1.02
	T F M	83	4	2.09	1.57	-0.70
	T F M τ	83	4	1.87	1.40	-0.15
	T F M S	100	4	2.21	1.27	0.12
	T F M τ S	100	4	2.00	1.34	0.50

Comparing the OLS and mixed-effects models against both the turkey and beef hot dog data put together (Table 24 and Table 26) it is evident that the mixed-effects versions fared better in almost all cases. For example, percentage of data points above the fail-dangerous PI band in the mixed-effects models was at least 83%, compared to a maximum 75% from the OLS models. Additionally, PI widths were practically the same across all models, giving the mixed-effects versions the performance advantage. Finally, RMSEs and bias were also better for the mixed-effects versions; with a maximum RMSE of 1.57 log CFU/g vs. a minimum of 1.69 log CFU/g from the OLS models, and all bias being fail-dangerous in the OLS versions vs. positive bias in ~50% of the cases in the mixed-effects models.

4.3.4.4 Overall model performance on all pilot-scale data

Table 27 shows OLS models performance against all pilot-scale data. It can be seen that PI width remains practically constant across models, while the percentage of data points above the fail-dangerous PI band increases. Although this change is not substantial (~10%), RMSE and bias do improve more notably: by 0.27 log CFU/g and 0.41 log CFU/g, for each parameter, respectively. Therefore, the statement from previous sections that a model accounting for fat content and sublethal history has a better overall performance is reinforced.

Table 27. OLS models performance against ALL pilot-scale data.

Data source for validation	Model	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL pilot-scale data n_{obs} = 210	T (g) + T (w)	69	62	1.83	2.44	-1.08
	T F (g) + T F (w)	76	66	1.85	2.28	-0.80
	T τ (g) + T τ (w)	75	67	1.83	2.31	-0.88
	T F τ (g) + T F τ (w)	78	67	1.83	2.17	-0.67

Table 28 shows the different versions of the mixed-effects models when validated against all pilot-scale data put together. It can be seen that average PI widths remain relatively constant (although it does reach its maximum values in the most complicated models), but the percentage of data points above the fail-dangerous PI band increases with model complexity, reaching the 100%. This does not necessarily mean that the model with the most parameters will always give the best predictions and/or is the most adequate to predict lethality in a certain process, but it

does show the possible positive implications of pooling data from products with differing characteristics and obtaining a model that could possibly account for most of the variability between them.

Table 28. Mixed-effects models performance against ALL pilot-scale data.

Data source for validation	Model*	% above low PI	% that fit in PI	Average 95% PI (+/- value)	RMSE	bias
				log (CFU/g)		
ALL pilot-scale data n_{obs} =210	T (g) + T (w)	86	77	2.40	2.32	-0.55
	T F (g) + T F (w)	77	70	2.11	4.40	-1.47
	T F τ (g) + T F τ (w)	78	67	2.02	2.20	-0.61
	T F S (g) + T F S (w)	89	77	2.36	1.91	0.02
	T F τ S (g) + T F τ S (w)	94	88	2.55	1.62	0.39
	T F M	91	78	2.37	1.93	-0.14
	T F M τ	92	77	2.15	1.69	0.30
	T F M S	100	91	2.95	2.03	-0.06
	T F M τ S	100	88	2.73	1.68	0.36

4.4 Conclusions

This study showed possible sources and methods that can be used to develop a multi-product, multi-factor model for *Salmonella* thermal inactivation in meat and poultry products. It provides insight on the challenges and difficulties of attempting to complete such a task with the existing experimental data and without making use of extremely complex statistical methods. Data were gathered, multiple models were developed with different techniques, and they were all validated against pilot-scale data to test their usefulness to industrial applications. While there were significant deviations between model predictions and experimental results, explaining them was not the purpose of this project, but rather to demonstrate the degree of the expected variability and the possible effects of applying the laboratory-developed models in industrial settings.

There is no individual model that can be deemed the “best”, as each would be able to perform differently under differing processing conditions, in which case the “best” model to use would be the one that accounts for the parameters important to the user’s cooking process and product characteristics. Therefore, while this project does not completely close the gap between scientific work and real-life applications, it does provide new information that should be helpful both in directing future research in this field and in improving utilization of inactivation models in industry.

5. OVERALL CONCLUSIONS

5.1 Sublethal injury

- Models not accounting for bacterial sublethal injury can overestimate kill in meat products cooked under heat regimes where this phenomenon occurs (i.e., slow cooking/roasting).

5.2 Model validation

- The literature on thermal inactivation data and models developed in laboratory settings is extensive. However, almost none of these models are validated. Those that are provide few quantitative measures of model performance.
- Very little work exists where thermal inactivation models are validated against pilot-scale data or industry-relevant conditions, and they illustrate the difficulties associated with this kind of work – largely scattered data and model predictions.
- These results indicate that the existing information on thermal inactivation has limitations in directly helping to improve “real time” food safety.

5.3 Use of models in industry

- Research shows that multiple product and pathogen factors affect thermal inactivation: temperature, fat content, muscle structure, meat species, pH, salt %, sublethal injury, etc. Processors should identify which of these are truly important to their specific product and choose a model accordingly.

- When choosing a model, industry users should match, to the extent possible, the process to be validated and the nature of the data under which the considered models were obtained, especially when allowing for the use of models developed from laboratory-based experiments.
- Although model complexity does not necessarily translate into better lethality predictions, using an overly simplified model that neglects key factors can lead to fail-dangerous predictions, depending on product and process characteristics.

5.4 The need for standardized testing methods

- Gathering data from different sources to develop more comprehensive models can be done with mixed-effects or other statistical methods. However, due to generally unquantified data variability, prediction intervals will inevitably be large, decreasing model utility in industry.
- The introduction of standardized microbiological and analytical methods for carrying out thermal inactivation studies would allow qualitative comparison of data across studies and the quantification of their differences.
- While the ComBase database is a useful resource, the thermal inactivation data presented cannot be pooled together to develop models or statistics without expecting high variability due to the diverse methodologies in the original studies.

5.5 Future work

5.5.1 Enhancement of the AMI lethality spreadsheet

As mentioned in section 2.4.1.3.2, the AMI lethality spreadsheet is a tool currently used by meat processors to aid in determining process lethality. The results from this project could potentially help to enhance this tool, as several models were developed and validated against industry-relevant data. From what was concluded in this study, it is recommended that the model chosen should account for product fat content, sublethal history, muscle structure, and species, as their incorporation always showed prediction improvements in some way or another (RMSE, bias, etc.). The question then remains as to which model version, from those presented in this study, should be incorporated into the tool, especially in the case of the mixed-effects varieties, as even those considering the four aforementioned factors could have been calibrated with different data sets. For example, the T F M S τ model and ground-muscle-beef-specific T F τ model are both equally appropriate to predict lethality in ground-beef products, but the T F M S τ model was parameterized with both ground- and whole-muscle data, and turkey, beef, and pork data sets, while only ground beef data was used to calibrate the T F τ version. However each model yielded different fitting statistics, factors that should also be considered when determining which model to use. This can lead to three possible solutions: (i) allow the user to choose the model based on validation statistics and a comparison of the process characteristics and the conditions under which the calibration and/or validation data were generated, (ii) have the tool predict lethality and PIs with all the validated models relevant to the product, and output the most conservative values, or (iii) a combination of (i) and (ii), where the user can choose the model, but the tool will additionally output predicted values from other relevant model versions and allow the user to confirm or change the initial model selection. While these choices do not

entirely solve the issue of validating a process with 100% certainty, the tool's outputs based on these recommendations could certainly be an improvement from the currently available options.

5.5.2 Methods comparison with other research groups

As stated throughout this project, one of the main issues when attempting to pool and manipulate data from different sources was that methodologies varied widely between them, causing high unexplained variability in the final results. Further studies, such as that carried out by Hildebrandt et al. (2012b) could potentially help quantify the effects of methodology variability and gain insight into possible ways to solve this problem for future studies.

5.5.3 The need for standardized testing methods

Related to the work described in the previous section, this proposed study could be the next step to possibly separately quantify the contributions of methodology and human/experimental error of data variability. A project where the importance of standardized testing methods is assessed can provide further insight into the problems and necessities for this, and hopefully lead to potential improvements across this field of research.

5.5.4 Statistical improvements and model modifications

While several versions of the multi-product multi-factor model were tested, there is definitely room for improvement to obtain a better mathematical relationship between all the parameters and/or variables in the exponential term in equation (7) (i.e., β_2, τ, β_3, F, etc) and process lethality. The current relationships are quite basic. For example, the way sublethal history (τ) is determined (equation (4)) assumes that there is a linear relationship between the sublethal region temperature and acquired bacterial resistance. However, taking into account the

biological aspect of bacterial adaptation, it is likely that the vegetative cells become more thermotolerant up to a certain temperature within the sublethal range and then the effect tails at the transition to the lethal temperature range (Diller 2006). A study analyzing this behavior could lead to improvement of equation (4).

Another example is fat content; the current model version implies that the difficulty of achieving a certain process lethality increases exponentially (and proportionally to β_3) with the product fat content. However, future research should test this rigorously, or whether a different mathematical relationship is needed. All of these parameters could potentially be improved with different mathematical relationships.

6. APPENDICES

6.1 MATLAB code for sublethal injury model parameter estimation (section 3)

```
%thank you to Dr. Dolan for providing the file template, modified by: Isabel Tenorio
% units:
% *time: minutes!!!
% *log reductions: CFU/g or CFU/ml, (-) in model function, and (-) in
% experimental observations (Excel file)
% *Temperature in Excel file in Celsius!!

%Column order in excel: T(C)--time(min)--Tau(sublethal injury)

clear all
nlinfitcheck = statset('nlinfit');
nlinfitcheck.FunValCheck='off';

global nsets nrows
nsets=36;
nrows=2401;

%read in data
%data format must be first column is temperature (C), second column is time (sec), 3rd column
is Tau (sublethal injury history), 3rd dimension is set number
%make it global so that modelTTau function can read it

xTTau1=zeros(nrows,4,nsets);
for k=1:nsets
   xTTau1(:,:,k)=xlsread('G Turkey 2009 calibration.xlsx',k); % sheets are read in order!!!!
end

%now put them together to make replicates
global xTTau
xTTau=zeros(2401,4,nsets*3);
xTTau(:,:,1:36)=xTTau1;
xTTau(:,:,37:72)=xTTau1;
xTTau(:,:,73:108)=xTTau1;

%initial estimates
param0=xlsread('G Turkey 2009 calibration.xlsx','beta0');
%experimental log reductions observed
XYobs = xlsread('G Turkey 2009 calibration.xlsx','X and Yobs');
Yobs=XYobs(:,2);

%X is the data set number
```

```
X=XYobs(:,1);

%estimate the parameters
%---only-T model
[param,resids,J,covar,mse] = nlinfit(X,Yobs,'modelTTau',param0);

%parameter 95% asymptotic confidence intervals
ci = nlparci(param,resids,J);

%final params, RMSE, and bias of fit
finals(1:3)=param;
finals(4)=sqrt(mse);
finals(5)=mean(resids);

% %asymptotic simultaneous CONFIDENCE intervals for Y
% T-only model
[ypred,delta] = nlpredci('modelTTau',X,param,resids,J,0.05,'on','curve');
asyCIup=ypred+delta;
asyCIdo=ypred-delta;

%asymptotic simultaneous PREDICTION intervals for Y (number 2 in ypred2
%and such is just to differentiate from CI's parameter).
% T-only model
[ypred2, deltaob] = nlpredci('modelTTau',X,param,resids,J,0.05,'on','observation');
asyPIup=ypred+deltaob;
asyPIdo=ypred-deltaob;
%time=xgtT(:,2); %for plotting later

 % Correlation between parameters
 %R is the correlation matrix for the parameters, sigma is the standard error vector
 [R,sigma]=corrcov(covar);
RTref=R(2,1);

 %Relative standard error for parameters
 %RSE=zeros(6,9);
 RSE(1,1)=sigma(1)/param(1,1);
 RSE(2,1)=sigma(2)/param(2,1);
 RSE(3,1)=sigma(3)/param(3,1);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% generic function estimating logN/No for data with T and Tau, nonisothermal
%inputs are: parameters and data set number

function result = modelTTau(params,X)
```

```
%params=param0;
global xTTau nsets nrows

%columns:
%xTTau(1)= Temperature (C)
%xTTau(2)= time(min)
%xTTau(3)= Tau
%xTTau(4)= total cook time (min) (value repeated through rows for MatLab to accept matrix)
%xTTau(5) = Tref (C) (value repeated through rows also)

bref=params(1);
B1=params(2);
B2=params(3); %Tau

%Predict b first
Tref=60; %same for all data sets 60 C
b=zeros(nrows,1,nsets*3);
pred=zeros(nrows,1,nsets*3);

for i=1:nrows; %row is b at t=i;
   for j=1:nsets*3; % 3rd dimension is set number (with replicates)
      b(i,1,j)=bref*exp(-B1*((1/(xTTau(i,1,j)+273.15))-(1/(Tref+273.15)))-B2*xTTau(i,3,j)); %b at
t=i
   end
end

% Get log reductions
pred(1,1,:)=0;
for i=2:nrows;  %row is log N/No prediction at t=i;
   for j=1:nsets*3;  % 3rd dimension is set number (with replicates)
      pred(i,1,j)=-((b(i,1,j)+b(i-1,1,j))/2).*(xTTau(i,2,j)-xTTau(i-1,2,j))+pred(i-1,1,j);
   end
end

%get result with total cooking time index (total cook time was different
%for each set)
%result has to be nsets*3 long (rows, 1 column)
cooktimeind(X,1)=round(xTTau(1,4,X)*12)+1;  %this is for first replicate, repeat to x3
cooktimeind=[cooktimeind;cooktimeind;cooktimeind];
result=zeros(nsets,1);
for i=1:nsets;
   result(i)=-pred(cooktimeind(i),i);
end
```

6.2 Correlation coefficients, and standard and relative errors for path-dependent model parameters (section 3)

Table 29. Correlation coefficients for path-dependent model parameters.

Species	Parameter*	b_{ref}	β_1
Turkey	β_1	0.18	-
	β_2	0.74	0.14
Beef	β_1	0.26	-
	β_2	0.77	0.24
Pork	β_1	0.14	-
	β_2	0.76	0.17

*Parameter units: b_{ref} = min^{-1}, β_1 =K, β_2 = K·min

Table 30. Standard and relative errors for path-dependent model parameters.

Species	Parameter*	Estimate	Standard error	Relative error (%)
Turkey	b_{ref}	0.9071	0.0214	2.35
	β_1	50,787	636	1.25
	β_2	0.0017	0.0001	5.47
Beef	b_{ref}	0.9389	0.0328	3.49
	β_1	44,710	878	1.96
	β_2	0.0018	0.0001	7.56
Pork	b_{ref}	0.7040	0.0248	3.52
	β_1	54,713	836	1.52
	β_2	0.0016	0.0001	8.39

*Parameter units: b_{ref} = min^{-1}, β_1 =K, β_2 = K·min

6.3 MATLAB and R programming codes for multi-product multi-factor model parameter estimation (section 4)

6.3.1 MATLAB code example (OLS method)

```
%thank you to Dr. Dolan for providing the file template
%modified by: Isabel Tenorio

% units:
% *time: minutes!!!
% *log reductions: CFU/g or CFU/ml, (-) in model function, and (-) in
% experimental observations (Excel file)
% *Temperature in Excel file in Celsius!!
%Column order in excel: T(C)--time(min)--logN/N0--muscle--turkey--beef—pork,
% (last 4 are either 0 or 1)

%This program will estimate parameters and produce confidence intervals and
%prediction intervals for a generic data set
clear
clear all
nlinfitcheck = statset('nlinfit');
nlinfitcheck.FunValCheck='off';

%read in data
%data format must be first column is temperature (K), second column is time
%(sec), 3rd column is log reductions (log N/No).
gt = xlsread('MatLab File.xls','Ground Turkey');
wt = xlsread('MatLab File.xls','Whole Turkey');

%initial estimates
initialparams=xlsread('MatLab File.xls','initial parameters');

%initial estimates for T-only model
param0gtT(1)= initialparams(1,1); %bref
param0gtT(2)= initialparams(2,1); %B1
param0wtT(1)= initialparams(1,4); %bref
param0wtT(2)= initialparams(2,4); %B1

%---Set up for T-only model
%---set up independent variables, ground turkey
xgtT(:,1)=gt(:,1);
xgtT(:,2)=gt(:,2);
```

```
xgtT(:,3)=initialparams(7,1); %Tref
%set up dependent variable, ground turkey
YobsgtT = gt(:,3);
%---set up independent variables, whole turkey
xwtT(:,1)=wt(:,1);
xwtT(:,2)=wt(:,2);
xwtT(:,3)=initialparams(7,4); %Tref
%set up dependent variable, whole turkey
YobswtT = wt(:,3);

%estimate the parameters
%---only-T model
[paramgtT,residsgtT,JgtT,covargtT,msegtT] = nlinfit(xgtT,YobsgtT,'modelT',param0gtT);
 [paramwtT,residswtT,JwtT,covarwtT,msewtT] = nlinfit(xwtT,YobswtT,'modelT',param0wtT);

%parameter 95% asymptotic confidence intervals
cigtT = nlparci(paramgtT,residsgtT,JgtT);
ciwtT = nlparci(paramwtT,residswtT,JwtT);

%RMSE of fits
RMSE(1,1)=sqrt(msegtT);
RMSE(1,4)=sqrt(msewtT);

%bias of fits
bias(1,1)=mean(residsgtT);
bias(1,4)=mean(residswtT);

 %asymptotic simultaneous CONFIDENCE intervals for Y
% T-only model
[ypredgtT, deltagtT] = nlpredci('modelT',xgtT,paramgtT,residsgtT,JgtT,0.05,'on','curve');
asyCIupgtT=ypredgtT+deltagtT;
asyCIdogtT=ypredgtT-deltagtT;
 [ypredwtT, deltawtT] = nlpredci('modelT',xwtT,paramwtT,residswtT,JwtT,0.05,'on','curve');
asyCIupwtT=ypredwtT+deltawtT;
asyCIdowtT=ypredwtT-deltawtT;

%asymptotic simultaneous PREDICTION intervals for Y (number 3 in ypredgtT3 and such is just
to differentiate from CI's parameter).
% T-only model
[ypredgtT3, deltaobgtT] =
nlpredci('modelT',xgtT,paramgtT,residsgtT,JgtT,0.05,'on','observation');
asyPIupgtT=ypredgtT+deltaobgtT;
asyPIdogtT=ypredgtT-deltaobgtT;
```

```
tgtT=xgtT(:,2); %for plotting later
[ypredwtT3, deltaobwtT] =
nlpredci('modelT',xwtT,paramwtT,residswtT,JwtT,0.05,'on','observation');
asyPIupwtT=ypredwtT+deltaobwtT;
asyPIdowtT=ypredwtT-deltaobwtT;
twtT=xwtT(:,2); %for plotting later

 % Correlation between parameters
 %R is the correlation matrix for the parameters, sigma is the standard error vector
 [RgtT,sigmagtT]=corrcov(covargtT);
 [RwtT,sigmawtT]=corrcov(covarwtT);

 %Relative standard error for parameters
 %RSE=zeros(6,9);
 RSE(1,1)=sigmagtT(1)/finalparams(1,1);
 RSE(2,1)=sigmagtT(2)/finalparams(2,1);
RSE(1,4)=sigmawtT(1)/finalparams(1,4);
 RSE(2,4)=sigmawtT(2)/finalparams(2,4);

 %Add B3 to parameter matrices for TF models
  B3s=xlsread('MatLab file.xls','B3s');
 paramgtTF=[paramgtT,B3s(1,:)];
paramwtTF=[paramwtT,B3s(1,:)];

 %Add B2 to parameter matrices for TTau models
 B2s=xlsread('MatLab file.xls','B2s');
 paramgtTTau=[paramgtT,B2s(1,:)];
paramwtTTau=[paramwtT,B2s(1,:)];

 %parameters with B2 and B3 for TFTau models
 paramgtTFTau=[paramgtT,B2s(1,:),B3s(1,:)];
 paramwtTFTau=[paramwtT,B2s(1,:),B3s(1,:)];
```

