Quantitative Microbial Process Model to Estimate the Risk of
Salmonellosis from Consuming Pork and Pork Products

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(Agriculture)

University of Tasmania

October, 2016
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Phillip Gurman
University of Tasmania
October, 2016
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Contents

Acknowledgments ix
Abstract xi
Abbreviations xix
List of Figures xx
List of Tables xxiii

1. Introduction 1
   1.1. Hazard Identification ................................. 2
       1.1.1. Epidemiology .................................... 2
       1.1.2. Source Attribution ................................ 3
       1.1.3. Outbreaks Associated with Pork Products .......... 4
       1.1.4. Differences between *Salmonella* serovars .......... 4
   1.2. Hazard Characterisation ................................ 6
   1.3. Pork Production, Supply and Consumption .................. 7
       1.3.1. Farm ............................................. 7
       1.3.2. Transport ....................................... 10
       1.3.3. Abattoir ....................................... 11
       1.3.4. Boning ........................................ 14
           1.3.4.1. Processing .................................... 14
## Contents

1.3.5. Supply Chain .................................................. 16
  1.3.5.1. Distribution ........................................... 16
  1.3.5.2. Retail .................................................. 16
  1.3.5.3. Consumer Transport .................................. 17
  1.3.5.4. Domestic Storage ...................................... 17
  1.3.5.5. Cooking ............................................... 17

1.4. New Industry Marketing Initiatives and Need for Risk Assessment .......... 18

1.5. Thesis Aims ..................................................... 18

1.6. Thesis Structure ................................................ 19

2. Methods ..................................................................... 21

  2.1. Microbial Risk Assessment ................................... 21
    2.1.1. Risk Assessment Approaches .......................... 23
    2.1.2. Variability, Uncertainty and Confidence in Model Predictions .... 25
    2.1.3. Quantitative Risk Assessment Tools ................ 25

  2.2. Predictive Microbiology ....................................... 26
    2.2.1. Primary Growth Models .................................. 28
      2.2.1.1. ‘Modified-Gompertz Model’ ...................... 29
      2.2.1.2. Baranyi ‘D-model’ ................................. 30
      2.2.1.3. ‘Three phase linear’ Model ..................... 31
      2.2.1.4. Primary Model Conclusion ....................... 32
    2.2.2. Secondary Growth Models ............................... 32
      2.2.2.1. Growth Rate ....................................... 32
        Bělehrádek-Type ........................................... 32
        Arrhenius-Type ............................................ 33
      2.2.2.2. Lag Phase Duration .................................. 34
      2.2.2.3. Maximum Population Density .................... 35
## Contents

2.2.3. Inactivation Models ........................................ 35
  2.2.3.1. Models Based on \( D \)- and \( z \)-Values .......... 36
  2.2.3.2. Cooking Method Validation Models ............... 37
  2.2.3.3. Inactivation Model Conclusions ................... 37
2.2.4. Growth and Inactivation Data ............................ 38
2.3. Dose-Response Models ...................................... 38
  2.3.1. Feeding Studies ......................................... 39
  2.3.2. Outbreak Studies ........................................ 42
  2.3.3. Surrogate Organisms ..................................... 44
  2.3.4. Combination Models ....................................... 47
  2.3.5. Dose-Response Conclusion ............................... 47

3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers 49

3.1. Abstract ..................................................... 49
3.2. Introduction ................................................ 50
3.3. Materials and Methods ...................................... 53
  3.3.1. *Salmonella* Strains ................................... 53
  3.3.2. Mince .................................................. 53
  3.3.3. Patty Preparation ....................................... 54
  3.3.4. Cooking of Pork Burger Patties ....................... 55
  3.3.5. *Salmonella* Enumeration ................................ 56
  3.3.6. Fat Level Analysis ..................................... 57
  3.3.7. pH Testing .............................................. 57
  3.3.8. Statistical Analysis .................................... 57
3.4. Results ...................................................... 61
  3.4.1. Fat Content, pH and Temperature Calibration .......... 61
  3.4.2. *Salmonella* Inactivation ............................... 66
  3.4.3. Temperature Profile ..................................... 67
3.5. Discussion ................................................................. 68
  3.5.1. Effect of Fat Content .......................................... 68
  3.5.2. Effect of Serovar ............................................... 69
  3.5.3. Temperature Profile ........................................... 70
  3.5.4. Predictive Modelling ........................................... 70
  3.5.5. Doneness and Colour .......................................... 73
  3.5.6. Practical Findings ............................................. 74
3.6. Conclusions ........................................................... 74
3.A. Statistical Fitting of the model ................................. 75

4. Growth of *Salmonella* in Moisture-Infused Pork ............ 77
  4.1. Abstract ............................................................. 77
  4.2. Introduction ....................................................... 78
  4.3. Methods ............................................................. 79
    4.3.1. Preparation of Pork Steaks ................................ 79
    4.3.2. Incubation Conditions ..................................... 81
    4.3.3. *Salmonella* Enumeration .................................. 81
    4.3.4. Sterilised Pork Experiment ................................. 82
    4.3.5. Statistical Analysis ........................................ 82
  4.4. Results ............................................................. 85
  4.5. Discussion ........................................................ 89

5. Quantitative Microbial Risk Assessment of Salmonellosis From the Con-
   sumption of Australian Pork: Minced Meat From Retail to Burgers Prepared
   and Consumed at Home ................................................ 95
  5.1. Abstract ............................................................. 95
  5.2. Introduction ....................................................... 96
  5.3. Methods ............................................................. 98
# Contents

6.3.1.5. *Salmonellae* per Primal ........................................... 137  
6.3.1.6. Brine Injection per Primal ........................................ 138  
6.3.1.7. Contamination Locations ........................................... 138  
6.3.1.8. Transfer Matrix .................................................... 139  
6.3.1.9. Processing .......................................................... 143  
6.3.1.10. Post Processing .................................................... 144  
6.3.2. Supply and Consumption Module ..................................... 145  
6.3.2.1. Initial Steak Conditions ......................................... 145  
6.3.2.2. Growth Model ....................................................... 146  
6.3.2.3. Retail and Consumer Transport .................................. 147  
6.3.2.4. Domestic Storage .................................................. 147  
6.3.2.5. Inactivation ....................................................... 147  
6.3.2.6. Consumption ....................................................... 149  
6.4. Results ........................................................................ 149  
6.4.1. Prevalence and Concentration in Infused Pork Loins .......... 149  
6.4.2. Risk Estimates ......................................................... 150  
6.4.3. Growth Observations .................................................. 150  
6.4.4. Sensitivity Analysis .................................................... 151  
6.4.5. Scenario Analysis ...................................................... 151  
6.5. Discussion .................................................................. 151  
6.6. Conclusion .................................................................. 159  
6.6.1. Comparison of Inactivation Rates ................................. 159  

7. **Discussion** .................................................................. 173  
7.1. Interpretation to Pork Products in General ....................... 173  
7.2. Outcomes for Industry .................................................. 175  
7.3. Risk Modelling Considerations ....................................... 177  
7.4. Further Work .............................................................. 178
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Abstract

Salmonellosis is the second largest cause of foodborne illness in Australia, with rates of salmonellosis increasing over recent time. Outbreaks of foodborne illness attributed to pork products are most often associated with \textit{Salmonella}. Pork industry risk managers, therefore, commissioned this study to develop robust risk assessment models to quantify the risks of salmonellosis to Australian consumers from pork burgers and moisture-infused pork, with the aim of informing risk managers in their decisions. Pork burgers are currently being marketed in Australia as an alternate serving suggestion for pork mince, while moisture-infused pork is also now being promoted as an alternative low-fat product with increased juiciness to offset the effects of reduced intramuscular fat. These products, however, may present increased risk compared to other pork products because of the potential internalisation of pathogens.

The risk assessments followed the approaches and guidelines recommended by FAO/WHO and Codex for microbiological risk assessment and used a stochastic modelling approach. An extensive review of literature relevant to these risk assessments identified many appropriate data sources, including \textit{Salmonella} prevalence after boning, storage conditions during retail storage, consumer transport and domestic storage; and dose-response models for \textit{Salmonella}. The lack of predictive models for thermal inactivation of \textit{Salmonella} spp. in pork burger patties and the growth of \textit{Salmonella} in moisture-infused pork, as well as the \textit{Salmonella} concentration after boning, endpoint cooking temperatures for pork products, and the effect of the infusion process, were all identified as data gaps. Accordingly, novel data on the behaviour of \textit{Salmonella} in pork products were also developed and incorporated into the modelling.
Abstract

Thermal inactivation of *Salmonella* in pork burgers was investigated by cooking pork patties made from pork mince with either ‘Low’ or ‘Regular’ levels of fat. Mince was inoculated with several strains of *Salmonella* and cooked to one of seven internal endpoint temperatures (46, 49, 52, 55, 58, 61, 64 °C) and *Salmonella* enumerated, with survival described by a generalised logistic regression model. The fat content of pork mince influenced *Salmonella* survival significantly (*p* = 0.043), with increasing fat levels resulting in increased *Salmonella* survival, though this effect became negligible as the internal endpoint temperature approached 62 °C. Survival of *Salmonella* serovars did not differ significantly (*p* = 0.86 and 0.10 for the intercept and slope of the fitted logistic regression curve, respectively).