6.3.2 R code example (mixed-effects method)

```
library(lme4)
#change data file for species/muscle data desired: GT, GB, GP, WT, WB, WP

dat=read.csv(file="WPmixeffdata, TFw.csv",header=F)
#dat = read.csv("C:/USER/sb/sm.csv",header=F)
colnames(dat)=c("Fat","Temp","Time","Y","Group")
```

```
dat2 = dat[dat$Time!=0, ]

model = function(bref,beta1,beta3,Time,Temp,Fat){
  const = -bref*exp(-beta1*(1/Temp-1/333.15)-beta3*Fat)*Time
  model = const
  gradient <- cbind(const/bref,-const*(1/Temp-1/333.15),-const*Fat)
  attr(model, "gradient") <- gradient
  model
}

res = nlmer( Y ~ model(bref,beta1,beta3,Time,Temp,Fat)~(bref|Group),data=dat2,
    start=c(bref=1.0818162302473487,beta1=43951.966921578,beta3=0.352765575011846))

res
fixef(res)
fitted(res)
resid(res)
# to get RMSE, change nobs as needed
SSE=resid(res)*resid(res)
SSE=sum(SSE)
RMSE=sqrt(SSE/(105-3))

#Function Coding# with assistance from CSTAT at MSU
pred<-function(res,n.sim,temp,time,fat,level=0.95) {
#Retrieve the standard deviation estimate for the random effect term bref
brefstdev<-sqrt(VarCorr(res)$Group[1,1])
#Retrieve the standard deviation estimate of the model error
errstdev<-attr(VarCorr(res),"sc")
#Retrive the fixed effect coefficient estimates
beta1<-fixef(res)[2]
beta3<-fixef(res)[3]

#Generate n.sim random effect of bref
bref<-rnorm(n.sim,fixef(res)[1],brefstdev)
#Number of time lags
n.time<-length(time)
Y<-rep(NaN,n.sim)
for(j in 1:n.sim) {
        #Compute the mean of cumulative predicted log reduction value
        cplr.mean<-0
        b=bref[j]*exp(-beta1*(1/(temp+273.15)-1/333.15)-beta3*fat)
   for(i in 1:(n.time-1)) {
        cplr.mean<-cplr.mean-(b[i+1]+b[i])/2*(time[i+1]-time[i])
```

```
                    }
    #Sample a new observation Y based on mean=cplr.mean and st.dev=model's standard
deviation
    Y[j]<-rnorm(1,cplr.mean,errstdev)
}
#Obtain the simulated sample mean of new Y
Y.mean=mean(Y)
#Obtain the simulated sample median of new Y
Y.median=median(Y)
#Obtain the Predictive interval of new Y
Y.predictive=quantile(Y,c((1-level)/2,(1+level)/2))
Y.output=list(Y.mean=Y.mean,Y.median=Y.median,Y.predintv=Y.predictive,n.sim=n.sim)
return(Y.output)
}
fat=1.53
validdata=read.csv(file="Tasha PR.csv",header=F)
#To tell R how many sets you have in total in the csv file, you can use col(validdata) to tell how
many columns in total then divide it by 2
n.set=ncol(validdata)/2
predInt<-matrix(NaN,n.set,2)
for(i in 1:n.set) {
set=na.omit(validdata[,(2*i-1):(2*i)])
colnames(set)=c("temp","t.min")
predInt[i,]<-pred(res,1000,set$temp,set$t.min,fat)$Y.predintv
}
```

6.4 Hot dog methods and data (section 4)

6.4.1 Preparation of Salmonella

An 8-serovar *Salmonella* cocktail consisting of S. Thompson FSIS 120 (chicken isolate), S. Typhimurium DT 104 H3380 (human isolate), S. Hadar MF60404 (turkey isolate), S. Copenhagen 8457 (pork isolate), S. Montevideo FSIS 051 (beef isolate), and S. Heidelberg F5038BGI (human isolate), was previously obtained from V.K. Juneja (Agricultural Research Service, Eastern Regional Research Center, USDA-ARS, Wyndmoor, PA). Each serovar was maintained separately at -80°C in vials containing tryptic soy broth (Difco Laboratories, Sparks, MD) with yeast extract (TSBYE) and 20% glycerol. Cultures were grown separately in TSBYE at 37°C with a minimum of two consecutive 24-hour transfers prior to inoculation.

6.4.2 Preparation of inoculated frankfurters

Emulsified beef and turkey frankfurter batter was obtained from a federally inspected commercial supplier. Emulsion was vacuum packaged in 1000 g packages, frozen (-10°C). Twenty-four hours prior to experiment, two packages of either beef or turkey batter were thawed in a refrigerator (4°C) until day of experiment.

Concentrated inoculum was prepared by combining 36 ml of each culture to yield a total of 288 ml. This cocktail was centrifuged (6000x*g*, 15 minutes) and the pellet was re-suspended in 14 ml of 1% sterile buffered peptone water (Difco Laboratories, Sparks, MD). In order to enumerate the inoculum, a 1 ml sample was taken from the 14 ml inoculum and was serially diluted and plated on modified Tryptic Soy Agar (mTSA*) (Difco Laboratories, Sparks, MD) plates (37°C, 48 hours). Meat and 13 ml of marinade (10^9 CFU/g) was added to a Kitchen Aid mixer and mixed for 180s at setting 1 and using the paddle.

Prior to stuffing, the inoculated emulsion (10^6 CFU/g) was vacuumed (101.325kPa of vacuum, 10 s) to reduce air bubbles. The emulsion was stuffed into cellulose casing using a hand-crank stuffer system to make three two-link hot dog chains with a mass of about 60 g (~15.5 cm long, 2 cm diameter) per hot dog. Frankfurters were tied in the center and on each end to form links. In order to place a probe in the center of each hot dog, a jig was created to insert a hypodermic needle (16G x 12.7cm) at a height of 1 cm into the length of the frankfurter, along its center axis. This needle was pushed all the way through the hot dog and out the other end; a wire thermocouple was inserted half-way (~7.75cm) into the length of the frankfurter, the needle was pulled out, and the thermocouple remained. This procedure was repeated for each of the six frankfurters per treatment.

*mTSA recipe: 2 L deionized distilled water, 80 g TSA, 12 g yeast extract, 1g ammonium iron citrate, 0.6g sodiun thiosulfate.

6.4.3 Frankfurter cooking and survivor recovery

The hot dogs were cooked in a pilot scale, moist air convection oven (Cres Cor, Mentor, OH) using a cook schedule for low-fat frankfurters similar to that used in industry. This cycle increased temperature and humidity over a period of ~140 min (Table 31). A predicted lethality of 4 log and 6 log was calculated real-time using a data logger (Datapaq Inc., Wilmington, MA) with inputs of D- and z- values from previously published laboratory data (Table 30) (Breslin 2009; Tuntivanich et al. 2008). When the frankfurter reached the end lethality, it was taken out of the oven and quenched in liquid nitrogen to terminate cooking. Additional experiments were run to end temperatures of 160°C and 165°C for beef and turkey, respectively. The goal of these cooks was to ensure no bacterial survival at these end temperatures, often used in industry. The center 5 cm of length of each hot dog was cut and cored (1.2 cm diameter), serially diluted, and plated on mTSA (37°C, 48 h) to enumerate survivors.

Table 31. Commercial cooking schedule for frankfurters.

Cumulative time (min)	Dry bulb temperature (°C)	% Relative Humidity
0 – 20	60	38
20 – 35	71.1	37
35 – 50	76.7	39
50 – 140	82.2	79

Table 32. D- and z- values from previous research used to predict real-time lethality.

Parameter	Ground Beef	Ground Turkey
D_{ref} (sec)	60.55	62.66
z (°C)	5.48	5.14
T_{ref} (°C)	60	60

6.4.4 Results

For each sample, the log reduction predicted with the D- and z- values from Table 32, along with the experimental lethality are presented in Table 33 and Table 34.

Table 33. Predicted and experimental log reductions for turkey hot dogs.

Sample	Log reduction predicted	Log reduction experimental
A1	3.83	1.10
A2	3.95	0.96
A3	3.65	1.09
A4	3.49	0.97
A5	2.13	1.36
A6	2.03	0.85
B1	5.23	2.89
B2	5.95	2.97
B3	5.65	3.62
B4	1.73	4.04
B5	1.87	3.41
B6	5.54	3.49

Table 34. Predicted and experimental log reductions for beef hot dogs.

Sample	Log reduction predicted	Log reduction experimental
A1	2.47	1.44
A2	3.59	1.74
A3	2.20	1.71
A4	3.62	1.56
A5	3.11	1.36
A6	2.90	1.39
B1	5.28	2.81
B2	5.79	3.48
B3	6.34	2.82
B4	5.04	2.62
B5	4.01	2.55
B6	4.82	2.80

6.5 Standard and relative errors for the multi-product, multi-factor model parameters (section 4)

6.5.1 OLS method

Table 35 shows the standard and relative errors for the parameters obtained when doing the regression on the fat-relevant data (Table 6), which would then lead to the use of solely β_3 in the multi-product multi-factor model (see section 4.2.3.1.2 for details).

Table 35. Parameter standard and relative errors for β_3 estimation.

Model	Species	Parameter	b_{ref}	β_1	β_3
			min^{-1}	K	fat $\%^{-1}$
T (g)	G turkey	Estimate	0.461	39,231	0.030
		Std error	0.010	542	0.003
		% Rel error	2.19	1.38	8.87
	G beef	Estimate	0.396	39,885	0.023
		Std error	0.011	509	0.002
		% Rel error	2.80	1.28	7.73
	G pork	Estimate	0.344	39,877	0.014
		Std error	0.009	721	0.001
		% Rel error	2.62	1.81	10.93

Since β_2 and β_3 were obtained separately, they cannot be included with the values from the b_{ref} and β_1 regressions. β_2's errors from its corresponding estimation are in Table 30 (section 6.2). β_3's errors are on Table 35 (above).

Table 36. Parameter standard and relative errors for OLS models (continued next page).

Model	Species	Parameter	b_{ref}	β_1
			min^{-1}	K
T (g)	G turkey	Estimate	1.11	52,269
		Std error	0.038	1,673
		% Rel error	3.43	3.20
	G beef	Estimate	0.83	44,242
		Std error	0.016	800
		% Rel error	1.98	1.81
	G pork	Estimate	0.63	41,750
		Std error	0.020	1,206
		% Rel error	3.20	2.89

Table 36 (cont'd). Parameter standard and relative errors for OLS models.

Model	Species	Parameter	b_{ref}	β_1
			min^{-1}	K
T (w)	W turkey	Estimate	0.37	48,589
		Std error	0.014	1,696
		% Rel error	3.78	3.49
	W beef	Estimate	0.44	44,799
		Std error	0.011	859
		% Rel error	2.44	1.92
	W pork	Estimate	0.45	47,164
		Std error	0.016	1,337
		% Rel error	3.54	2.84

6.5.2 Mixed-effects method

Table 37 shows the parameter standard and relative errors for the mixed-effects models. Notice that because β_2 was estimated from a different regression (section 4.2.3.1.2), its errors cannot be presented in the same table. However, β_2's errors from its corresponding estimation are in Table 30 (section 6.2).

Table 37. Parameter standard and relative errors for mixed-effects models (continued next page).

Model	Species	Parameter	b_{ref}	β_1	β_3	β_4	β_5	β_6	β_7
			min^{-1}	K	fat $\%^{-1}$	•	•	•	•
T (g)	G turkey	Estimate	0.672	50,750	-	-	-	-	-
		Std error	0.052	4,131	-	-	-	-	-
		% Rel error	7.74	8.14	-	-	-	-	-
	G beef	Estimate	0.555	44,710	-	-	-	-	-
		Std error	0.034	2,260	-	-	-	-	-
		% Rel error	6.13	5.05	-	-	-	-	-
	G pork	Estimate	0.430	53,950	-	-	-	-	-
		Std error	0.042	2,591	-	-	-	-	-
		% Rel error	9.77	4.80	-	-	-	-	-

Table 37 (cont'd). Parameter standard and relative errors for mixed-effects models (continued next page).

Model	Species	Parameter	b_{ref}	β_1	β_3	β_4	β_5	β_6	β_7
			min^{-1}	K	fat $\%^{-1}$	•	•	•	•
T (w)	W turkey	Estimate	0.593	50,750	-	-	-	-	-
		Std error	0.024	1,761	-	-	-	-	-
		% Rel error	4.05	3.47	-	-	-	-	-
	W beef	Estimate	0.538	44,710	-	-	-	-	-
		Std error	0.020	1,500	-	-	-	-	-
		% Rel error	3.72	3.35	-	-	-	-	-
	W pork	Estimate	0.654	53,950	-	-	-	-	-
		Std error	0.045	2,637	-	-	-	-	-
		% Rel error	6.88	4.89	-	-	-	-	-
T F (g)	G turkey	Estimate	1.582	36,470	0.2094	-	-	-	-
		Std error	0.089	1,944	0.007	-	-	-	-
		% Rel error	5.63	5.33	3.34	-	-	-	-
	G beef	Estimate	0.949	36,320	0.0627	-	-	-	-
		Std error	0.057	1,833	0.004	-	-	-	-
		% Rel error	6.01	5.05	6.38	-	-	-	-
	G pork	Estimate	0.742	35,940	0.0635	-	-	-	-
		Std error	0.045	1,713	0.004	-	-	-	-
		% Rel error	6.06	4.77	6.30	-	-	-	-
T F (w)	W turkey	Estimate	1.363	44,710	0.897	-	-	-	-
		Std error	0.133	1,658	0.098	-	-	-	-
		% Rel error	9.76	3.71	10.93	-	-	-	-
	W beef	Estimate	0.796	44,710	0.128	-	-	-	-
		Std error	0.092	1,424	0.037	-	-	-	-
		% Rel error	11.56	3.18	28.91	-	-	-	-
	W pork	Estimate	1.045	53,950	0.188	-	-	-	-
		Std error	0.311	3,076	0.121	-	-	-	-
		% Rel error	29.76	5.70	64.36	-	-	-	-
T F S (g)	G turkey, beef, pork	Estimate	1.534	49,800	0.065	-	0.832	0.776	1.089
		Std error	0.172	1,778	0.004	-	0.087	0.100	0.106
		% Rel error	11.21	3.57	6.15	-	10.46	12.89	9.73
T F S (w)	W turkey, beef, pork	Estimate	5.200	45,690	0.127	-	2.145	1.881	1.791
		Std error	0.877	1,166	0.046	-	0.190	0.211	0.200
		% Rel error	16.87	2.55	36.22	-	8.86	11.22	11.17

Table 37 (cont'd). Parameter standard and relative errors for mixed-effects models.

Model	Species	Parameter	b_{ref}	β_1	β_3	β_4	β_5	β_6	β_7
			min^{-1}	K	fat $\%^{-1}$	•	•	•	•
T F M	G + W turkey	Estimate	0.516	36,860	0.210	-1.140	-	-	-
		Std error	0.034	1,723	0.008	0.075	-	-	-
		% Rel error	6.59	4.67	3.81	-6.58	-	-	-
	G + W beef	Estimate	0.612	37,490	0.062	-0.453	-	-	-
		Std error	0.034	1,433	0.004	0.071	-	-	-
		% Rel error	5.56	3.82	6.45	-15.67	-	-	-
	G + W pork	Estimate	0.674	40,300	0.066	-1.355	-	-	-
		Std error	0.044	1,575	0.004	0.083	-	-	-
		% Rel error	6.53	3.91	6.06	-6.13	-	-	-
T F M S	G + W turkey, beef, pork	Estimate	1.094	49,410	0.062	-0.506	0.652	0.651	0.855
		Std error	0.064	1,160	0.003	0.062	0.085	0.074	0.073
		% Rel error	5.85	2.35	4.84	-12.25	13.04	11.37	8.54

6.6 Correlation coefficients for multi-product multi-factor model parameters (section 4)

6.6.1 OLS models

Table 38. Parameter correlation coefficients for β_3 estimation.

Model	Species	Parameter*	b_{ref}	β_1
T (g)	G turkey	β_1	-0.042	-
		β_3	0.848	-0.046
	G beef	β_1	0.0005	-
		β_3	0.922	-0.0002
	G pork	β_1	-0.061	-
		β_3	0.825	-0.073

*Parameter units: $b_{ref} = min^{-1}$, β_1 =K, β_3 = fat $\%^{-1}$

Table 39. Correlation coefficients for OLS models parameters.

Model	Species	Parameter	b_{ref}
T (g)	G turkey	β_1	2.84e-06
	G beef	β_1	5.47e-06
	G pork	β_1	1.41e-05
T (w)	W turkey	β_1	-6.77e-06
	W beef	β_1	-1.33e-05
	W pork	β_1	-1.61e-05

*Parameter units: $b_{ref} = min^{-1}$, β_1 =K

6.6.2 Mixed-effects models

Table 40 shows the parameter correlation coefficients for the mixed-effects models. Notice that because β_2 was estimated from a different regression (section 4.2.3.1.2), its correlation with the other parameters cannot be presented in the same table. Table 29 (section 6.2) shows the correlation of β_2 with the parameters from its original estimation.

Table 40. Correlation coefficients for mixed-effects models parameters (continued next page).

Model	Species	Parameter	b_{ref}	β_1	β_3	β_4	β_5	β_6
			min^{-1}	K	fat $\%^{-1}$	•	•	•
T (g)	G turkey	β_1	0.065	-	-	-	-	-
	G beef	β_1	0.058	-	-	-	-	-
	G pork	β_1	0.185	-	-	-	-	-
T (w)	W turkey	β_1	0.318	-	-	-	-	-
	W beef	β_1	-0.011	-	-	-	-	-
	W pork	β_1	0.251	-	-	-	-	-
	G pork	β_1	0.063	-	-	-	-	-
		β_3	0.669	0.295	-	-	-	-

Table 40 (cont'd). Correlation coefficients for mixed-effects models parameters (continued next page).

Model	Species	Parameter	b_{ref}	β_1	β_3	β_4	β_5	β_6
			min^{-1}	K	fat $\%^{-1}$	•	•	•
T F (g)	G turkey	β_1	0.003	-	-	-	-	-
		β_3	0.658	0.331	-	-	-	-
	G beef	β_1	-0.194	-	-	-	-	-
		β_3	0.689	-0.248	-	-	-	-
T F (w)	W turkey	β_1	~ 0	-	-	-	-	-
		β_3	1.000	~ 0	-	-	-	-
	W beef	β_1	-0.067	-	-	-	-	-
		β_3	0.955	-0.062	-	-	-	-
	W pork	β_1	-0.451	-	-	-	-	-
		β_3	0.973	-0.515	-	-	-	-
T F S (g)	G turkey, beef, pork	β_1	-0.362	-	-	-	-	-
		β_3	-0.004	-0.062	-	-	-	-
		β_5	0.614	-0.245	-0.092	-	-	-
		β_6	0.668	-0.395	-0.490	-	0.460	-
		β_7	0.624	-0.331	-0.411	-	0.424	0.644
T F S (w)	W turkey, beef, pork	β_1	0.032	-	-	-	-	-
		β_3	0.072	-0.050	-	-	-	-
		β_5	0.846	-0.032	-0.176	-	-	-
		β_6	0.739	0.059	-0.596	-	0.779	-
		β_7	0.783	0.031	-0.571	-	0.801	0.952
T F M	G + W turkey	β_1	0.116	-	-	-	-	-
		β_3	0.111	0.267	-	-	-	-
		β_4	0.669	0.079	-0.477	-	-	-
	G + W beef	β_1	-0.113	-	-	-	-	-
		β_3	0.152	-0.232	-	-	-	-
		β_4	0.589	0.059	-0.515	-	-	-
	G + W pork	β_1	-0.038	-	-	-	-	-
		β_3	0.094	0.252	-	-	-	-
		β_4	0.626	-0.142	-0.519	-	-	-

Table 40 (cont'd). Correlation coefficients for mixed-effects models parameters.

Model	Species	Parameter	b_{ref}	β_1	β_3	β_4	β_5	β_6
			min^{-1}	K	fat $\%^{-1}$	•	•	•
T F M S	G + W turkey, beef, pork	β_1	-0.306	-	-	-	-	-
		β_3	0.053	-0.103	-	-	-	-
		β_4	0.013	-0.020	-0.389	-	-	-
		β_5	0.495	-0.169	0.209	-0.610	-	-
		β_6	0.627	-0.273	-0.138	-0.449	0.621	-
		β_7	0.611	-0.259	-0.085	-0.372	0.559	0.683

6.7 OLS method prediction interval calculations (section 4)

6.7.1 Ellipse, bootstrapping, and Monte Carlo (PI methods) contours for each calibration product

As described in section 4.2.3.1.3, contours were developed for each calibration data set with the PI methods to determine the best approach for calculating PIs. The contours for the ground turkey data set are shown in the main text (section 4.3.2, Figure 10).

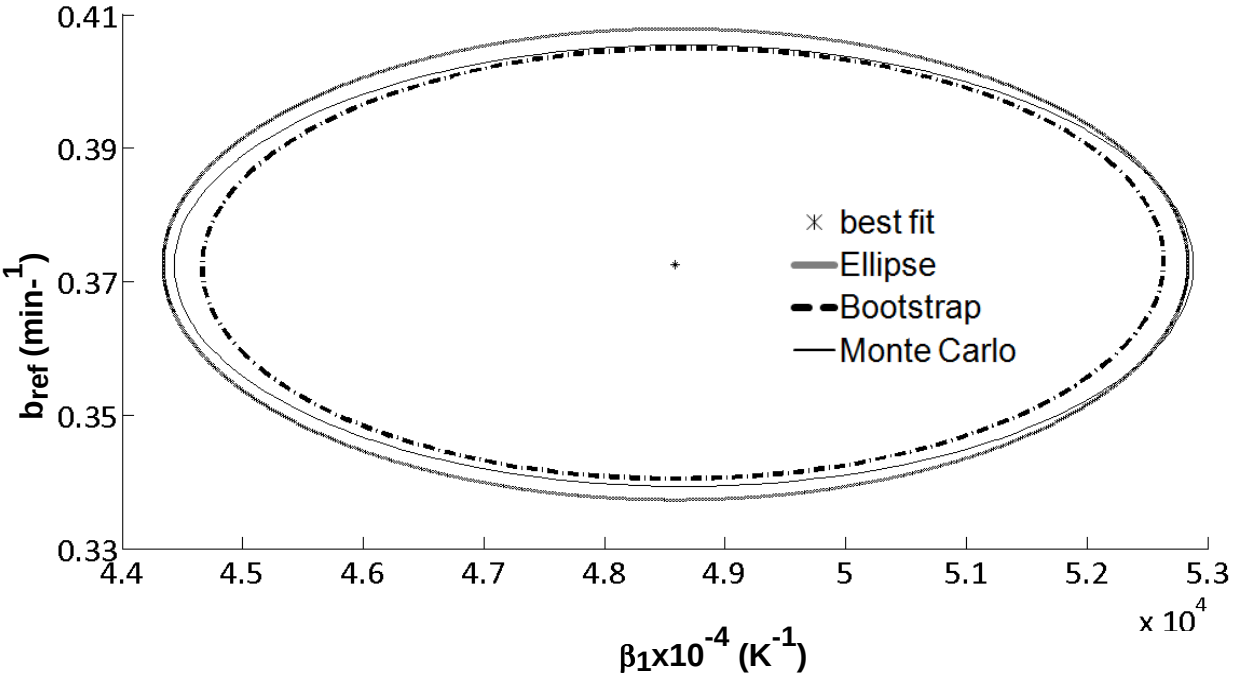

Figure 32. PI methods parameter contours for whole turkey calibration set.

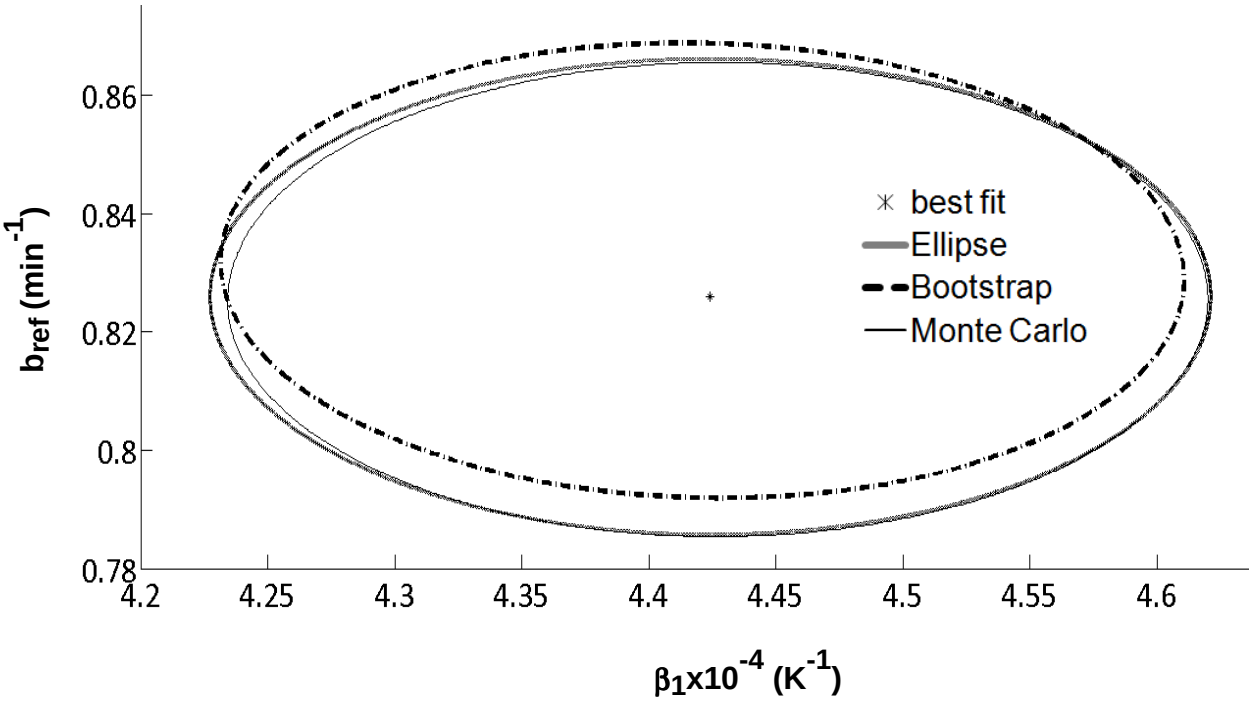

Figure 33. PI methods parameter contours for ground beef calibration set.

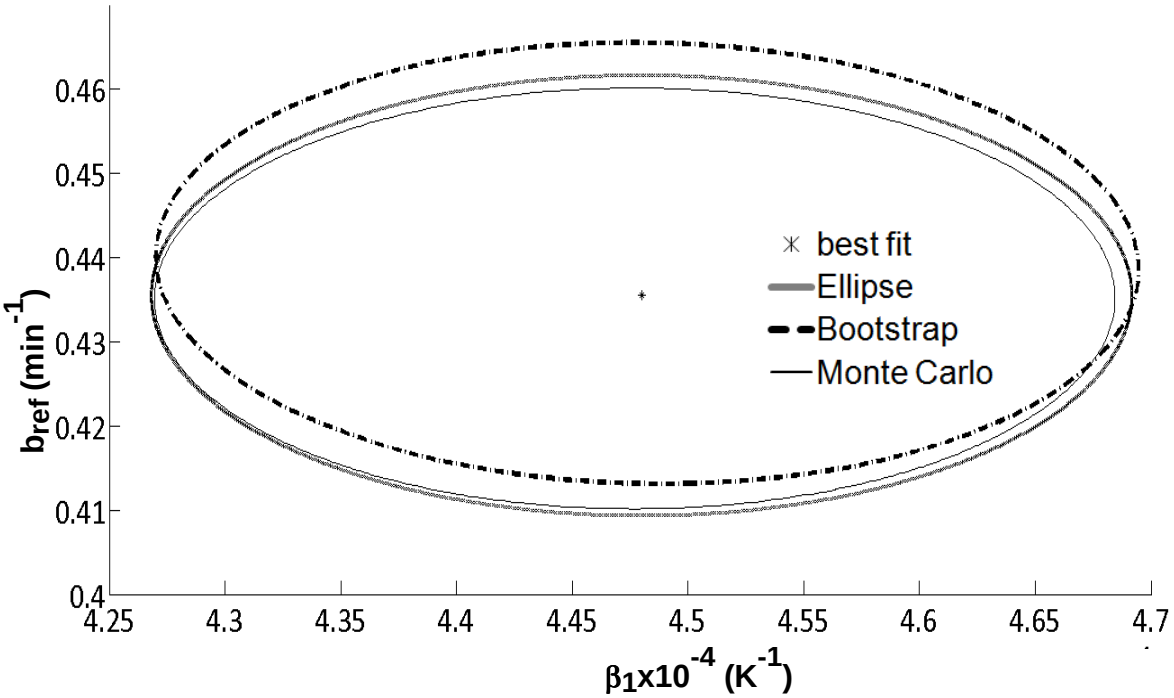

Figure 34. PI methods parameter contours for whole beef calibration set.

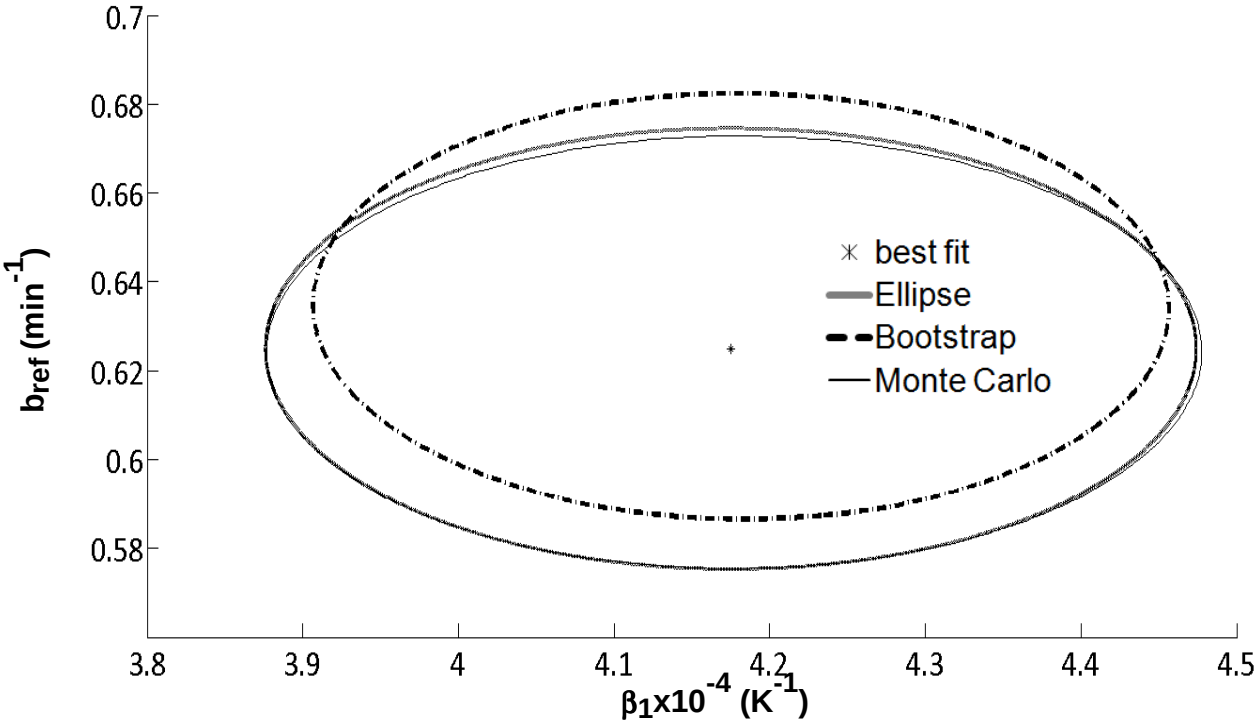

Figure 35. PI methods parameter contours for ground pork calibration set.

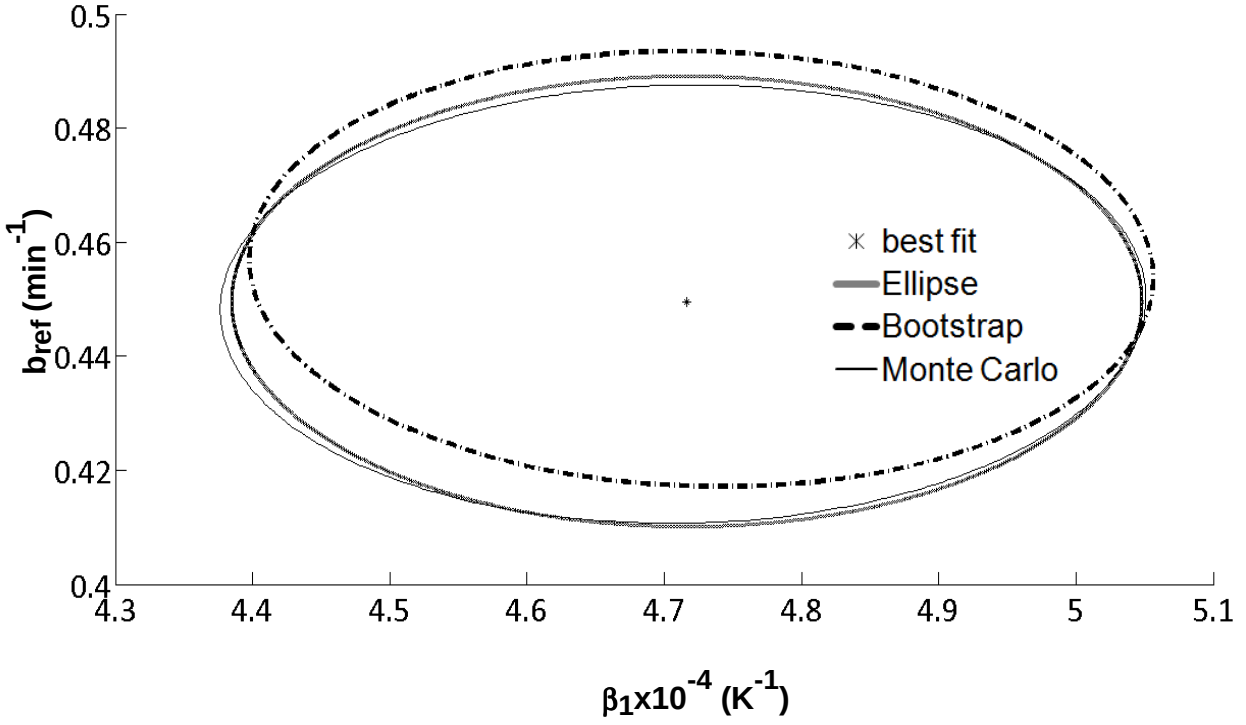

Figure 36. PI methods parameter contours for whole pork calibration set.