The growth of *Salmonella* in moisture-infused pork as a function of temperature was assessed by injecting intact pork loin steaks with a cocktail of *Salmonella* serovars. The steaks were cut into strips and incubated at nominal temperatures of 8, 15, 20, 25 or 30 °C, and *Salmonella* enumerated at various time intervals. Primary and secondary growth models were fitted to these observations using both one-step and two-step procedures to develop predictive growth models. The fits obtained using both procedures were compared with those obtained from ComBase data for meat products and were found to be similar, with the one-step method in closer agreement with the ComBase fits than the two-step fits. These results indicate that *Salmonella* growth is not significantly retarded by moisture infusion brines.

Stochastic risk assessment models were constructed to estimate the changes in *Salmonella* prevalence and concentration, between retail and consumption, with the probability of illness per meal estimated. These models were constructed in the statistical programming language ‘R’ using the ‘mc2d’ package, with models constructed in two dimensions, allowing variability and uncertainty to be separated, quantified and assessed independently. This separation is not currently implemented in commercial stochastic modelling software packages. The mean risk of illness from the consumption of a pork burger was estimated at $1.54 \times 10^{-8}$ (95% uncertainty credible interval $7.2 \times 10^{-10}$, $4.96 \times 10^{-8}$) and for a moisture-infused pork steak at $4.12 \times 10^{-8}$ (95% uncertainty credible interval $9.85 \times 10^{-9}$, $7.75 \times 10^{-8}$). Sensitivity analysis was performed.
in both risk assessments using Spearman rank correlation, which identified the low prevalence and concentration of *Salmonella* on pork in Australia and the high temperatures to which Australian consumers cook pork as having the greatest influence on the low risk experienced by consumers. Hypothetical scenarios were also investigated to determine their effect on risk, including changes to mean retail temperatures and consumer pork ‘doneness’ preferences.

The work described in this thesis has produced predictive models for *Salmonella* inactivation in pork burgers and growth in moisture-infused pork, providing novel information on factors that influence these processes. The risk assessments of salmonellosis from consumption of pork burgers and moisture-infused pork in Australia provide valuable information to industry on these risks, which will inform risk-based decisions, including industry risk management strategies, for pork products in Australia.
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Abstract

Co-Authorship Statement

Chapter 3 has been published in the *International Journal of Food Microbiology*. Chapter 5 has been submitted to the journal Risk Analysis and is currently undergoing revision based on reviewers comments.

The following people and institutions contributed to the publication of work undertaken as part of this thesis:

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**Chapter 3: Thermal Inactivation of *Salmonella* spp. in Pork Burgers**


Candidate contributed 40% (performed large portions of laboratory work, conducted the statistical analysis of the data and wrote the majority of the manuscript). Author 3 and Author 4 contributed 20% each (contributed to the idea, its formalisation and development while also revising the manuscript). Author 1 contributed 10% (assisted with statistical analysis). Author 2 contributed 10% (provided laboratory assistance).
Chapter 5, ‘Quantitative Microbial Risk Assessment of Salmonellosis From the Consumption of Australian Pork: Minced Meat From Retail to Burgers Prepared and Consumed at Home’:

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Located in Chapter 5

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Author 3 and Author 4 contributed 15% each (contributed to the idea, its formalisation and development while also revising the manuscript).

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University of Tasmania  University of Tasmania  University of Tasmania
Other publications and communications arising from this research


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### Abbreviations

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<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>APL</td>
<td>Australian Pork Limited</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Units</td>
</tr>
<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>FSANZ</td>
<td>Food Standards Australia New Zealand</td>
</tr>
<tr>
<td>GR</td>
<td>Growth Rate</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of Detection</td>
</tr>
<tr>
<td>LPD</td>
<td>Lag Phase Duration</td>
</tr>
<tr>
<td>MPD</td>
<td>Maximum Population Density</td>
</tr>
<tr>
<td>MPN</td>
<td>Most Probable Number</td>
</tr>
<tr>
<td>QMRA</td>
<td>Quantitative Microbial Risk Assessment</td>
</tr>
<tr>
<td>RMSE</td>
<td>Root Mean Squared Error</td>
</tr>
<tr>
<td>SARDI</td>
<td>South Australian Research &amp; Development Institute</td>
</tr>
<tr>
<td>TVC</td>
<td>Total Viable Counts</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
</tr>
<tr>
<td>ZTP</td>
<td>Zero Truncated Poisson</td>
</tr>
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</table>
List of Figures

1.1. Flowchart for overall pig production chain based on Cutler and Holyoake (2007) and Pointon et al. (2008) .......................................................... 8

1.2. Flowchart for Farm processes and transport based on Cutler and Holyoake (2007). 9

1.3. Flowchart of slaughter floor processes in Australian abattoirs based on Pointon et al. (2008). .......................................................... 12

2.1. Generalised growth curve for microorganisms depicting the lag, growth and stationary phases. ......................................................... 28

2.2. Dose-response model for Salmonella spp. of FAO/WHO (2002). The darker grey represents the 95% confidence interval for the model uncertainty and the lighter band represents the maximum and minimum bands on the model uncertainty. 45

2.3. Dose-response model for Salmonella spp. of USDA-FSIS (1998). The grey bands represent the 95% confidence interval on the model uncertainty. ............... 46

2.4. Comparison of dose-response relationships for Salmonella. Where uncertainty estimates of the parameter estimates are provided, the mean value was used for that parameter. The model of Teunis et al. (2010) is not included as it could not be reproduced in R. ......................................................... 48
3.1. *Salmonella* concentration after cooking ($\log_{10} \text{CFU/g}$) versus the final internal cooking temperature. Individual regression fits are presented for mince with a fat content of 2.99% and 12.35% fat. These two values represent the mean of the two distinct groups of fat content observed, those with less than 4% fat ($n = 12$) and those with greater than 10% fat ($n = 6$). .................................................. 62

3.2. *Salmonella* concentration after cooking ($\log_{10} \text{CFU/g}$) versus the final internal cooking temperature. The *Salmonella* concentrations at 4 °C represent the uncooked patties. For each *Salmonella* serovar, the regression model estimated is presented for the mean fat level (6.11%) accompanied by the notional concentrations estimated for each serovar. .................................................. 64

3.3. Temperature profiles for cooked burger patties. Temperatures were recorded every 90 seconds starting 90 seconds into cooking. The final temperature point is the final temperature recorded as the patty was removed from the skillet. . . . . 68

4.1. The fit of the growth model to *Salmonella* enumeration data for the four experiments where growth was observed. Observations removed from the data are indicated by triangles. ................................. 88

4.2. Comparison of growth rate fits obtained using the one-step and two-step fitting procedures and the data obtained from the ComBase database. ................................. 89

4.3. Comparison of the maximum population density fits obtained using the one-step and two-step fitting procedures. These fits are compared to the data obtained from the ComBase database, and associated model. ................................. 90

5.1. Flowchart showing the overall structure of the quantitative risk assessment model. Inputs to the model are depicted as trapeziums. Each stage of the supply chain modelled is depicted as a rectangle. ................................. 100
List of Figures

5.2. Tornado plot of the Spearman rank correlations between inputs of the model and the probability of illness in the variability dimension. For each input, the mean correlation, with 95% credible intervals are given. 115

5.3. Tornado plot of the Spearman rank correlations between the inputs of the model and the probability of illness in the uncertainty dimension. For nodes with variability and uncertain, correlations were calculated for the mean, standard deviation and 97.5% quantiles of the variability. 116

6.1. Tornado plot of the Spearman rank correlations between inputs of the model and the probability of illness in the variability dimension. For each input, the mean correlation, with 95% credible intervals are given. 152

6.2. Tornado plot of the Spearman rank correlations between the inputs of the model and the probability of illness in the uncertainty dimension. For nodes with variability and uncertainty, correlations were calculated of the mean, standard deviation, 75% and 97.5% quantiles of the variability. 153

6.3. Spider plot of the effect of changes to the transfer coefficients on the Salmonella prevalence. 154

6.4. Spider plot of the effect of changes to the transfer coefficients on the Salmonella concentration per gram. 154
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# List of Tables