6.7.2 Prediction intervals for each PI method and each calibration product.

Just as shown for the ground turkey data set in section 4.3.2 (Figure 11 and Figure 12), the following plots represent the PIs generated for the rest of the data sets.

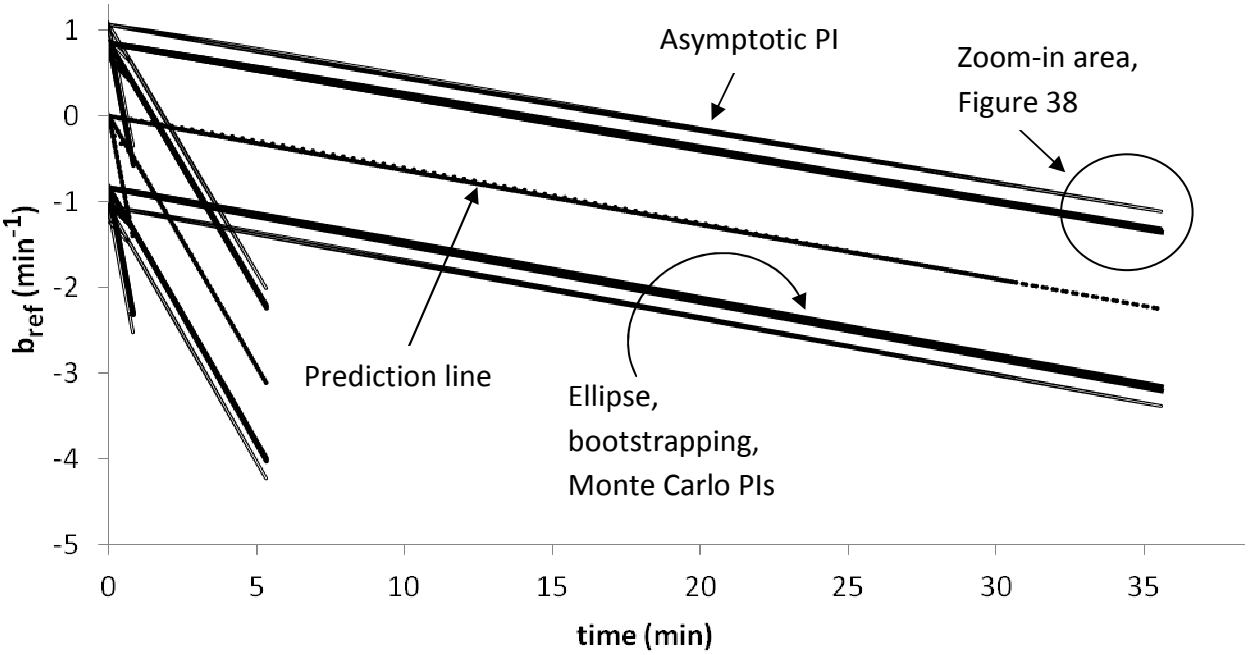

Figure 37. PIs with all methods for whole turkey calibration data set.

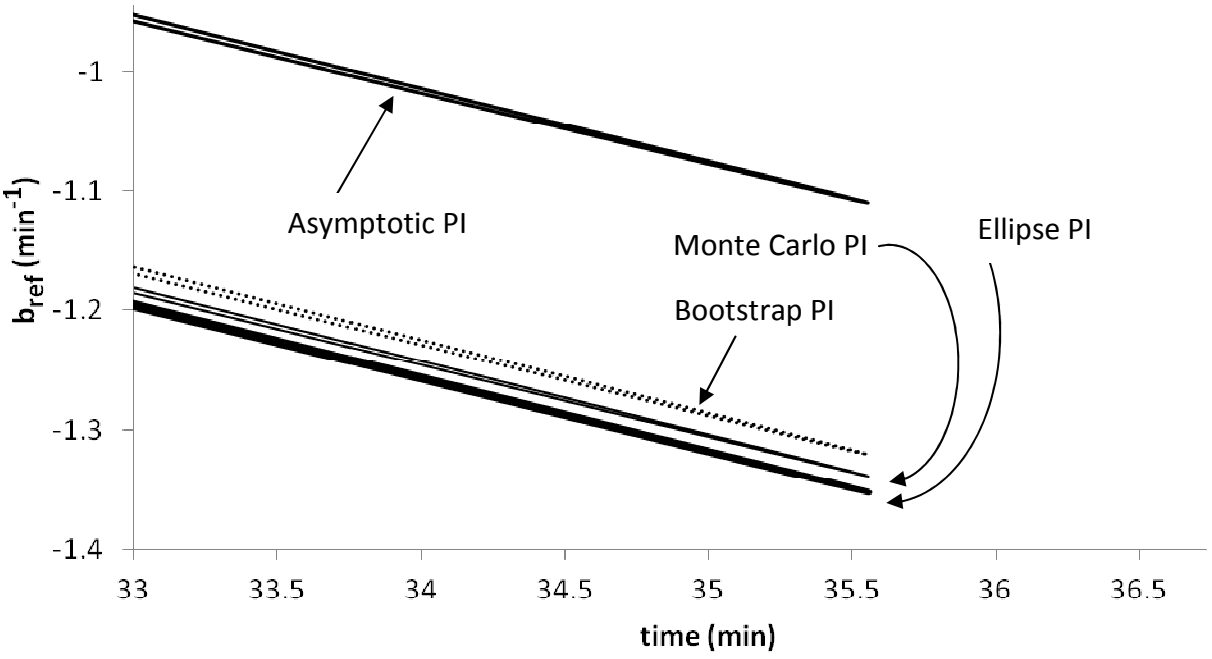

Figure 38. Zoom-in section from Figure 37.

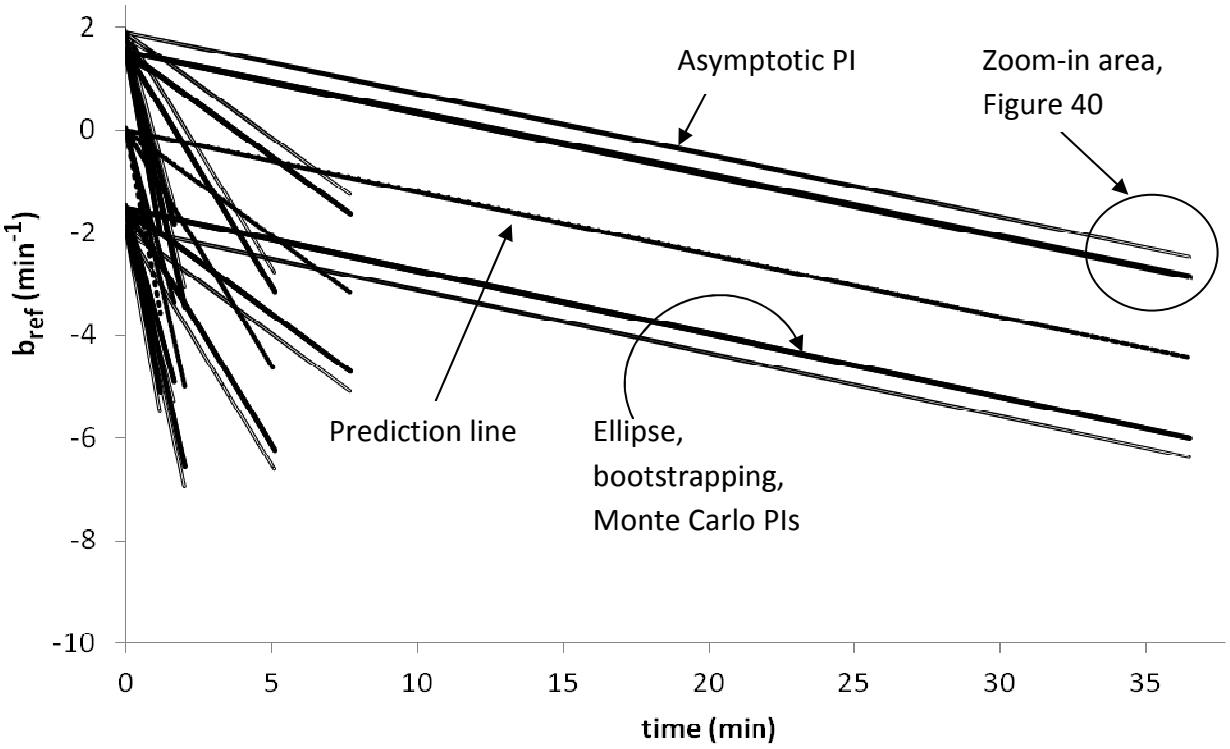

Figure 39. PIs with all methods for ground beef calibration data set.

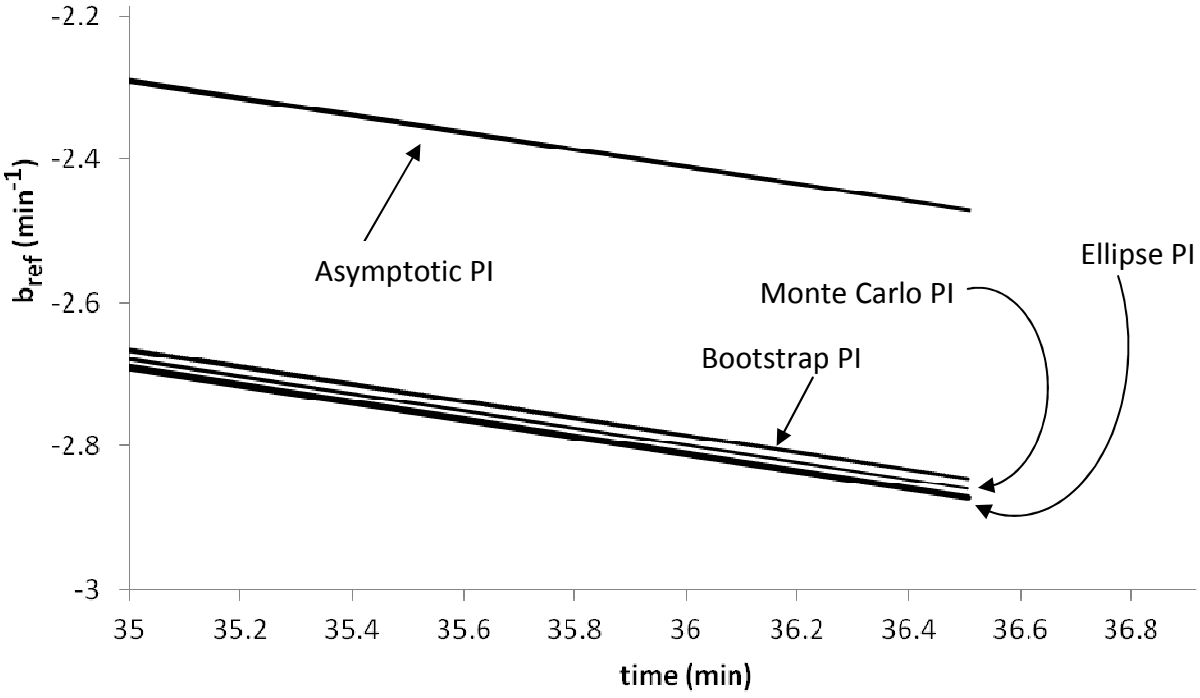

Figure 40. Zoom-in section from Figure 39.

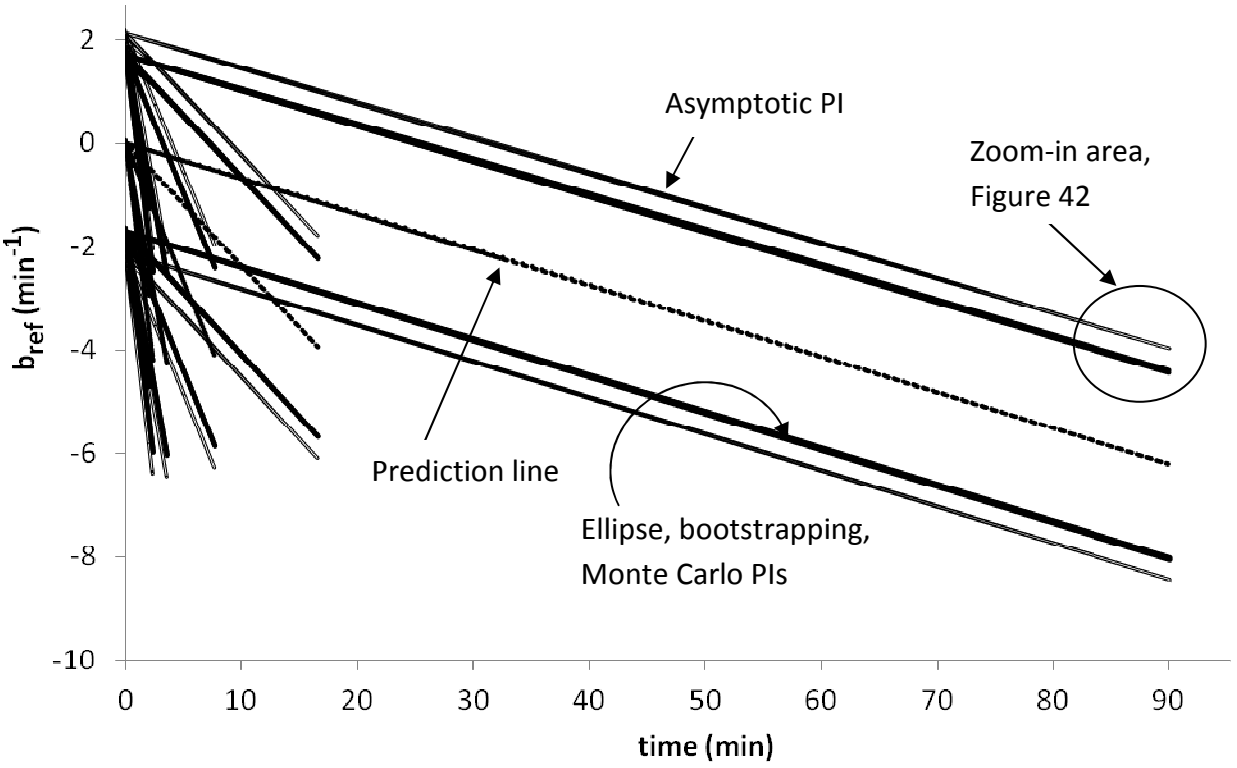

Figure 41. PIs with all methods for whole beef calibration data set.

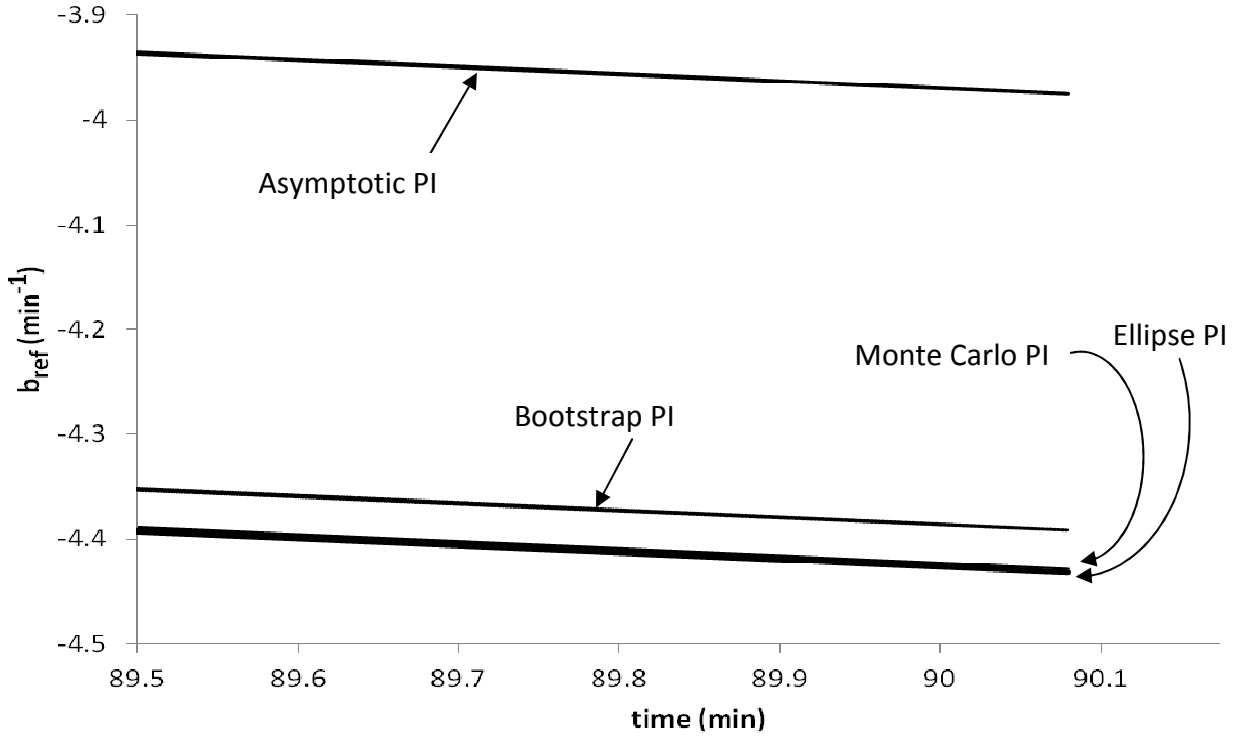

Figure 42. Zoom-in section from Figure 41.

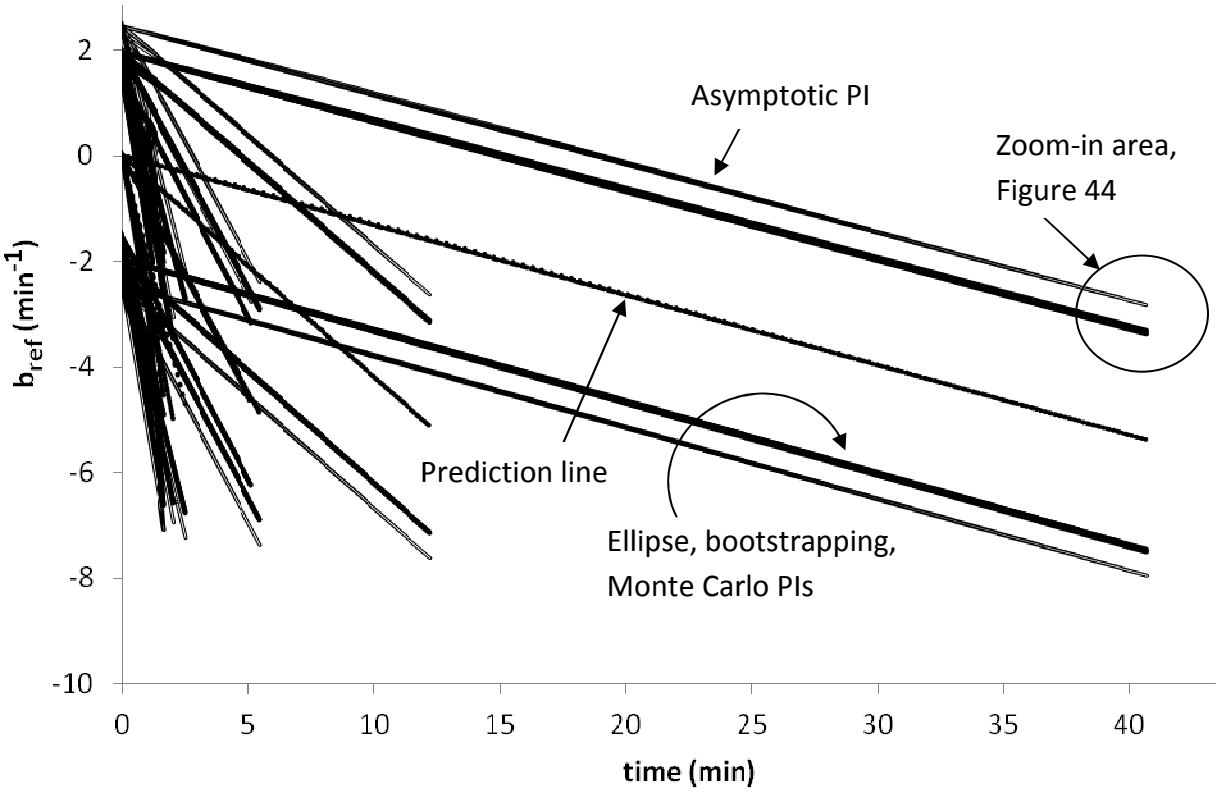

Figure 43. PIs with all methods for ground pork calibration data set.

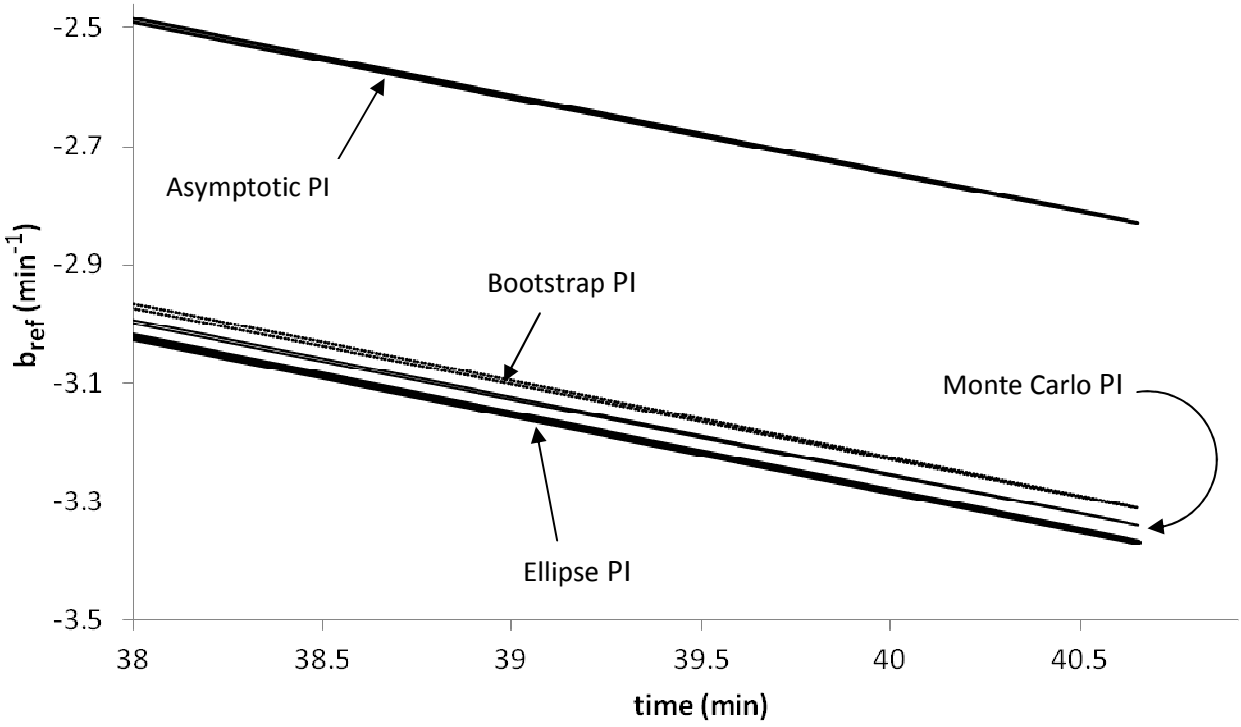

Figure 44. Zoom-in section from Figure 43.

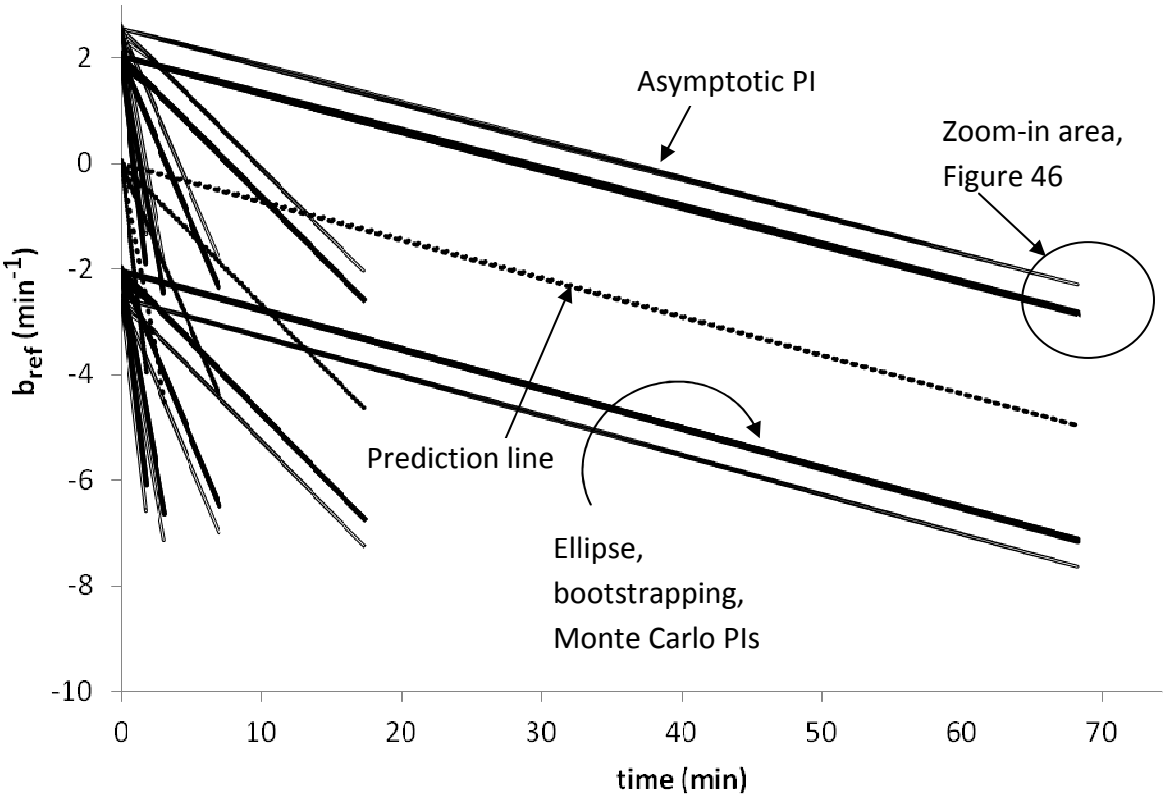

Figure 45. PIs with all methods for whole pork calibration data set.

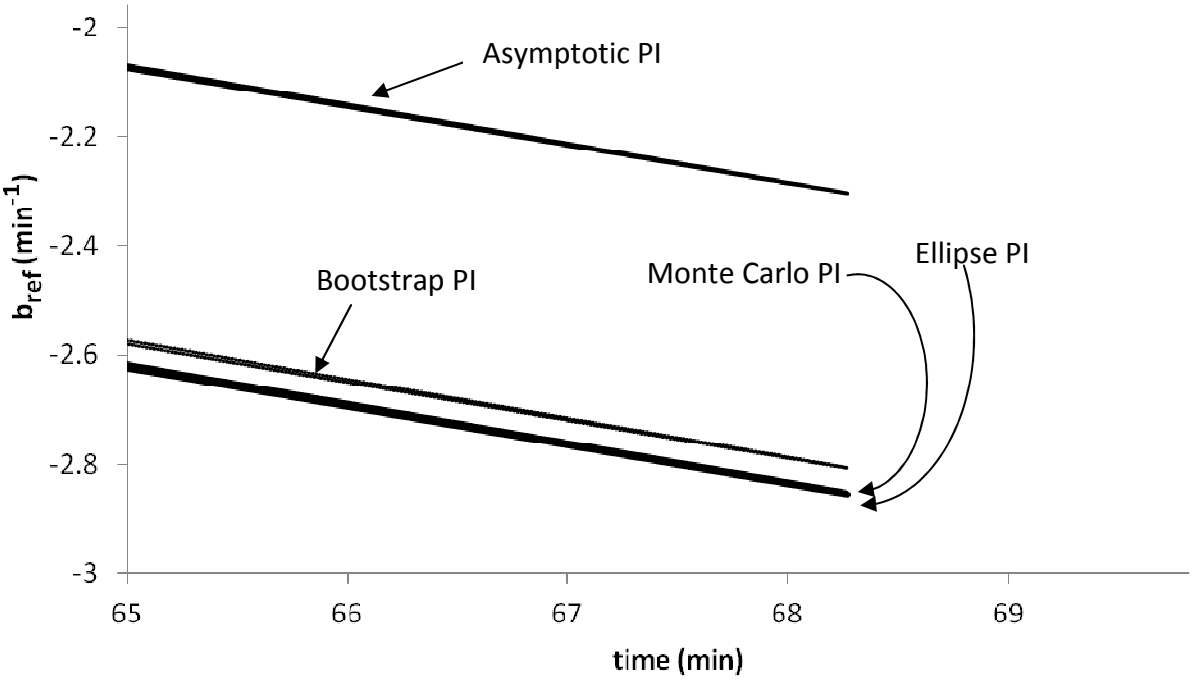

Figure 46. Zoom-in section from Figure 45.

6.8 Random effects variance for the mixed-effects models (section 4)

Table 41. Random effects variance for mixed-effects models.

Model	Species	Random effect variance
T (g)	G turkey	0.13198
	G beef	0.08530
	G pork	0.078252
T (w)	W turkey	0
	W beef	0.0074619
	W pork	0.023054
T F (g)	G turkey	0.19477
	G beef	0.12499
	G pork	0.047815
T F (w)	W turkey	4.9773e-14
	W beef	0.012638
	W pork	0.058894
T F S (g)	G turkey, beef, pork	1.36663
T F S (w)	W turkey, beef, pork	0.64025
T F M	G + W turkey	0.018597
	G + W beef	0.040271
	G + W pork	0.039966
T F M S	G + W turkey, beef, pork	0.20553

6.9 Validation against pilot-scale data (section 4)

6.9.1 Minimum and maximum errors for each model

6.9.1.1 Impingement oven data

Table 42. OLS models prediction errors chicken fillets and turkey patties (impingement cooked).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground turkey + Whole chicken n_{obs} = 44	T (g)	-0.12	-9.50
	T F (g)	0.05	-9.15
	T τ (g)	-0.04	-9.29
	T F τ (g)	0.13	-8.95
	T (g) + T (w)	2.20	-9.50
	T F (g) + T F (w)	2.23	-9.15
	T τ (g) + T τ (w)	2.23	-9.29
	T F τ (g) + T F τ (w)	2.25	-8.95
Ground turkey n_{obs} = 23 fat = 1.05% τ_{avg} = 8.77 K·min	T (g)	-0.12	-9.50
	T F (g)	0.05	-9.15
	T τ (g)	-0.04	-9.29
	T F τ (g)	0.13	-8.95
Whole chicken n_{obs} = 21 fat = 0.33% τ_{avg} = 7.92 K·min	T (g)	-0.61	-8.72
	T F (g)	-0.57	-8.62
	T τ (g)	-0.55	-8.59
	T F τ (g)	-0.51	-8.50
	T (w)	2.20	-2.89
	T F (w)	2.23	-2.86
	T τ (w)	2.23	-2.85
	T F τ (w)	2.25	-2.81

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 43. OLS models prediction errors on beef steaks and patties (impingement cooked).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground + Whole beef n_{obs} = 44	T (g)	2.76	-5.45
	T F (g)	2.96	-5.05
	T τ (g)	2.84	-5.39
	T F τ (g)	3.03	-4.99
	T (g) + T (w)	2.76	-3.39
	T F (g) + T F (w)	2.96	-3.13
	T τ (g) + T τ (w)	2.84	-3.29
	T F τ (g) + T F τ (w)	3.03	-3.03
Ground beef n_{obs} = 19 fat = 2.32% τ_{avg} = 9.28 K·min	T (g)	2.76	-3.39
	T F (g)	2.96	-3.13
	T τ (g)	2.84	-3.29
	T F τ (g)	3.03	-3.03
Whole beef n_{obs} = 25 fat = 2.68% τ_{avg} = 8.18 K·min	T (g)	0.87	-5.45
	T F (g)	1.10	-5.05
	T τ (g)	0.95	-5.39
	T F τ (g)	1.17	-4.99
	T (w)	2.47	-2.68
	T F (w)	2.60	-2.43
	T τ (w)	2.51	-2.64
	T F τ (w)	2.65	-2.40

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 44. OLS models prediction errors on pork steaks and patties (impingement cooked).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground + Whole pork n_{obs} = 56	T (g)	2.91	-6.12
	T F (g)	2.42	-5.01
	T τ (g)	2.02	-6.00
	T F τ (g)	2.52	-4.91
	T (g) + T (w)	2.69	-6.12
	T F (g) + T F (w)	2.77	-5.01
	T τ (g) + T τ (w)	2.72	-6.00
	T F τ (g) + T F τ (w)	2.77	-4.91
Ground pork n_{obs} = 27 fat = 10% τ_{avg} = 9.34 K·min	T (g)	1.61	-6.12
	T F (g)	2.42	-5.01
	T τ (g)	1.73	-6.00
	T F τ (g)	2.52	-4.91
Whole pork n_{obs} = 29 fat = 1.53% τ_{avg} = 8.21 K·min	T (g)	2.91	-3.66
	T F (g)	2.05	-4.78
	T τ (g)	2.02	-4.85
	T F τ (g)	2.09	-4.71
	T (w)	2.69	-3.97
	T F (w)	2.77	-3.77
	T τ (w)	2.72	-3.91
	T F τ (w)	2.77	-3.78

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 45. OLS models prediction errors on ALL impingement oven data.

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
ALL impingement oven data	T (g) + T (w)	2.76	-9.50
	T F (g) + T F (w)	2.96	-9.15
	T τ (g) + T τ (w)	2.84	-9.29
n_{obs} = 144	T F τ (g) + T F τ (w)	3.03	-8.95

*In all cases, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data. In addition, models used were species specific, that is, turkey models predicted for turkey data, and so on.