1.1. The number of notified cases of disease or infections commonly transmitted by food products, 2008-2015. Diseases listed are ordered by the total notified cases in 2015. Source: Department of Health Australia (2016) .......................... 3

1.2. Reported pork and pork-associated outbreaks of foodborne illness in the food service sector where the hazard was identified in Australia. Based on Pointon (2016). .............................................................. 5

1.3. Pork carcase regulatory monitoring results for *Salmonella* spp. – overall prevalence and proportional occurrence of *S. Typhimurium* 193 and monophasic *Salmonella* spp. (Reproduced from Pointon (2016)) ................................. 15

2.1. Parameter estimates for the dose-response model presented by FAO/WHO (2002), including uncertainty estimates. ................................................................. 44

3.1. Sequence that each fat level and serovar combination was cooked. Nominal fat levels are the descriptions on the mince packages. ........................................ 54

3.2. *Salmonella* concentrations in raw and cooked patties with associated cooking times required to achieve the target internal endpoint temperature. Values given are the mean and standard deviation for each group of concentrations (log\(_{10}\) CFU/g) and times (minutes). Temperatures have been corrected based on the thermometer calibration results. ................................................................. 63

3.3. Analysis of deviance table for the inactivation of *Salmonella* in pork burgers. 64
3.4. Regression table for the inactivation model. All coefficients given in this table are in ln CFU/g. ................................................. 65

3.5. The correlation matrix for the eight parameters of the logistic regression component of the model. ............................................ 67

4.1. Nominal temperatures of incubators (°C) with the number of temperatures recorded for that incubator and the mean and standard deviation of those temperatures. 86

4.2. Growth model parameter estimates with associated standard errors, 95% confidence intervals (CI) for fits obtained using one-step and two-step fitting procedures. The fits obtained from the ComBase database are also provided for comparison. Asymptotic confidence intervals based on log-likelihood were calculated using the “confint2” function of the “nlstools” R package (Baty et al., 2015). ........ 87

5.1. Results from the scenario analysis. Ratio to baseline values are the ratio of the mean probability of illness for each scenario against the mean of the probability of illness for the baseline scenario. .................................................. 101

5.2. Concentrations of Salmonella (MPN/g) estimated on samples where Salmonella was detected. The limit of quantification was 0.3 MPN/g for the belly strip excision samples and 3 MPN/g for the trim samples. .................. 102

5.3. Consumer preference for pork products. .................................................. 111

5.4. Results from the scenario analysis. Ratio to baseline values are the ratio of the mean probability of illness for each scenario against the mean of probability of illness for the baseline scenario. .................................................. 117

5.5. Comparison of risk estimate to VLA/DTU/RIVM (2011) results for pork burgers. MS stands for member state of the European Union. .................. 118
5.6. Parameters used in the stochastic model. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions. 122

6.1. Scenarios explored to better understand relative risk, and most influential contributing factors, for salmonellosis from moisture-infused pork steaks in Australia. 135

6.2. Results from the scenario analysis for scenarios denoted by letters. Ratio to baseline values are the ratio of the scenario with the baseline for the prevalence and concentration estimates. 152

6.3. Results from the scenario analysis for numbered scenarios. Ratio to baseline values are the ratio of the mean probability of illness for each scenario against the mean of the probability of illness for the baseline scenario. 155

6.4. Parameters used in the infusion module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions. 161

6.5. Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions. 167
7.1. Comparison of estimates of the risk of salmonellosis from pork products per serving presented here and from VLA/DTU/RIVM (2011) for four European Union member countries. .......................... 174

A.1. Inactivation data for *Salmonella* in pork burger patties. Column headings are the same as those required by the R code in Listing A.1 ......................... 207

B.1. Data extracted from the ComBase database used to generate growth rate and maximum population density predictive models ......................... 217
1. Introduction

Global foodborne hazards caused an estimated 600 million cases of illness in 2010, of which 550 million were caused by infectious agents that cause diarrhoeal diseases (WHO, 2015). In Australia, circa 2010, an estimated 4.1 million cases of gastroenteritis were caused by contaminated food (Kirk et al., 2014a). Salmonellosis is the largest cause of notified foodborne illness annually in the US (CDC, 2015) and second largest cause in the EU (EFSA, 2013) and Australia (Department of Health Australia, 2016; OzFoodNet Working Group, 2012a). Cases of salmonellosis are also increasing annually in Australia (Department of Health Australia, 2016). Various food products have been implicated in salmonellosis outbreaks in Australia, including pork products (Delpech et al. 1998, Table 1.2). For these reasons, the Australian pork industry has invested in research since the 1990s into factors affecting Salmonella on pigs and pork between farm and fork (Coates et al., 1997; Hamilton et al., 2004; Hamilton et al., 2011. Hamilton et al., 2006; Hamilton et al., 2002, 1999). Changes in marketing directions by the Australian pork industry have raised questions about the safety of products and preparation/serving methods currently being promoted. Two products, pork burgers and moisture-infused pork, are non-intact products, in which pathogens, normally limited to the meat surface, may potentially be transferred to the interior of the product, thereby increasing the risk of salmonellosis compared to other pork serving methods.

Accordingly, risk assessments of pork burgers and moisture-infused pork steaks were commissioned by Australian Pork Limited to provide information for risk management decisions by industry. These risk assessments were to be conducted following the Codex methodology for

1.1. Hazard Identification

Salmonella is a rod-shaped, gram-negative bacterium that belongs to the family Enterobacteriaceae and is a pathogen of humans and animals. It is widely dispersed in nature and can be carried by mammals, reptiles, amphibians and birds, including livestock.

1.1.1. Epidemiology

In Australia, since the 1990s, salmonellosis has regularly ranked second in the annual number of notified cases of foodborne illness, with only campylobacteriosis ranked higher. In Table 1.1 are presented the number of notified cases of foodborne diseases, based on data from the Department of Health Australia (2016). In 2015, there were 17,015 notified cases of salmonellosis in Australia, at a rate of 72.7 cases per 100,000 population. Notification rates of salmonellosis are seasonal, with increases occurring during summer months. The general trend for notified cases of both campylobacteriosis and salmonellosis is increasing yearly. This compares to 7,439 cases of salmonellosis in the US during 2014 at a rate of 15.3 per 100,000 (CDC, 2014), 95,548 cases across 27 European Union member states at a rate of 20.7 per 100,000 inhabitants (EFSA, 2013), 1,122 cases of salmonellosis in Denmark at a rate of 19.9 cases per 100,000 (Anonymous, 2015). Comparing these rates directly should be made with care, however, as each may have different under-reporting rates (see below) and use different reporting methods.

In Table 1.1 only notified cases are included. There are various reasons why cases go unreported. People who experience only mild symptoms from foodborne diseases often do not seek medical...
1. Introduction

Table 1.1.: The number of notified cases of disease or infections commonly transmitted by food products, 2008-2015. Diseases listed are ordered by the total notified cases in 2015. Source: Department of Health Australia (2016)

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</tr>
</thead>
<tbody>
<tr>
<td>Campylobacteriosis</td>
<td>15,562</td>
<td>16,105</td>
<td>16,992</td>
<td>17,725</td>
<td>15,672</td>
<td>14,688</td>
<td>19,943</td>
<td>22,570</td>
</tr>
<tr>
<td>Salmonellosis</td>
<td>8,225</td>
<td>9,440</td>
<td>11,829</td>
<td>12,202</td>
<td>11,171</td>
<td>12,723</td>
<td>16,284</td>
<td>17,016</td>
</tr>
<tr>
<td>Shigellosis</td>
<td>830</td>
<td>617</td>
<td>552</td>
<td>493</td>
<td>547</td>
<td>538</td>
<td>1,049</td>
<td>1,096</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>275</td>
<td>563</td>
<td>267</td>
<td>145</td>
<td>166</td>
<td>190</td>
<td>231</td>
<td>178</td>
</tr>
<tr>
<td>Listeriosis</td>
<td>68</td>
<td>92</td>
<td>71</td>
<td>70</td>
<td>93</td>
<td>76</td>
<td>80</td>
<td>70</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>44</td>
<td>33</td>
<td>37</td>
<td>41</td>
<td>32</td>
<td>34</td>
<td>57</td>
<td>41</td>
</tr>
<tr>
<td>HUS</td>
<td>32</td>
<td>13</td>
<td>9</td>
<td>13</td>
<td>20</td>
<td>15</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>Botulism</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

HUS: Haemolytic-Uraemic Syndrome, caused by Enterohaemorrhagic E. coli.

treatment and if they do, diagnostic testing may not be considered necessary. In some cases, the results of diagnostic tests are not communicated to public health officials. Results of studies in the United States (Mead, 1999; Scallan et al., 2011) and Australia (Hall et al., 2008; Kirk et al., 2014a) allow estimates of the proportion of cases that go unreported each year, to enable a more accurate estimation of the true burden of salmonellosis in those countries. These studies found that the true number of cases of salmonellosis is greater than that reported by one to two orders of magnitude. Scallan et al. (2011) estimated there were approximately 1.02 million domestically acquired foodborne cases of salmonellosis in USA annually, which is around 24.5 times higher than the 41,930 estimated laboratory confirmed cases, circa 2006. In Australia, it was estimated that when taking into account under reporting and under diagnosis, circa 2010, there were 56,200 cases of salmonellosis of which 39,600 were foodborne (Kirk et al., 2014a).

1.1.2. Source Attribution

Salmonellosis contributes a large proportion of the total foodborne disease burden worldwide. In Denmark, pork (domestic and imported) was estimated to be the largest source of salmonellosis, representing 22.9% of laboratory-confirmed salmonellosis cases, followed by broilers (4.9%) and eggs (3%) (Anonymous, 2015). In New Zealand, pork was identified as the major source
of salmonellosis over three years, followed by poultry, beef and veal (Mullner et al., 2009). A recent source attribution study for the Australian state of South Australia estimated that, of the total cases of salmonellosis, 2.5% could be attributed to porcine sources, ovine 6.7–7.4%, chicken 34.6–28.9%, eggs 37.14–44.3% and 13.4% is of unknown origin (Glass et al., 2016). It appears that in contrast to other countries, pork is estimated to have a relatively low source of foodborne salmonellosis in Australia compared to other food products. It is important to note with each of these source attribution studies that commonly consumed foods in each country will tend to cause more outbreaks but the absolute risk per serving for that food may be low.

1.1.3. Outbreaks Associated with Pork Products

Information on the outbreaks of foodborne illness that have been attributed to pork products in Australia is presented in Table 1.2. Only one outbreak described was attributed to a pathogen other than *Salmonella*, highlighting *Salmonella* as the foodborne hazard of most significance to the pork industry.

1.1.4. Differences between *Salmonella* serovars

*Salmonella* spp. can be grouped (serotyped) based on their surface and flagellar antigens using the White-Kauffmann-Le Minor scheme (Grimont and Weill, 2007). Only some *Salmonella* serovars are routinely associated with foodborne illness, and of those, some serovars are more regularly associated with certain foods (Glass et al., 2016; Table 1.2). OzFoodNet Working Group (2015) noted that of the 12,271 cases of salmonellosis in 2011, 48% were caused by *S*. *Typhimurium*. There also appears to be a shift in serovars commonly detected in Australia. Since 2005–2006, there has been an increase in *S. enterica* Serotype 4,[5],12:i:- among human, bovine and porcine isolates (Pointon, 2016). This shift also appears to be occurring in the serovars attributed to human salmonellosis in Australia, with outbreaks from the last few years being attributed to *S. enterica* 4,[5],12:i:-, whereas earlier outbreaks were largely associated with *S. Typhimurium*
Table 1.2: Reported pork and pork-associated outbreaks of foodborne illness in the food service sector where the hazard was identified in Australia. Based on Pointon (2016).

<table>
<thead>
<tr>
<th>Year</th>
<th>Product</th>
<th>Hazard</th>
<th>Cases</th>
<th>Evidence*</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009</td>
<td>Uncooked pork sausage</td>
<td><em>S. Anatum</em></td>
<td>5</td>
<td>D</td>
<td>South Australian Health (2010)</td>
</tr>
<tr>
<td>2010</td>
<td>BBQ pork</td>
<td><em>S. Typhimurium</em> 204</td>
<td>4</td>
<td>M</td>
<td>OzFoodNet Working Group (2012a)</td>
</tr>
<tr>
<td>2011</td>
<td>Spit roast pig</td>
<td><em>S. Typhimurium</em> 135</td>
<td>5</td>
<td>D</td>
<td>OzFoodNet Working Group (2011)</td>
</tr>
<tr>
<td>2011</td>
<td>Home-made pork salami</td>
<td>*S. subsp 1 ser 4,[5],12:i:- PT193</td>
<td>4</td>
<td>D</td>
<td>OzFoodNet Working Group (2012b)</td>
</tr>
<tr>
<td>2014</td>
<td>Roast pork</td>
<td>*S. subsp 1 ser 4,[5],12:i:- MLVA profile 04-15-12-00-490</td>
<td>8</td>
<td>M</td>
<td>South Australian Health (2014, p. 24)</td>
</tr>
<tr>
<td>2014</td>
<td>Spit roasted pork</td>
<td>*S. subsp 1 ser 4,[5],12:i:- MLVA profile; 04-15-12-00-490</td>
<td>18</td>
<td>D</td>
<td>South Australian Health (2015, pp. 25, 29)</td>
</tr>
<tr>
<td>2015</td>
<td>Spit roasted pork and lamb</td>
<td>*S. subsp 1 ser 4,[5],12:i:-</td>
<td>6</td>
<td>D</td>
<td>South Australian Health (2015, pp. 28, 29)</td>
</tr>
</tbody>
</table>

*A = analytical epidemiological association between illness and vehicle, D = descriptive evidence implicating vehicle, M = microbiological confirmation of aetiology in vehicle and cases, C = Cohort study; CC = Case-control study.

**S. Typhimurium 170 and 108 reported as the same organism.

NA – Not available
1.2. Hazard Characterisation

All serovars of *Salmonella* can cause non-typhoidal salmonellosis upon ingestion but only some serovars have been associated with foodborne illness, and of those, some serovars are more frequently associated with certain foods, with the exception of *S. Typhi* and *S. Paratyphi A* (FDA, 2012), which result in Typhoid Fever. Symptoms of salmonellosis include nausea, vomiting, abdominal cramps, diarrhoea, fever and headaches, with the onset of symptoms occurring between 6 and 72 hours after ingestion, with symptoms usually lasting between four and seven days (FDA, 2012). Invasive non-typhoidal salmonellosis symptoms do not necessarily include diarrhoea, though they do include bloodstream infections (Ao et al., 2015). In Australia, salmonellosis results in an estimated 2,100 hospitalisations and 15 deaths, with salmonellosis estimated as the joint largest cause of foodborne deaths alongside listeriosis (Kirk et al., 2014a). Salmonellosis can also cause sequelae, including in Australia an estimated 3,500 cases of Irritable Bowel Syndrome and 3,250 cases of Reactive Arthritis, based on 2010 data (Ford et al., 2014). The elderly, young and immuno-compromised are at most risk of serious complications from salmonellosis. Treatment can be hindered if the pathogen is resistant to the antimicrobials used, with some antimicrobials now not considered in salmonellosis treatment (Klochko, 2016). Non human usage of antimicrobials has been linked to adverse human health outcomes. With increasing treatment failure rates and increased severity of diseases, significant attention has been placed on managing the non-human usage of antimicrobials (FAO/OIE/WHO, 2003; 2004; FAO, 2016; WHO, 2011). Not all people that consume a quantity of *Salmonella* will become ill, because the probability of salmonellosis is linked to the number of organisms consumed and the specific physiology and health status of the consumer. Knowledge of this dose-response relationship, and variables affecting it, is required to complete a hazard characterisation and this relationship is considered
1. Introduction

in Section 2.3.

1.3. Pork Production, Supply and Consumption

Many factors influence the prevalence and concentration of *Salmonella* in the supply chain and consumption of pork. Knowledge of these factors is required to accurately describe the changes to product contamination, for example, as part of a risk assessment model. Production and consumption can be broken up into the steps outlined in Figure 1.1 and explained below.

1.3.1. Farm

The following overview of farm practices is based on Cutler and Holyoake (2007). The farm is the first step in production and supply. At the farm, pigs are born, weaned and grown until they reach market weight, a process typically taking 5–6 months. A flowchart of this process is presented in Figure 1.2. Weaning, where the piglets are transitioned from their mother’s milk to feed, typically occurs around two to four weeks from birth. Weaners are placed into a pen with between 15 and 1000 other weaners, ranging in weight from 6 to 25 kg, and provided a feed specifically designed for weaners. After eight to ten weeks, pigs are moved to a ‘grower’ facility, with between 5 and 200 pigs and their feed is changed to a grower formulation. Males and females are normally separated at this stage. The final stage is called ‘finishing’, where the diet is changed to one with a lower energy content (Morgan, 2013) to reduce fat deposition (Carter et al., 2008).