Table 46. Mixed-effects models prediction errors on chicken steaks and turkey patties (impingement cooked) (continued next page).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground Turkey + Whole Chicken n_{obs} = 44	T (g)	2.73	-3.75
	T F (g)	1.52	-5.55
	T F τ (g)	1.58	-5.46
	T F S (g)	2.97	-3.58
	T F τ S (g)	3.00	-3.52
	T (g) + T (w)	2.73	-3.62
	T F (g) + T F (w)	1.52	-5.03
	T F τ (g) + T F τ (w)	1.58	-5.46
	T F S (g) + T F S (w)	2.97	-3.09
	T F τ S (g) + T F τ S (w)	3.00	-3.00
	T F M	3.05	-5.22
	T F M τ	3.07	-5.09
	T F M S	2.21	-5.04
	T F M τ S	2.24	-4.91

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 46 (cont'd). Mixed-effects models prediction errors on chicken steaks and turkey patties (impingement cooked).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground turkey n_{obs} = 23 fat = 1.05% τ_{avg} = 8.77 K·min	T (g)	2.73	-3.62
	T F (g)	1.52	-5.03
	T F τ (g)	1.58	-4.90
	T F S (g)	2.97	-3.09
	T F τ S (g)	3.00	-3.00
	T F M	1.42	-5.22
	T F M τ	1.48	-5.09
	T F M S	1.98	-5.04
	T F M τ S	2.03	-4.91
Whole chicken n_{obs} = 21 fat = 0.33% τ_{avg} = 7.92 K·min	T (g)	1.79	-3.75
	T F (g)	0.02	-5.55
	T F τ (g)	0.07	-5.46
	T F S (g)	1.84	-3.58
	T F τ S (g)	1.87	-3.52
	T (w)	2.05	-3.22
	T F (w)	0.97	-4.81
	T F τ (w)	1.01	-4.73
	T F S (w)	2.29	-2.70
	T F τ S (w)	2.32	-2.66
	T F M	3.05	-1.50
	T F M τ	3.07	-1.47
	T F M S	2.21	-2.90
	T F M τ S	2.24	-2.86

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 47. Mixed-effects models prediction errors on beef steaks and patties (impingement cooked) (continued next page).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground + Whole beef n_{obs} = 44	T (g)	4.24	-2.80
	T F (g)	3.49	-3.14
	T F τ (g)	3.56	-3.10
	T F S (g)	3.82	-4.02
	T F τ S (g)	3.88	-3.97
	T (g) + T (w)	4.24	-2.67
	T F (g) + T F (w)	3.49	-2.88
	T F τ (g) + T F τ (w)	3.56	-2.84
	T F S (g) + T F S (w)	3.82	-3.03
	T F τ S (g) + T F τ S (w)	3.88	-2.99
	T F M	3.41	-2.08
	T F M τ	3.47	-2.01
	T F M S	2.91	-3.65
	T F M τ S	2.98	-3.54
Ground beef n_{obs} = 19 fat = 2.32% τ_{avg} = 9.28 K·min	T (g)	4.24	-1.40
	T F (g)	3.49	-1.91
	T F τ (g)	3.56	-1.84
	T F S (g)	3.82	-2.30
	T F τ S (g)	3.88	-2.22
	T F M	3.41	-2.08
	T F M τ	3.47	-2.01
	T F M S	2.91	-3.65
	T F M τ S	2.98	-3.54

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 47 (cont'd). Mixed-effects models prediction errors on beef steaks and patties (impingement cooked).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Whole beef n_{obs} = 25 fat = 2.68% τ_{avg} = 8.18 K·min	T (g)	2.39	-2.80
	T F (g)	1.61	-3.14
	T F τ (g)	1.67	-3.10
	T F S (g)	2.07	-4.02
	T F τ S (g)	2.12	-3.97
	T (w)	2.47	-2.67
	T F (w)	2.35	-2.88
	T F τ (w)	2.40	-2.84
	T F S (w)	2.33	-3.03
	T F τ S (w)	2.37	-2.99
	T F M	2.70	-1.64
	T F M τ	2.74	-1.61
	T F M S	2.58	-2.95
	T F M τ S	2.62	-2.90

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 48. Mixed-effects models prediction errors on pork steaks and patties (impingement cooked) (continued next page).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Ground + Whole pork n_{obs} = 56	T (g)	4.08	-4.68
	T F (g)	5.51	-1.65
	T F τ (g)	5.56	-1.61
	T F S (g)	5.59	-1.75
	T F τ S (g)	5.64	-1.76
	T (g) + T (w)	3.73	-4.68
	T F (g) + T F (w)	5.51	-5.82
	T F τ (g) + T F τ (w)	5.56	-5.75
	T F S (g) + T F S (w)	5.59	-3.04
	T F τ S (g) + T F τ S (w)	5.64	-2.97
	T F M	5.23	-1.70
	T F M τ	5.28	-1.66
	T F M S	4.50	-3.09
	T F M τ S	4.44	-3.01
Ground pork n_{obs} = 27 fat = 10% τ_{avg} = 9.34 K·min	T (g)	3.73	-4.68
	T F (g)	5.51	-0.66
	T F τ (g)	5.56	-0.66
	T F S (g)	5.59	-1.18
	T F τ S (g)	5.64	-1.13
	T F M	5.23	-1.07
	T F M τ	5.28	-1.02
	T F M S	4.37	-3.09
	T F M τ S	4.44	-3.01

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 48 (cont'd). Mixed-effects models prediction errors on pork steaks and patties (impingement cooked).

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
Whole pork n_{obs} = 29 fat = 1.53 % τ_{avg} = 8.21 K·min	T (g)	4.08	-2.06
	T F (g)	3.90	-1.65
	T F τ (g)	3.92	-1.61
	T F S (g)	4.41	-1.75
	T F τ S (g)	4.12	-1.76
	T (w)	3.08	-4.44
	T F (w)	2.50	-5.82
	T F τ (w)	2.54	-5.75
	T F S (w)	3.97	-3.04
	T F τ S (w)	4.00	-2.97
	T F M	3.95	-1.70
	T F M τ	3.98	-1.66
	T F M S	4.50	-1.36
	T F M τ S	4.32	-1.37

*where (g)+(w) models appear, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data.

Table 49. Mixed-effects models prediction errors on ALL impingement oven data.

Data source for validation	Model*	Max error	Min error
		log (CFU/g)	
ALL impingement oven data n_{obs} =144	T (g) + T (w)	4.24	-4.68
	T F (g) + T F (w)	5.51	-5.68
	T F τ (g) + T F τ (w)	5.56	-5.75
	T F S (g) + T F S (w)	6.03	-3.09
	T F τ S (g) + T F τ S (w)	5.64	-3.00
	T F M	5.23	-5.22
	T F M τ	5.28	-5.09
	T F M S	4.50	-5.04
	T F M τ S	4.44	-4.91

*In all cases, ground-muscle calibrated models predicted lethality for ground-muscle data and whole-muscle calibrated models did for whole-muscle data. In addition, models used were species specific, that is, turkey models predicted for turkey data, and so on.

6.9.1.2 Big roasts

Table 50. OLS models prediction errors on roasts.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
Turkey roasts n_{obs} = 9 fat = 0.27% τ_{range} = 200 – 500 K·min	T (g)	-10.96	-14.32
	T F (g)	-10.83	-14.19
	T τ (g)	-9.08	-13.16
	T F τ (g)	-8.96	-13.04
	T (w)	-2.06	-5.30
	T F (w)	-2.00	-5.25
	T τ (w)	-1.21	-4.79
	T F τ (w)	-1.16	-4.74
Beef roasts n_{obs} = 13 fat = 2.68% τ_{range} = 100 – 480 K·min	T (g)	-2.23	-15.19
	T F (g)	-1.75	-14.14
	T τ (g)	-1.78	-13.87
	T F τ (g)	-1.33	-12.90
	T (w)	1.15	-6.12
	T F (w)	1.45	-7.14
	T τ (w)	1.43	-6.98
	T F τ (w)	1.69	-6.41
Pork roasts n_{obs} = 20 fat =1.53% τ_{range} = 100 – 600 K·min	T (g)	-0.11	-4.64
	T F (g)	-0.01	-4.50
	T τ (g)	0.03	-3.80
	T F τ (g)	0.14	-3.68
	T (w)	1.60	-1.77
	T F (w)	1.71	-1.65
	T τ (w)	1.70	-1.30
	T F τ (w)	1.81	-1.16

Table 51. Mixed-effects models prediction errors on roasts (continued next page).

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
Turkey roasts n_{obs} = 9 fat = 0.27% τ_{range} = 200 – 500 K·min	T (g)	-2.97	-6.28
	T F (g)	-15.90	-22.66
	T F τ (g)	-6.51	-12.02
	T F S (g)	-9.06	-15.21
	T F τ S (g)	1.03	-3.54
	T (w)	-1.78	-5.21
	T F (w)	-15.90	-22.66
	T F τ (w)	-1.98	-7.22
	T F S (w)	-2.39	-5.72
	T F τ S (w)	1.26	-3.13
	T F M	-1.58	-5.93
	T F M τ	1.45	-2.62
	T F M S	-3.56	-8.17
	T F M τ S	1.64	-2.75
Beef roasts n_{obs} = 13 fat = 2.68% τ_{range} = 100 – 480 K·min	T (g)	1.00	-8.13
	T F (g)	-1.97	-13.48
	T F τ (g)	0.89	-8.32
	T F S (g)	0.92	-8.81
	T F τ S (g)	3.33	-5.21
	T (w)	-1.97	-13.47
	T F (w)	0.91	-8.32
	T F τ (w)	3.08	-4.62
	T F S (w)	0.97	-8.29
	T F τ S (w)	3.15	-4.60
	T F M	0.89	-7.68
	T F M τ	2.66	-4.21
	T F M S	1.83	-6.67
	T F M τ S	3.65	-3.72

Table 51 (cont'd). Mixed-effects models prediction errors on roasts.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
Pork roasts n_{obs} = 20 fat = 1.53% τ_{range} = 100 – 600 K·min	T (g)	2.96	-0.02
	T F (g)	0.88	-4.47
	T F τ (g)	1.46	-1.21
	T F S (g)	2.58	-0.56
	T F τ S (g)	3.27	0.71
	T (w)	1.81	-1.02
	T F (w)	1.14	-1.77
	T F τ (w)	1.46	-1.21
	T F S (w)	1.09	-2.53
	T F τ S (w)	2.02	-0.48
	T F M	1.47	-2.84
	T F M τ	2.15	-0.42
	T F M S	2.81	-0.36
	T F M τ S	3.44	0.82

Table 52. OLS models prediction errors on ALL roast data.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
ALL roast data n_{obs} = 42	T (w)	1.60	-6.12
	T F (w)	1.71	-7.14
	T τ (w)	1.70	-6.98
	T F τ (w)	1.81	-6.41

Table 53. Mixed-effects models prediction errors on ALL roast data.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
ALL roast data n_{obs} = 42	T (w)	1.81	-13.48
	T F (w)	1.14	-22.66
	T F τ (w)	3.08	-7.22
	T F S (w)	1.09	-8.29
	T F τ S (w)	3.15	-4.60
	T F M	1.47	-7.68
	T F M τ	2.66	-4.21
	T F M S	2.81	-8.17
	T F M τ S	3.65	-3.72

6.9.1.3 Hot dogs

Table 54. OLS models prediction errors on hot dogs.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
Turkey hot dogs n_{obs} = 12 fat = 4.28% τ_{range} = 50 – 125 K·min	T (g)	1.88	-4.85
	T F (g)	2.14	-3.90
	T τ (g)	2.22	-3.61
	T F τ (g)	2.44	-3.06
Beef hot dogs n_{obs} = 12 fat =15.42% τ_{range} = 100 – 275 K·min	T (g)	-0.49	-3.50
	T F (g)	0.16	-1.64
	T τ (g)	0.35	-1.80
	T F τ (g)	0.75	-0.46

Table 55. OLS models predictions errors on ALL hot dog data.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
ALL hot dog data n_{obs} = 24	T (g)	1.88	-4.85
	T F (g)	2.14	-3.90
	T τ (g)	2.22	-3.61
	T F τ (g)	2.44	-3.06

Table 56. Mixed-effects models prediction errors on hot dogs.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
Turkey hot dogs n_{obs} = 12 fat =4.28% τ_{range} = 50 – 125 K·min	T (g)	2.90	-1.68
	T F (g)	2.17	-3.05
	T F τ (g)	5.92	-2.67
	T F S (g)	3.16	-1.19
	T F τ S (g)	5.04	-0.89
	T F M	6.13	-3.06
	T F M τ	2.47	-2.68
	T F M S	5.82	-2.00
	T F M τ S	5.23	-1.73
Beef hot dogs n_{obs} = 12 fat = 15.42% τ_{range} = 100 – 275 K·min	T (g)	0.41	-0.96
	T F (g)	0.63	-0.37
	T F τ (g)	2.72	-0.04
	T F S (g)	2.07	-1.01
	T F τ S (g)	3.81	0.77
	T F M	3.72	-0.33
	T F M τ	3.07	0.0
	T F M S	2.83	0.35
	T F M τ S	2.33	0.52

Table 57. Mixed-effects models prediction errors on ALL hot dog data.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
ALL hot dog data n_{obs} = 24	T (g)	2.90	-1.68
	T F (g)	2.17	-3.05
	T F τ (g)	2.47	-2.67
	T F S (g)	3.16	-1.08
	T F τ S (g)	3.30	-0.89
	T F M	2.17	-3.06
	T F M τ	2.47	-2.68
	T F M S	2.76	-2.00
	T F M τ S	2.96	-1.73

6.9.1.4 Overall model performance on pilot-scale data

Table 58. OLS models prediction errors on ALL pilot-scale data.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
ALL pilot-scale data n_{obs} = 210	T (g) + T (w)	2.76	-9.50
	T F (g) + T F (w)	2.96	-9.15
	T τ (g) + T τ (w)	2.84	-9.29
	T F τ (g) + T F τ (w)	3.03	-8.95

Table 59. Mixed-effects models prediction errors on ALL pilot-scale data.

Data source for validation	Model	Max error	Min error
		log (CFU/g)	
ALL pilot-scale data n_{obs} = 210	T (g) + T (w)	4.24	-13.48
	T F (g) + T F (w)	5.51	-22.66
	T F τ (g) + T F τ (w)	5.56	-7.22
	T F S (g) + T F S (w)	6.03	-8.29
	T F τ S (g) + T F τ S (w)	5.64	-4.60
	T F M	5.23	-7.68
	T F M τ	5.28	-5.09
	T F M S	4.50	-8.17
	T F M τ S	4.44	-4.91

6.9.2 Plots showing model predictions and PIs for representative data sets and models

Given the large number of validated models and data sets, this project produced ~320 plots (one for each validation). However, it was decided to present the plots of only a representative data set across all models, in addition to any other that may have shown special features.

6.9.2.1 OLS models

To show the effect of the addition of the fat and sublethal injury parameters (β_3 and β_2, represented by F and τ in model names) and the importance of using whole-muscle calibrated models (represented by (w)) to predict lethality in whole-muscle data (instead of ground-muscle calibrated models, represented by (g)), the impingement-cooked whole-muscle pork steaks were chosen. Notice the slight improvement created by the addition of β_2 and β_3, but the significant difference caused by the use of the whole-muscle-calibrated models (w) instead of the ground-muscle-calibrated versions (g).

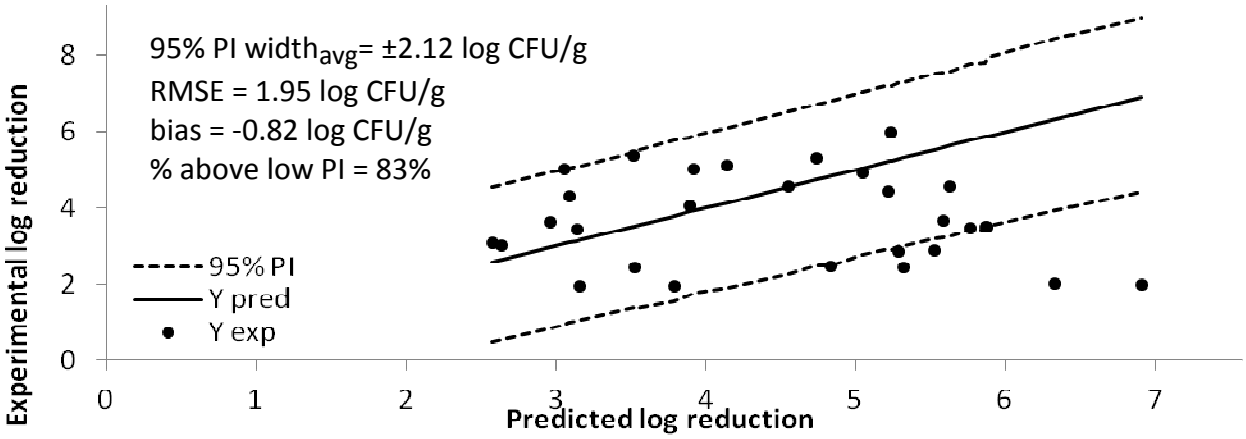

Figure 47. OLS T (g) model validated with whole-muscle pork steaks.

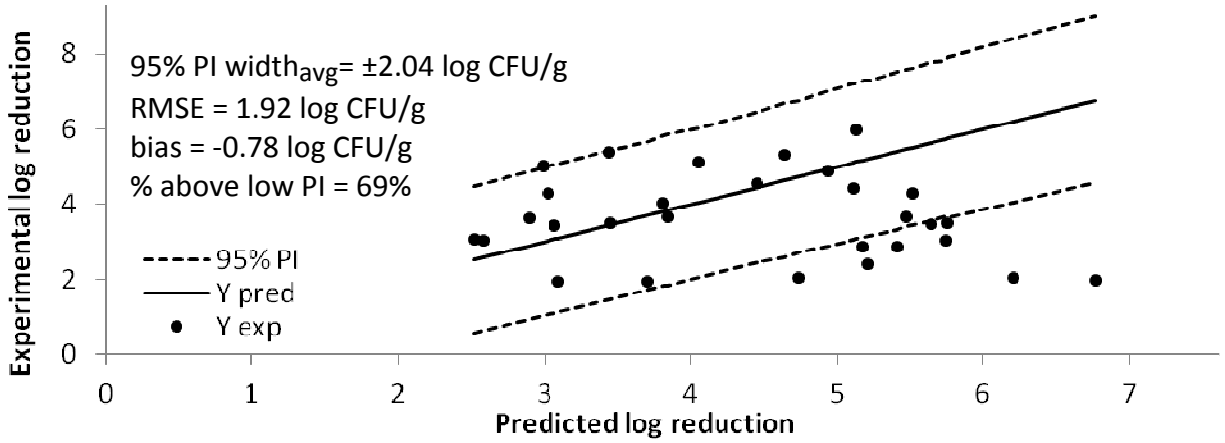

Figure 48. OLS T F (g) model validated against whole-muscle pork steaks.