Pigs are normally housed in one of two types of housing: traditional buildings or eco-shelters. Traditional buildings consist of partially slatted or fully slatted floors, which allow urine to pass through and faeces to be trodden through the floor. This style of building allows the waste products to be sent to an effluent pond, which allows for methane capture. Eco-shelters use straw or sawdust to capture the effluent as a solid product, which is then removed from the eco-shelter after the pigs are removed from the shed.
Figure 1.1.: Flowchart for overall pig production chain based on Cutler and Holyoake (2007) and Pointon et al. (2008)
Figure 1.2.: Flowchart for Farm processes and transport based on Cutler and Holyoake (2007).
Pigs in either housing type can be managed in either an ‘all-in all-out’ (batch) system or a continuous system. The advantage of managing pigs in batches is that, under ideal circumstances, housing can be pressure cleaned, disinfected and allowed to dry before the next batch of pigs is added. This reduces the chances of diseases being transferred between batches through the housing. Continuous systems require cleaning with the structure partially populated.

Since the early 1990s, Australian pig production has shifted from small farms with fewer than 100 sows to larger farms with 1000 or more sows (Pointon et al., 2008). From 1994 to 2003, the proportion of the breeding stock held by large producers increased from 31% to 52%. In this same period, the proportion of breeding stock held by smaller producers fell from 30% to 12% (Pointon et al., 2008). In 2012, 63% of Australian pig production was attributable to farms with greater than 1000 sows while the proportion of production attributable to small producers (<100 sows) was only 7% (Piazza Research Pty Ltd, 2012).

There has also been a shift since the early 1990s from ‘farrow-to-finish’ production, where the animal is born, weaned, grown and finished on the same farm, towards multi-site production. This latter style of farming can consist either of a farmer who owns multiple farms and transports livestock between various locations, or contract farming, where some farmers specialise in raising pigs of a specific age. Transportation of pigs is often required between the multiple sites, the significance of which is discussed in Section 1.3.2.

### 1.3.2. Transport

Once pigs reach market weight, they are transported by truck to the abattoir. The transportation of livestock in Australia poses greater challenges than in other nations due to the distances between farms and abattoirs. Piazza Research Pty Ltd (2010) found that the average journey length for a pig travelling to the abattoir was 3.3 hours, with standard deviation of 2.5 hours, with some pigs travelling in excess of 6 hours. Transportation causes stress to pigs, resulting in *Salmonella* shedding (cross contamination of *Salmonella* between pigs via faeces and contact) and increasing
prevalence after transport, with factors including transport duration and time-off-feed associated with this increase (Hamilton et al., 2002; Williams and Newell, 1970).

On arrival at the abattoir, pigs are unloaded into the lairage, where they are given a spray wash and an ante-mortem inspection is performed to detect any pigs that require special processing. Animals that are severely ill or injured may require emergency slaughter. Depending on their condition, they will be sent to the main slaughter line immediately or sent for rendering if deemed unfit for human consumption.

1.3.3. Abattoir

The usual processes that occur during slaughter are outlined in Figure 1.3 and are described below, based on Pointon et al. (2008). The design of an abattoir varies depending on the size of the abattoir and its throughput. Larger abattoirs will be more automated, requiring less manual handling of the carcase, while smaller abattoirs will involve more manual operations, with workers performing many processes on each carcase. The processes that occur in Australian abattoirs are similar to those that occur in most skin-on, international abattoirs.

Once ready for slaughter, pigs are moved inside the abattoir and stunned by either a low voltage electrical current or, in most modern abattoirs, ‘stunning’ by exposure to very high concentrations of carbon dioxide. Bleeding is then performed via a small stick wound to the throat. Pigs in medium to large abattoirs are then hung by either the Achilles tendon or aitchbone to a roof mounted conveyor system. Aitchbone hanging improves the eating quality of pork (Channon et al., 2014).

Once bled, carcases are scalded to soften the hair by passing the carcase through a scalding tank filled with water heated to 60 °C for around eight minutes, or through a steam cabinet.

The now softened hair is removed in a dehairing machine, which consists of rubber flails that scrape the hair and skin debris from the carcase, which is removed from the carcase with recirculated wash water. This process is not completely effective and some hair normally remains.
1.3. Pork Production, Supply and Consumption

Figure 1.3.: Flowchart of slaughter floor processes in Australian abattoirs based on Pointon et al. (2008).
1. Introduction

Carcases are then passed through a singeing machine, consisting of numerous gas burners that apply flames at 1200 °C to the skin of the carcase. This process removes any remaining hair. Some abattoirs apply a manual singeing process to the carcases, where areas known to be missed by the automated process are singed. Small abattoirs perform this process manually with a hand blowtorch.

‘Polishing’ is then performed in all but the smallest abattoirs. Carcases are passed, over a period of around five minutes, through a series of flails and stiff brushes, designed to remove carbonised/dried dirt, dried hair or loose remnants of the carcase.

The anus is separated from the carcase, through ‘bunging’, which allows the internal organs to be removed. A plastic bag placed over the anus or a plug in the anus is used by some abattoirs to reduce faecal leakage. An excision is made around the intestinal tract before being passed back into the pelvic cavity. The abdominal cavity is then opened by a knife by either a robot or worker. Once open, the pluck (organs from the thorax) is removed, the abdominal viscera removed for inspection and the edible offal processed separately. This process needs to be performed with care to prevent the puncturing of the gut, which can lead to contamination of the carcase with faecal material, potentially including enteric pathogens, and condemnation.

Carcases are then divided in half by saw, and the sides inspected to ensure that they are fit for human consumption. Unfit sides that contain gross abnormalities, including abscesses, lesions on organs, lymph node inflammation (Pointon et al., 2000), are either deemed as recoverable for animal food or pharmaceutical materials, or condemned. Some sides with visually detectable abnormalities are passed to the retain rail, where the abnormalities are excised and sides returned to the main production line.

While not currently performed in Australia, a microbial decontamination step, e.g. hot water and/or disinfectant, can be performed at this stage to lower microbial load on sides. Such interventions have been investigated for use in Australia (Hamilton et al., 2010). Some plants in Australia do perform a cold or warm water rinse to remove sawdust from carcasses, which may
have a microbial decontamination effect.

Sides are then taken into a chiller where they typically remain overnight before boning. Blast chilling is not currently performed in Australia. Chilling reduces the temperature of the sides to a safe level that precludes growth of bacterial pathogens, though cooling rates can vary widely in the cooler, with some sides exposed to temperatures close to zero and some to temperatures around 10 °C. Some sides are sent directly to retail butchers, who perform the boning process in-house, as described in Section 1.3.4, below.

*Salmonella* serovars detected on carcases in Australia by year are outlined in Table 1.3. Detections of *S. Typhimurium* 193 or monophasic *Salmonella* have only occurred since 2007.

### 1.3.4. Boning

During boning, pork sides are transformed into retail portions. The boning room consists of a series of conveyor belts that move pork portions between operators, who have a specific task to perform on each portion that reaches their work area. Cross contamination of microbes can occur inside the boning room between pork portions and the conveyor belts, knives, gloves and other surfaces that make contact with the pork. This contamination can then be spread to other pork portions. Knives are sterilised in hot water at breaks in production and the whole boning room, including knives and mesh gloves, are cleaned down at the end of the day with disinfectant (Pointon et al., 2008). Surfaces are not disinfected during breaks in production, which is in contrast to the practices of some red meat boning rooms and may increase the risk of cross contamination via these surfaces (D. Hamilton, 2016, South Australian Research and Development Institute, Pers. Comm.).

#### 1.3.4.1. Processing

Some pork products undergo further processing before retail, including smallgoods and moisture-infused pork. Moisture infusion pork is injected with a brine solution to increase the juiciness of
Table 1.3: Pork carcase regulatory monitoring results for *Salmonella* spp. – overall prevalence and proportional occurrence of *S. Typhimurium* 193 and monophasic *Salmonella* spp. (Reproduced from Pointon (2016))

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
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<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Typhimurium</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>S. Typhimurium</em> 193</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> monophasic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other isolates**</td>
<td>6</td>
<td>27</td>
<td>17</td>
<td>32</td>
<td>20</td>
<td>14</td>
<td>13</td>
<td>12</td>
<td>15</td>
<td>15</td>
<td>7</td>
<td>14</td>
<td>22</td>
<td>12</td>
</tr>
<tr>
<td>Total number</td>
<td>7</td>
<td>28</td>
<td>18</td>
<td>32</td>
<td>23</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>18</td>
<td>17</td>
<td>8</td>
<td>19</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td>No. of samples</td>
<td>586</td>
<td>1023</td>
<td>1147</td>
<td>1315</td>
<td>1284</td>
<td>1031</td>
<td>1066</td>
<td>1018</td>
<td>1051</td>
<td>987</td>
<td>891</td>
<td>1074</td>
<td>1025</td>
<td>858</td>
</tr>
</tbody>
</table>

*Excluding *S. Typhimurium* 193 and monophasic *Salmonella* spp.

**Most commonly isolated over this period in descending order are *S. London*, *Derby* and *Infantis.*
cooked pork and also to improve the eating qualities of cooked pork (Moore et al., 2012). During the infusion process, pork primals are placed under a grid of needles, which pierce the primals and inject a brine solution to the interior of the product. Common brine ingredients include sodium chloride, to enhance flavour, increase shelf life and increase water holding capacity (the ability of the meat to retain water under the influence of gravity and cooking) and sodium phosphates, used to increase the water holding capacity and antioxidant properties of the pork. Sodium and potassium lactate, sodium acetate, lemon juice, organic acids and other flavourings may also be included (APL, 2009). A potential hazard with moisture-infused pork is that the injection process may introduce pathogens to the interior of the product via the injected brine.

1.3.5. Supply Chain

1.3.5.1. Distribution

There is limited research describing the supply chain for pork products between the boning room and retail. Australian pork products can visit multiple sites between boning and retail, including commercial butchers that further process pork products and distribution centres that organise distribution of products to retailers. This process may result in products being transported interstate, lengthening Australian supply chains considerably and potentially increasing the risk of pathogen growth during distribution (Hamilton et al., 2012).

1.3.5.2. Retail

Australian pork products are sold at retail by two types of retailers: supermarkets or butchers. Supermarkets either have products sent to them pre-packaged or they have butchers on-site who portion the meat. Product sold by butchers is mostly portioned on-site. Pork is then displayed for retail sale in refrigerated cabinets.
1. Introduction

1.3.5.3. Consumer Transport

Once purchased from the retailer, consumers transport pork to their homes. Temperature abuse can occur during this time if product is left unrefrigerated, e.g. in cars, which can be exacerbated by periods of high ambient temperature, when internal spaces in the car can become very warm. Product conditions during transport by car have been examined previously in South Korea by Kim et al. (2013), who found that car ‘trunks’ (or ‘boots’) were, on average, warmer than the outside ambient temperature, due to solar absorption by the trunk (or boot) lid. These periods of temperature abuse may have the potential to increase pathogens levels on foods due to microbial growth.

1.3.5.4. Domestic Storage

Pork is usually stored in domestic refrigerators until cooking but may be cooked immediately on arrival at the home. Temperature abuse of products can occur if the door of the refrigerator is left open for extended periods, or if the product is left at ambient temperatures for extended periods prior to storage in the refrigerator, or during defrosting cycles. Raised refrigerator temperatures can also occur if the temperature control in the refrigerator is not set to the correct temperature, i.e. 4 °C.

1.3.5.5. Cooking

Pork is then cooked before consumption, with cooking usually resulting in the death of pathogens that may contaminate the product. Foods in which pathogens can be internalised require more thorough cooking than foods with surface-only contamination (Van, 2011) to ensure that potentially internalised pathogens are inactivated. Pork products with potentially internalised pathogens include pork burger patties, rolled pork roasts and moisture-infused pork due to the grinding of the pork, the rolling of the roast and the injection of the pork with brine, respectively.
1.4. New Industry Marketing Initiatives and Need for Risk Assessment

At the time this work was conducted, the Australian pork industry was marketing a number of products to consumers that were either less commonly consumed, or were considered as ‘value added’ products, in which ingredients are added to increase the quality of the end-product. For this reason, industry risk managers commissioned this thesis, in which, the risk posed by pork burgers and moisture-infused pork steaks is assessed. Both of these products are non-intact, with pathogens potentially internalised. There are gaps in the literature for assessment of public health risk for each of these products. The first of these gaps involves the ability to predict the survival of Salmonella in pork burgers after cooking. The second gap concerns Salmonella growth in moisture-infused pork steaks because the addition of salt in injected brine may retard the growth of Salmonella in this product and ameliorate the increased public health risk from the internalisation of pathogens.

1.5. Thesis Aims

This thesis aims to assess quantitatively the risk of salmonellosis from the consumption of pork and pork products in Australia. To this end, two quantitative microbial risk assessments have been conducted for salmonellosis from the consumption of pork burgers and for moisture-infused pork steaks. These assessments will answer the questions:

1. What is the risk of salmonellosis from the consumption of pork burgers and moisture-infused pork in Australia?

2. What factors have the greatest influence on the risk?

3. What is the expected effect of changes to current production and consumer practices on the risk?
1. Introduction

1.6. Thesis Structure

This thesis comprises seven chapters with the first chapter an Introduction to the thesis and review of salmonellosis and pork meat production, processing and distribution. The second chapter outlines methods and concepts required to conduct a quantitative microbial risk assessment. Chapters 3 and 4 describe experiments addressing significant identified data gaps which needed to be filled before robust risk assessments could be conducted. Risk assessments of salmonellosis in pork burgers and moisture-infused pork are presented in Chapters 5 and 6, followed by a discussion and summary of the overall significance and contribution of the thesis results to the body of knowledge on this topic.

For this thesis, only changes to *Salmonella* prevalence and concentration post-boning are considered. While a recent risk assessment model (VLA/DTU/RIVM, 2011) could be adapted to estimate the risks posed by infused pork chops and pork burgers in Australia, much of the data required is not available for Australia. That model also does not consider the effects of moisture infusion on salmonellosis risk from pork consumption.
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2. Methods

In this Chapter, tools, methods and models required to perform quantitative microbial risk assessment are presented. Risk assessments are performed in a variety of fields where minimising the risk of a negative event is of interest, including workplace health and safety, finance, natural disasters etc. Types of microbial risk assessments that can be conducted, frameworks that exist for conducting risk assessments, as well as concepts pertaining to growth and inactivation of pathogens in foods and dose-response modelling for the probability of illness upon enteral exposure to Salmonella are summarised and discussed.

2.1. Microbial Risk Assessment

The microbiological risk assessment process as described by Codex (Codex Alimentarius Commission, 1999) first requires ‘Hazard Identification’, where the microbiological agents capable of causing adverse health effects are identified and characterised, as well as evidence of their association with the food of interest. This step is then followed by ‘Hazard Characterisation’, where the health effects associated with a hazard are assessed, either qualitatively or quantitatively. ‘Exposure Assessment’ is then performed to determine, either qualitatively or quantitatively, the frequency and amount of consumption (‘dose’) of the hazard(s) identified, including estimates of the changes that occur over time, in the food, before consumption. To explain, data about pathogen loads and frequency at the time of consumption are rarely available and have to be inferred from data available at some earlier point in the product’s history and the microbial
2.1. Microbial Risk Assessment

ecology of the food, including expected changes in microbial numbers over time and the changes in the product environment of the product. Finally, ‘Risk Characterisation’ determines, qualitatively or quantitatively, the probability of occurrence and severity of adverse health effects. This step combines information from the previous three steps and also captures information about the uncertainties that are inherent in the data and assumptions of the models used in the risk assessment.

Trade barriers were used historically, in part, to protect domestic industries from cheaper imports from developing nations, and restricting developing nations from trading internationally and growing their economies (FAO/WHO, 1997). The rules that govern international trade and which apply to members of the World Trade Organisation (WTO) are the agreements on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) and Technical Barriers to Trade (TBT Agreement). These rules allow countries to impose trade barriers only to protect the lives and health of their citizens, or their domestic flora and fauna, but not for domestic economic reasons, sometimes termed ‘protectionism’. Codex Alimentarius Commission standards and guidelines reflect international consensus on how to best manage foodborne hazards. Risk-based standards are developed with advice from international expert panels convened by the Food and Agriculture Organisation and the World Health Organisation of the United Nations, under the auspices of the Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment (JEMRA) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA). These expert bodies are convened specifically to provide science-based guidance to Codex committees but their deliberations are usually made publicly available.

Risk assessment is one of three elements in the ‘risk analysis’ paradigm as described by Codex Alimentarius Commission (1999), the other elements being ‘risk management’ and ‘risk communication’. The information and insights that are generated from a risk assessment should support risk managers in their decision-making processes. The overall risk analysis approach is believed to offer social, political and economic benefits (FAO/WHO, 2009). Firstly, the approach allows risk managers to implement strategies to reduce the risk of illness to society, based on the findings
of a risk assessment. The approach also provides information that can be used by regulatory bodies to make informed, science-based, market access decisions. Economic benefits also accrue because interventions can be targeted towards harvest, production, distribution or food preparation process with the greatest influence on risk, thereby maximising the benefits of the costs incurred in risk management. While the risk assessment approach is often dependent upon limited and inadequate data, and relies on assumptions about the system being studied, the approach promotes transparency to allow for informed decision-making and subsequent refinement as more data and knowledge are made available. Due to their mathematical complexity, full expert peer review of risk assessments can be difficult. A full risk assessment can also over-complicate some risk management questions, where a simpler approach can provide the desired answers without unnecessary complexity (see Section 2.1.1).