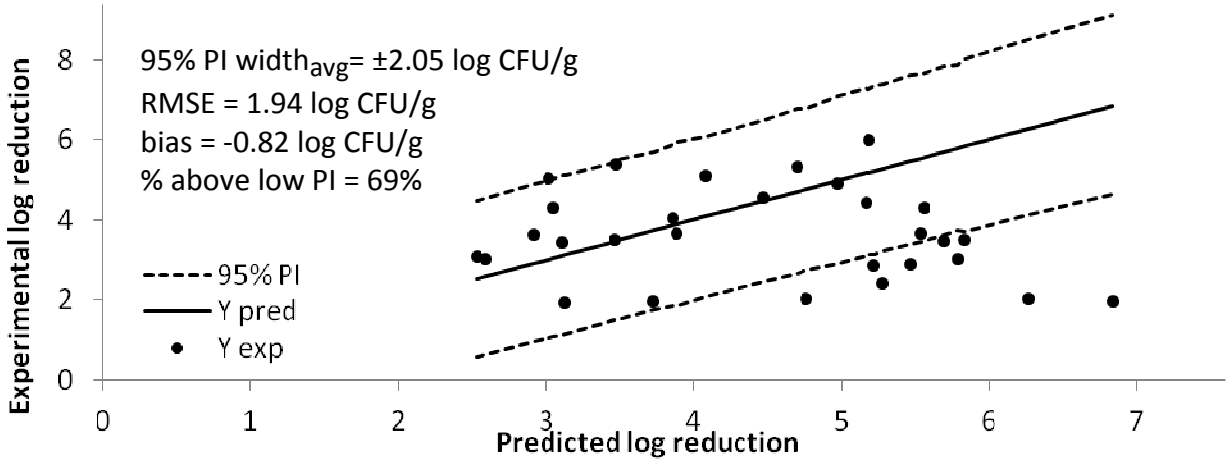

Figure 49. OLS T τ (g) model validated against whole-muscle pork steaks.

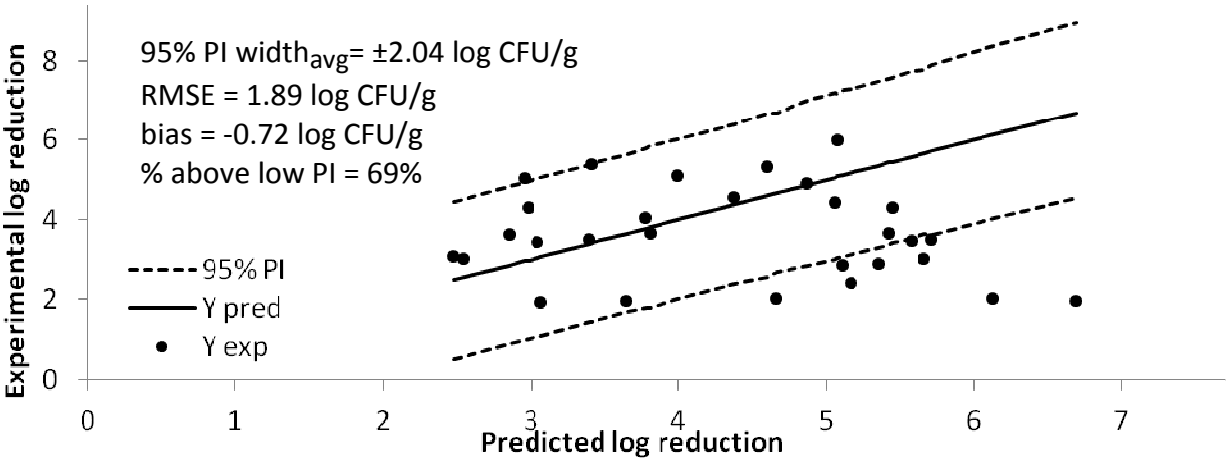

Figure 50. OLS T F τ (g) model validated against whole-muscle pork steaks.

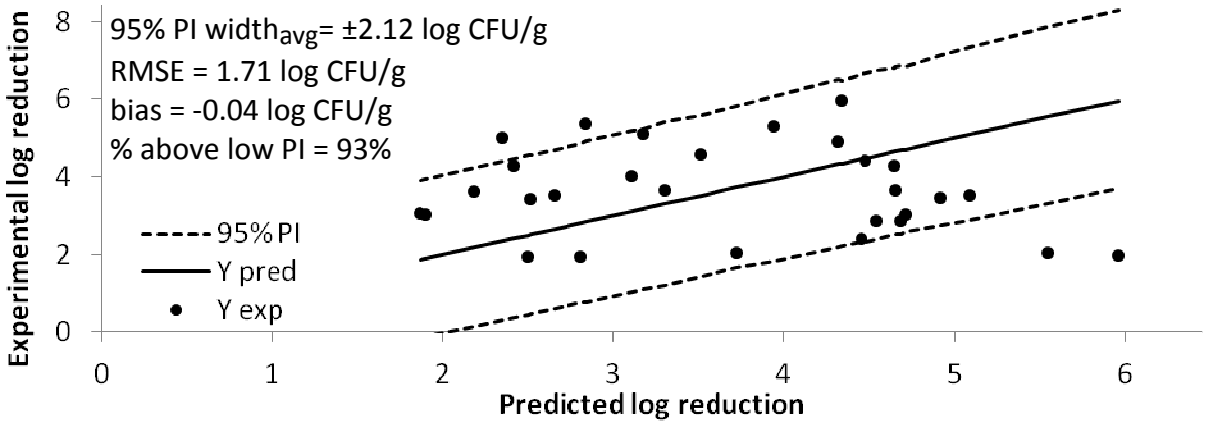

Figure 51. OLS T (w) model validated against whole-muscle pork steaks.

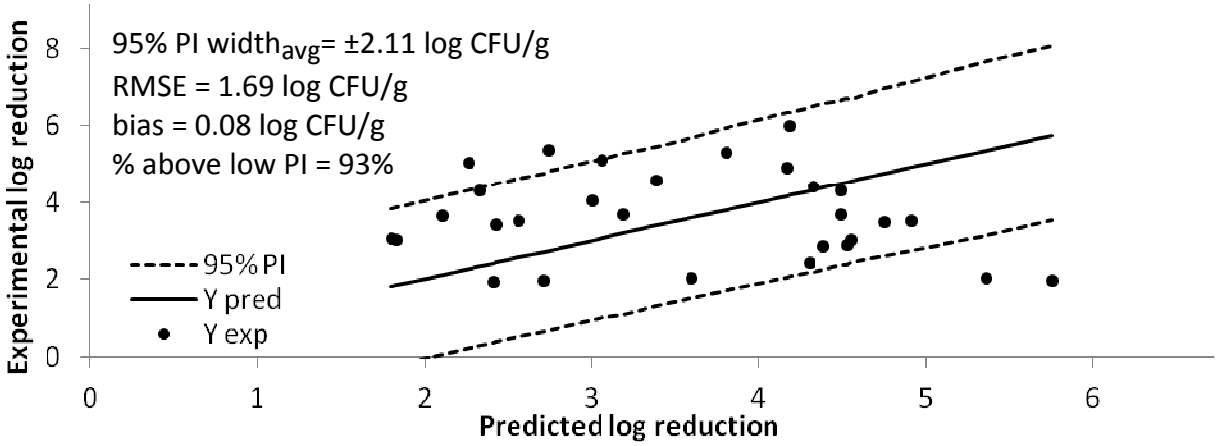

Figure 52. OLS T F (w) model validated against whole-muscle pork steaks.

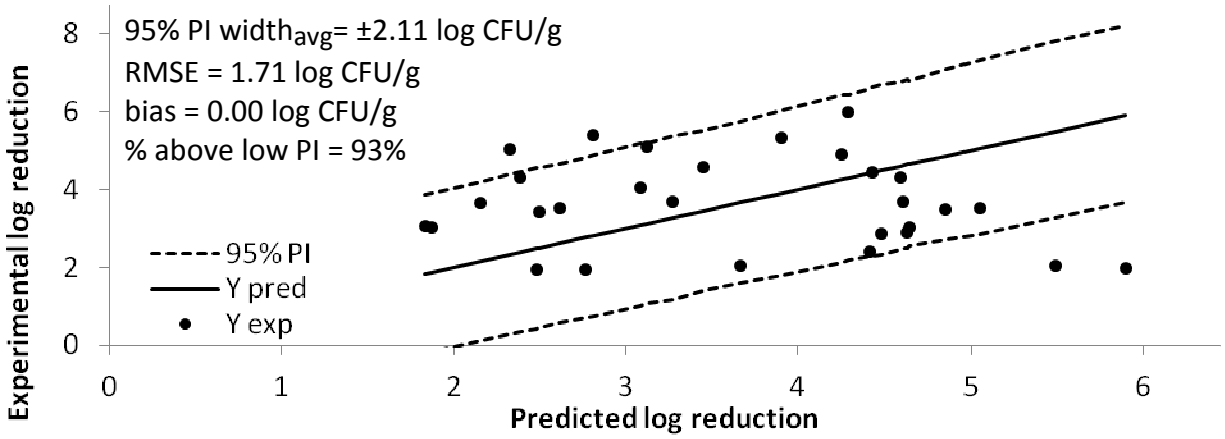

Figure 53. OLS T τ (w) model validated against whole-muscle pork steaks.

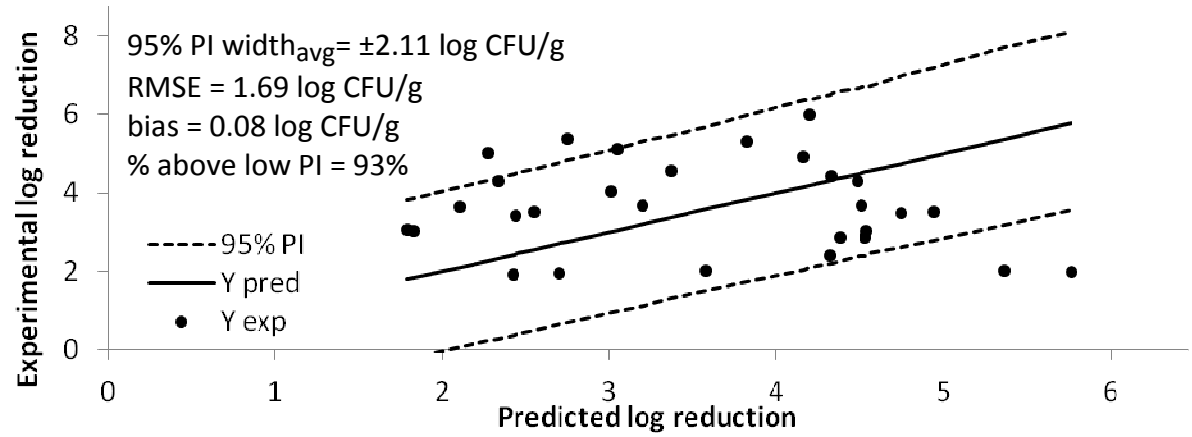

Figure 54. OLS T F t (w) model validated against whole-muscle pork steaks.

6.9.2.2 Mixed-effects models

For the mixed-effect models, the impingement-cooked whole-muscle chicken breast samples were chosen because of the significant changes noted with the addition of parameters and with the use of the ground-muscle-calibrated model (represented by (g)) versus the whole-muscle-calibrated versions (represented by (w)).

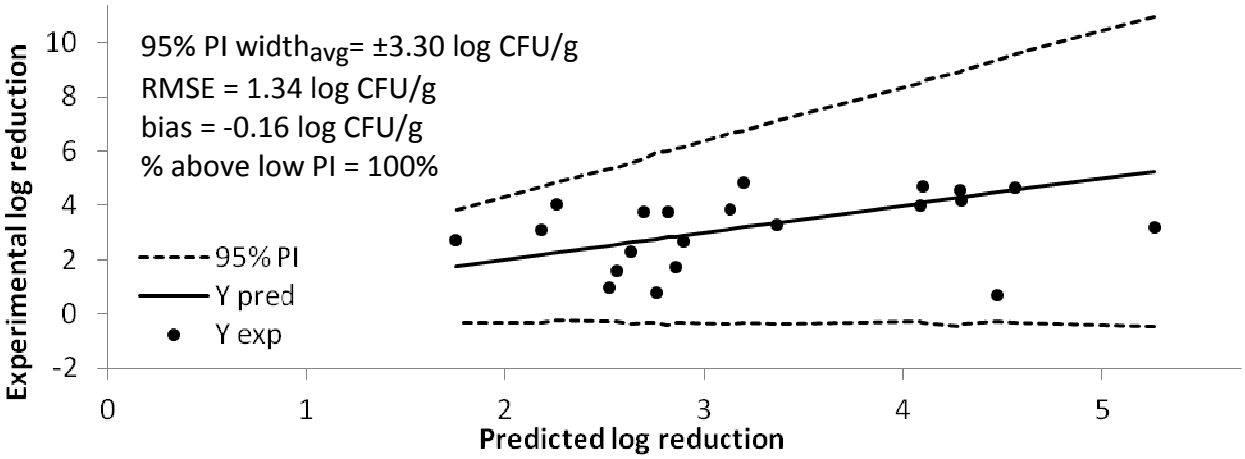

Figure 55. Mixed-effects T (g) model validated against whole-muscle chicken breasts.

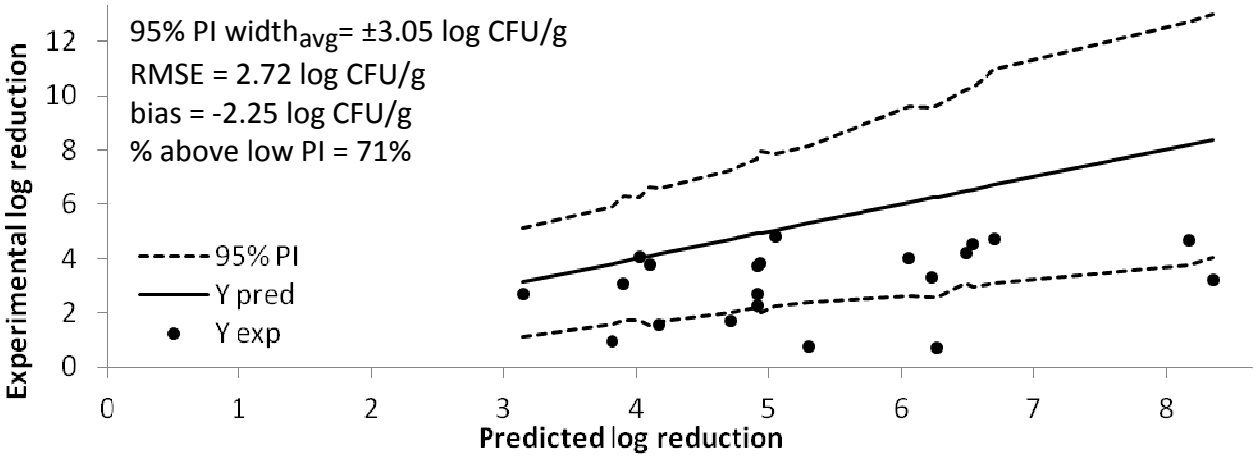

Figure 56. Mixed-effects T F (g) model validated against whole-muscle chicken breasts.

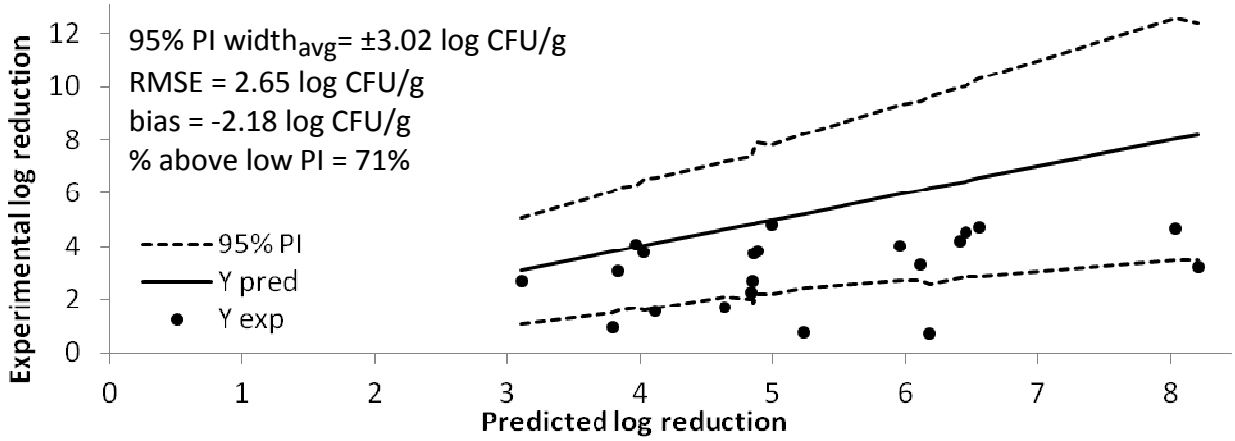

Figure 57. Mixed-effects T F τ (g) model validated against whole-muscle chicken steaks.

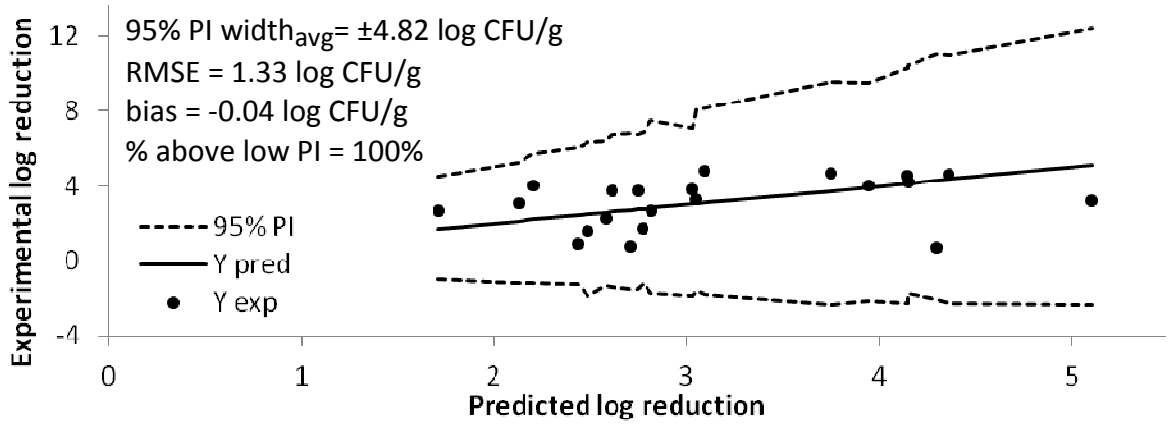

Figure 58. Mixed-effects T F S (g) model validated against whole-muscle chicken breasts.