### 2.1.1. Risk Assessment Approaches

Risk assessments can be conducted at three levels of complexity, either qualitatively, semi-quantitatively or fully quantitatively. A short description of these three types is provided here, based on information by FAO/WHO (2009).

Qualitative risk assessments are the simplest way of assessing risk, resulting in descriptive measures of risk such as ‘negligible’, ‘very low’, ‘low’, ‘medium’ or ‘high’. This type of risk assessment usually requires the shortest duration to complete compared to quantitative methods and can be used to rank potential hazards, and associated risks, but not to quantify them. These assessments can also be used to assess the need for a quantitative risk assessment. These risk assessments rely on subjective descriptions of quantities and often involve expert opinions, requiring risk assessors to combine these subjective descriptions together to infer a risk description.

Semi-quantitative risk assessments use scores instead of descriptors or primary data to evaluate risks. These models require less mathematical skills to be able to conduct than a fully quantitative
2.1. Microbial Risk Assessment

(stochastic) risk assessment and require less data to complete. These assessments provide a middle ground between the fully-quantitative and qualitative approach. A framework in Microsoft Excel exists for conducting semi-quantitative risk assessments (Ross and Sumner, 2002) and other generalised frameworks also exist (DAFF, 2011). Another platform for conducting semi-quantitative risk assessments is iRisk (FDA/CFSAN et al., 2015), which provides a web-based interface for development of models and extends the concepts introduced by Ross and Sumner (2002).

Fully quantitative risk assessments use numerical information and stochastic modelling approaches to estimate the risk to consumers and are the most mathematically complex and difficult to construct. Numerical estimates can be based on relevant data, meta-analysis of data, and expert opinion and can combine these sources to strengthen the certainty around these opinions. Fully-quantitative risk assessments produce a probability estimate for the mean probability of the event of interest occurring, including estimates of credible intervals/probability of specific outcomes of interest. While semi-quantitative risk assessments can also estimate the probability of illness, estimates produced through fully quantitative risk assessments will be of greater accuracy. The approach also allows changes in current supply chain and consumer behaviours to be examined through ‘what if’ scenarios, estimating the mean risk based on the changes investigated, e.g. by simulating a change in only one factor at a time and assessing the consequence of that change. These methods also allow for ‘sensitivity analysis’, in which the influence of each input in the risk assessment model on the final risk estimate can be quantified. Results of sensitivity analyses can be used by risk managers to best allocate available resources to reduce risk to consumers.

For some risk analysis questions, it is not necessary to model the complete farm-to-fork system, nor to adopt the entire four stage risk assessment paradigm proposed by FAO/WHO and adopted by CAC. For instance, for food products where an inactivation step is not performed, an exposure assessment is sufficient to inform risk managers about areas of the supply chain with the greatest influence on risk. Similarly, many risk management strategies are based on reducing the risk to consumers by a certain amount or proportion, e.g. reducing the initial concentration by 50% to
2. Methods

reduce disease incidence. Even without consideration of the Hazard Characterisation component, that required amount of risk reduction can be apportioned to different stages in the farm-to-fork continuum so that the overall Food Safety Objective is achieved. Methods similar to these have been used in previous risk assessments (FAO/WHO, 2004).

2.1.2. Variability, Uncertainty and Confidence in Model Predictions

In risk assessment models, variation in model parameters can be categorised as either ‘variability’ or ‘uncertainty’ (see FAO/WHO, 2008, Chapter 5). Variability represents the differences between each serving consumed, including differences between individuals (serving preferences etc.) and random processes (storage durations etc.). Uncertainty captures, qualitatively or quantitatively, our lack-of-knowledge of the system, including measurement error. In some instances, uncertainty might be reduced by increased observation of the system (Vose, 2000) or found to be due to genuine, inherent variability in the data, which may be irreducible without increased complexity of the model. Increased data collection can also reduce variability, in some instances, or further increased our confidence in the magnitude of the variability (Vose, 2000). In a risk assessment, distinction needs to be made between variability and uncertainty to allow the uncertainty to be reported accurately. Due to these concepts being linked, the complete separation of these two concepts is difficult, with some variability labelled as uncertainty and vice versa. Problems can occur in predictions if these two concepts are combined in risk assessment (Nauta, 2000).

2.1.3. Quantitative Risk Assessment Tools

Many ‘add-ins’ for Microsoft Excel are available that allow risk assessment stochastic models to be constructed and evaluated. Vose (2014) reviewed these add-ins, comparing the features and benefits of ‘@Risk’, ‘Crystal Ball’, ‘ModelRisk’ and other add-ins. As most researchers are familiar with the use of Microsoft Excel, these tools simplify the construction of a variety of risk assessment models. These software packages are also well supported, either by other users of
the add-ins or by their developers. These packages, however, are often commercial, requiring a licence for their use and other users sometimes cannot use and evaluate models created using these add-ins without purchase of the add-in itself. These add-ins make it difficult to separate variability and uncertainty and report each independently.

Julia (Bezanson et al., 2014, 2012) is a relatively new programming language that has been designed to provide performance benefits compared to other, more mature programming languages. The first version of the language was released in 2012. The language is free to use and extend, with user-contributed packages implementing many specialist functions. While this language provides promise, its immaturity and lack of specific tools for implementing risk assessment models makes its use for risk assessment challenging.

R (R Core Team, 2016) is a programming environment that can be used to implement risk assessment models. Like Julia, R has a large number (approaching 9,000) of user-contributed packages that have been designed to extend the capabilities of the language. The ‘mc2d’ package (Pouillot and Delignette-Muller, 2010) provides a framework for implementing risk assessment models in R and allows models to be constructed in two dimensions and hence allows separation of variability and uncertainty. This package has been used in previously published risk assessments (Brookes et al., 2014; Pouillot et al., 2012; Vásquez et al., 2014).

Based on the above information and considerations, R with the ‘mc2d’ package was chosen as the best tool to construct the risk assessment models. It has the largest range of user-contributed packages and has tools available for constructing risk assessment models in two dimensions and reporting their results.

### 2.2. Predictive Microbiology

Predictive microbiology is a field of microbiology that deals with estimating changes in microbial populations in foods due to the environmental changes over time via mathematical models. The
2. Methods

field covers the growth and inactivation of specific microbial populations in foods, as well as determination of combinations of factors that allow, or preclude, growth. These models and their development have gained prominence due to the difficulties, including expense and time, in directly measuring these changes in microbial populations in foods in real time. Predictive microbiological models also have utility in risk assessment models. In this section, a precis of predictive microbiology models and methods are presented. Many reviews and monographs on predictive microbiology have been written (FAO/WHO, 2008; McKellar and Lu, 2004; Pérez-Rodríguez and Valero, 2013; Ross and McMeekin, 1994; Ross and McMeekin, 2003; Whiting, 1995) and readers are directed to these for more information about this field.

Pathogen growth and inactivation can potentially occur at many points in the pork supply chain. Factors that are reported to influence pathogen growth and inactivation rates include temperature, pH, water activity, and differences between serovars. The range for growth for one environmental factor can be reduced if another environmental factor is not optimal. Mathematical models have been developed to estimate the change in pathogen concentrations over time, for a given set of environmental conditions, based on experimental data (the richest source of which is ComBase, see Section 2.2.4). These models normally are based on two equations: the ‘primary model’ and the ‘secondary model’ which, when combined and integrated into computer software, can be described as the ‘tertiary model’ (Whiting and Buchanan, 1993). The primary model describes microbial concentration as a function of time. It describes lag phase duration (LPD, the time required for pathogens to adjust to their new environment and begin multiplying), exponential or ‘specific’ growth rate (GR, the maximal rate at which the log10 population of microorganisms is increasing) and maximum population density (MPD, the maximum concentration of microorganisms that can be sustained by the foodstuff). The ‘secondary model’ describes either the lag phase duration, growth rate or maximum population density as a function of one or more environmental factors. Primary and secondary growth models can also be used to describe pathogen inactivation. Primary models for inactivation describe the lag time before inactivation commences, the exponential rate of inactivation and, in some cases,
2.2. Predictive Microbiology

Figure 2.1.: Generalised growth curve for microorganisms depicting the lag, growth and stationary phases.