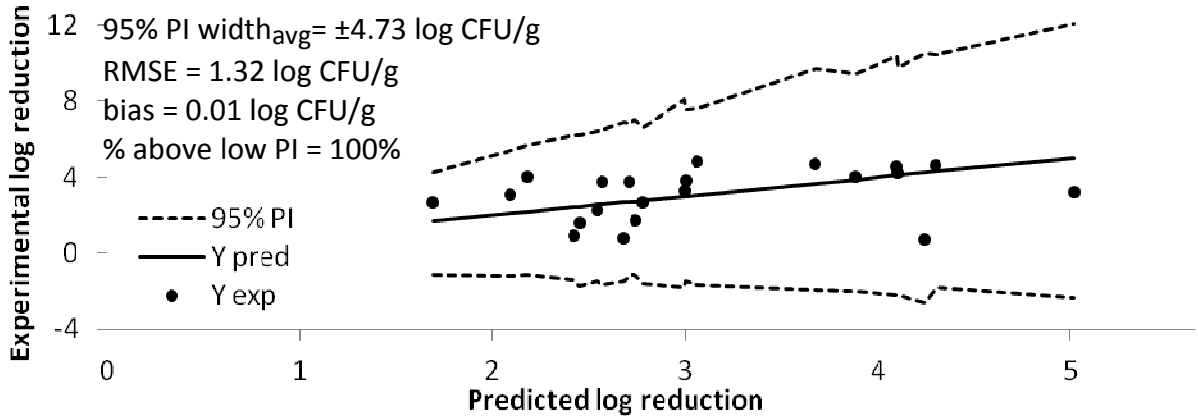

Figure 59. Mixed-effects T F S τ (g) model validated against whole-muscle chicken breasts.

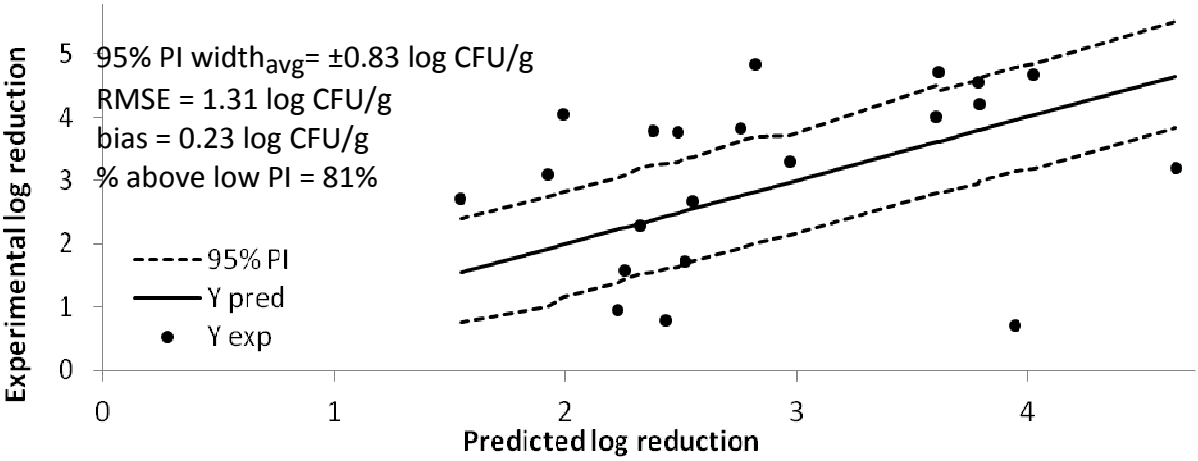

Figure 60. Mixed-effects T (w) model validated against whole-muscle chicken breasts.

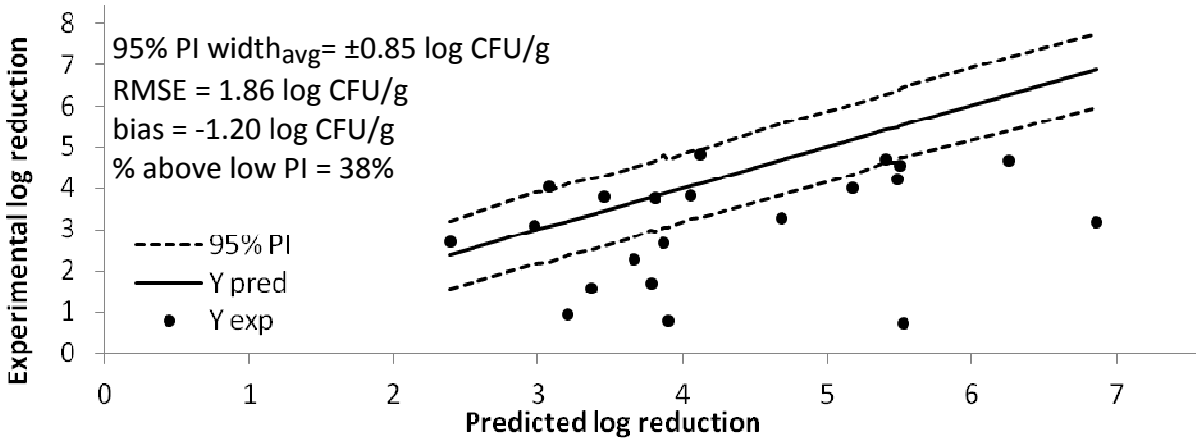

Figure 61. Mixed-effects T F (w) model validated against whole-muscle chicken breasts.

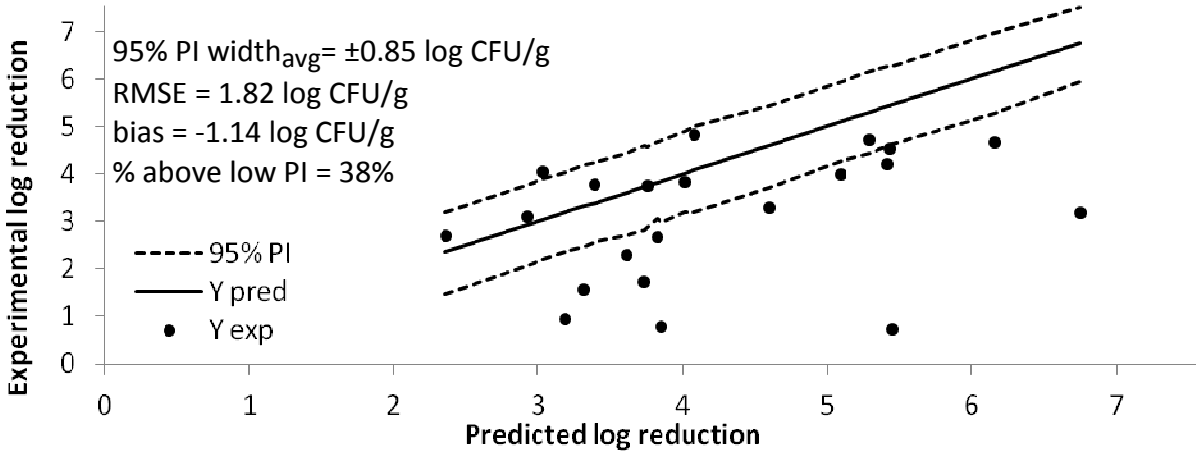

Figure 62. Mixed-effects T F τ (w) model validated against whole-muscle chicken breasts.

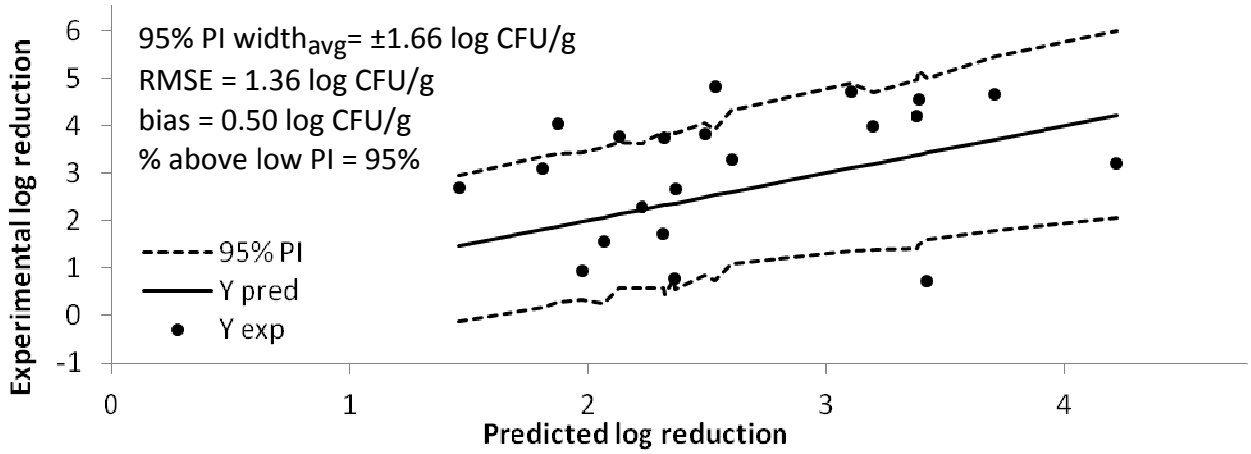

Figure 63. Mixed-effects T F S (w) model validated against whole-muscle chicken breasts.

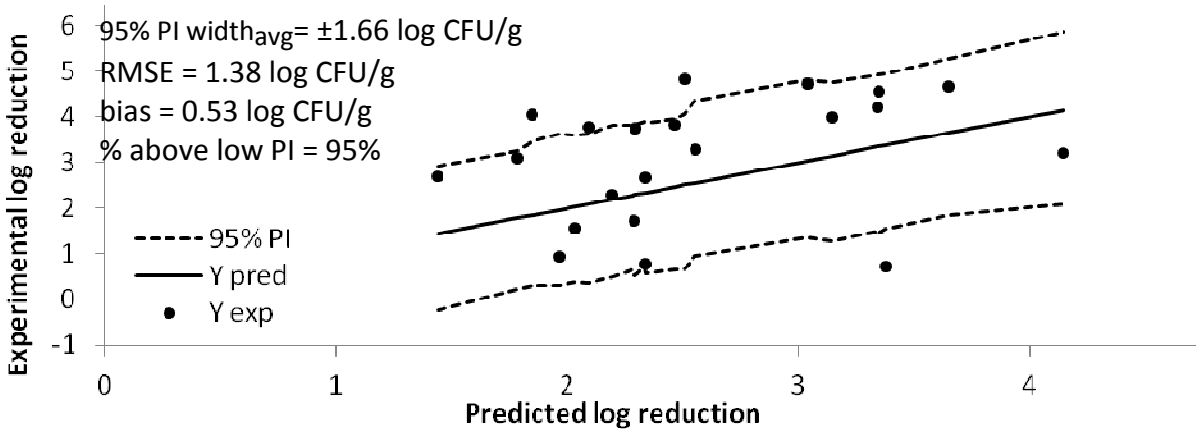

Figure 64. Mixed-effects T F S τ (w) model validated against whole-muscle chicken breasts.

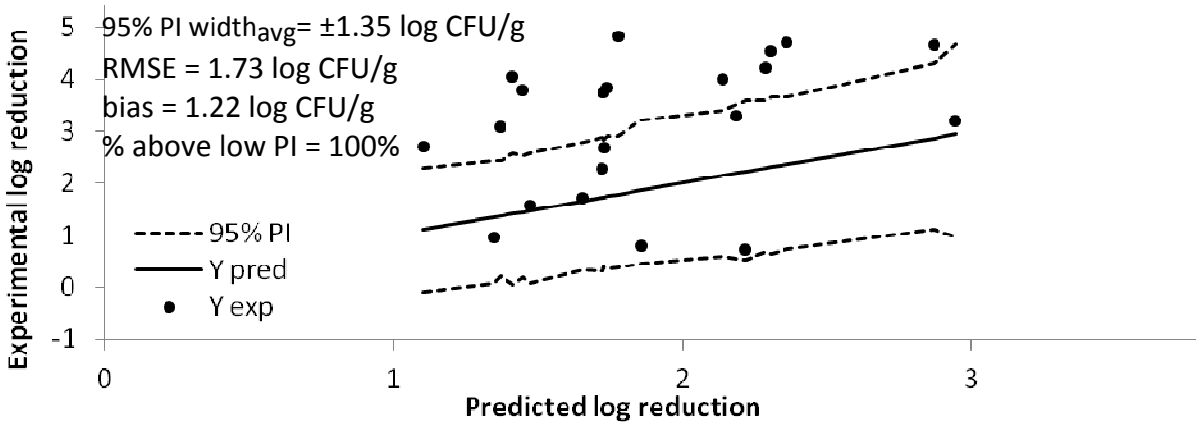

Figure 65. Mixed-effects T F M model validated against whole-muscle chicken breasts.

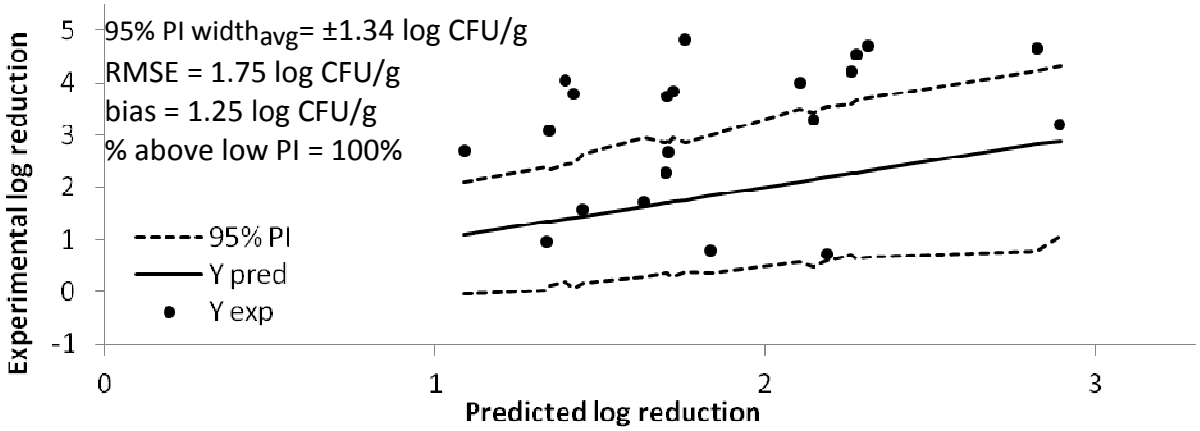

Figure 66. Mixed-effects T F M τ model validated against whole-muscle chicken breasts.

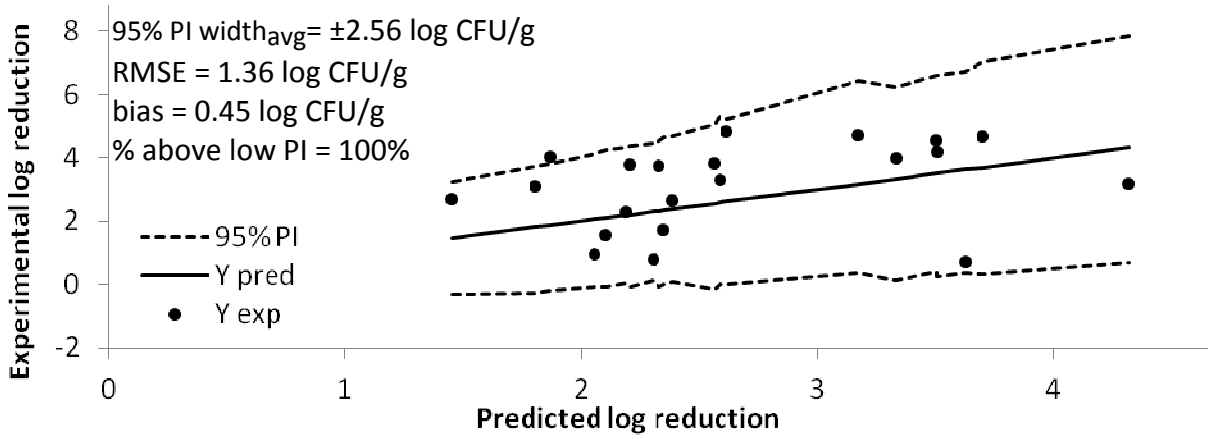

Figure 67. Mixed-effects T F M S model validated against whole-muscle chicken breasts.

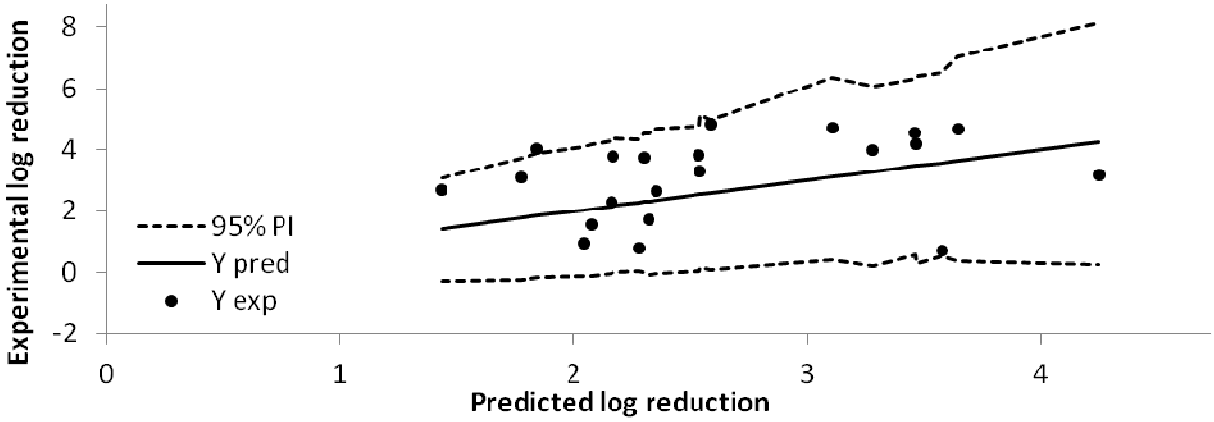

Figure 68. Mixed-effects T F M S τ model validated against whole-muscle chicken breasts.

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