The maximum level of inactivation achieved (if ‘tailing’ occurs). A generalised growth curve is depicted in Figure 2.1. In this section, we talk about data sources and models used in predictive microbiology.

2.2.1. Primary Growth Models

Primary growth models describe the change in the number of organisms over time resulting from growth. Many mathematical models have been proposed to describe the growth curve but the three most widely-adopted primary growth models are the modified-Gompertz, Baranyi and the Buchanan ‘three-phase linear’ models. The parameterisations of these models presented below include both the lag phase and the stationary phase though they can be parameterised without either one, depending on the available data. In the following model definitions, CFU/unit is used to denote both CFU/g and CFU/cm², as appropriate.
2. Methods

2.2.1. ‘Modified-Gompertz Model’

The Gompertz model was not originally designed for the purpose of modelling the growth of microorganisms and this is used by some as an argument against its use for growth modelling. Nevertheless, the history of this model in predictive microbiology is long (Ross et al., 2003). The ‘modified-Gompertz’ model that is most widely used in predictive modelling is the parameterisation due to Zwietering et al. (1990):

\[
\ln \left( \frac{N}{N_0} \right) = A \exp \left\{ - \exp \left[ \frac{\mu_{\text{max}} \times \exp(1)}{A} (\lambda - t) + 1 \right] \right\} \tag{2.1}
\]

where \( N_0 \) is the initial concentration at time \( t = 0 \) (\( \log_{10} \) CFU unit\(^{-1} \)), \( N \) is the concentration at time \( t \) (\( \log_{10} \) CFU unit\(^{-1} \)), \( A \) is the asymptotic level \( \ln \left( \frac{N_{\infty}}{N_0} \right) \) (\( \log_{10} \) CFU unit\(^{-1} \)), \( \mu_{\text{max}} \) is the maximum specific growth rate and \( \lambda \) is the lag phase duration (time). This equation does not estimate the absolute numbers of microorganisms but provides the relative change from the original population. Subsequent parameterisations exist, with one provided in the ‘nlsMicrobio’ R package (Baty and Delignette-Muller, 2014) in a more readily usable format:

\[
N = N_0 + (N_{\text{max}} - N_0) \exp \left\{ - \exp \left[ \frac{\mu_{\text{max}} e^{(\lambda - t)}}{(N_{\text{max}} - N_0) \ln (10)} + 1 \right] \right\} \tag{2.2}
\]

where \( e \), \( t \), \( N \) and \( N_0 \) have the same meaning as in Eqn. 2.1 and \( N_{\text{max}} \) is the concentration of organisms at stationary phase (\( \log_{10} \) CFU unit\(^{-1} \)). This parameterisation allows calculation of the actual concentration at time \( t \), not the relative population.

The Gompertz model is not symmetrical and estimates a more gradual transition from the exponential growth to maximum population phases compared to the transition from lag phase to growth. This transition is also more gradual compared to the Baranyi model (described in Section 2.2.1.2). It also systematically over-predicts the exponential growth rate by approximately 13\% (Dalgaard et al., 1994; Membré et al., 1999; Ross, 1993a; Whiting and Cygnarowicz-Provost, 1999).
1992) compared to estimates based on the assumption of constant exponential growth rate during
the growth phase, as is expected from theory.

2.2.1.2. Baranyi ‘D-model’

This eponymous model developed by Dr. József Baranyi was proposed as an alternate growth
model based on the idea of a constant rate of exponential growth (Baranyi et al., 1993a; Baranyi
and Roberts, 1994; Baranyi et al., 1993b). This model, when fully articulated in the literature,
is given as a series of differential equations (see Baranyi and Roberts (1994) and Velugoti et
al. (2011)). The model has also been included by Baty and Delignette-Muller (2014) in the
‘nlsMicrobio’ R package as

\[ N = N_{\text{max}} + \log_{10} \left\{ \frac{\exp (A\mu_{\text{max}}) + \exp (t\mu_{\text{max}}) - 1}{\exp (t\mu_{\text{max}}) + \exp (A\mu_{\text{max}}) \times 10^{N_{\text{max}}-N_0}} \right\} \] (2.3)

where \( N \) is the concentration of microorganisms (log_{10} CFU unit\(^{-1}\)) at time \( t \); \( t \) is the time (h); \( N_0 \)
is the concentration of microorganisms (log_{10} CFU unit\(^{-1}\)) at \( t = 0 \); \( N_{\text{max}} \) is the maximum popu-
lation density (log_{10} CFU unit\(^{-1}\)) and \( \mu_{\text{max}} \) is the maximum growth rate of the microorganisms
(log_{10} CFU unit\(^{-1}\) h\(^{-1}\)).

The Baranyi model is used by the ComBase predictor (http://www.combase.cc/) to fit growth
curves to experimental data stored in the database and is also available in the Microsoft Excel
add-in DMFit (http://www.combase.cc/tools/). While this model is the most complex of the
models presented here, it is also the most flexible, with the differential equation version of the
model designed to model variable temperatures i.e., temperature changing as a function of time
(Gonzales-Barron et al., 2010a; Velugoti et al., 2011).
2. Methods

2.2.1.3. ‘Three phase linear’ Model

The ‘three-phase’ linear model (Buchanan et al., 1997) is a simple extension of the ‘two-phase’ model presented by Einarsson (Einarsson, 1992; Einarsson, 1994). This model was proposed as an alternative to the above models for reasons of simplicity. Phases of the growth curve are described by discrete linear functions, with ‘elbows’ occurring where the exponential growth phases change. The model is defined as:

\[
N_t = \begin{cases} 
  N_0 & \text{if } t \leq t_{\text{lag}} \\
  N_0 + \mu_{\text{max}}(t - t_{\text{lag}}) & \text{if } t_{\text{lag}} \leq t \leq t_{\text{max}} \\
  N_{\text{max}} & \text{if } t \geq t_{\text{max}}
\end{cases}
\] (2.4)

where \(N_t\) is the concentration of microorganisms at time \(t\) (log\(_{10}\) CFU unit\(^{-1}\)); \(N_0\) is the initial concentration of microorganisms in the medium (log\(_{10}\) CFU unit\(^{-1}\)); \(N_{\text{max}}\) is the maximum concentration of microorganisms in the medium (log\(_{10}\) CFU unit\(^{-1}\)); \(\mu_{\text{max}}\) is the maximum growth rate (log\(_{10}\) CFU unit\(^{-1}\) h\(^{-1}\)); \(t_{\text{lag}}\) is the duration of the lag phase (h) and \(t_{\text{max}}\) is the time at which \(N_{\text{max}}\) is reached (h).

While the Buchanan model is less accurate than the Gompertz or Baranyi models (Buchanan et al., 1997) because it does not describe the gradual change of exponential growth phase, its advantages are its ease of use and flexibility. Buchanan et al. (1997) compared their model with the Gompertz and Baranyi models, stating that their model was more robust, especially where experimental data are few. The piecewise nature of the model also lends itself to use in risk assessments because the flexibility of the model allows growth under constant and dynamic temperatures to be estimated simply.
2.2. Primary Model Conclusion

All three of the models presented above have benefits depending on the application. The Baranyi model is the most accurate model presented, especially around the change of exponential growth phase, though it is the most complex formula. This accuracy makes the Baranyi model the best for modelling experimental growth and inactivation data and was adopted for this purpose in this thesis. The Buchanan model is, by design, the most inaccurate around the changes of exponential growth phase. The flexibility of the Buchanan model lends itself to risk assessment applications, especially when modelling dynamic temperatures without the use of differential equations, and was adopted for this purpose in this thesis.

2.2. Secondary Growth Models

Secondary models are used in predictive microbiology to describe the dependence of the growth rate, lag phase duration or maximum population density as a function of other, measurable, environmental conditions to which organisms are exposed. In many situations, temperature is the most influential condition but water activity, pH, salt content and other factors also influence the growth rate, lag phase duration and maximum population density.

2.2.1. Growth Rate

**Bělehrádek-Type**  Ratkowsky et al. (1982) proposed a model that predicts the maximum growth rate, \( \mu_{\text{max}} \), based on the temperature, \( T \), experienced by the organism. This model does not include parameters to describe the maximum temperature at which growth can occur and should not be used to describe growth at temperatures around or above the optimal for the organism’s growth because, at those temperatures, growth deviates from that predicted by this model. The model is

\[
\sqrt{\mu_{\text{max}}} = b(T - T_{\text{min}})
\]  

(2.5)
2. Methods

where \( \mu_{\text{max}} \) is the exponential growth rate; \( b \) is a regression coefficient; \( T_{\text{min}} \) is the theoretical minimum temperature for growth of the organism, derived from the regression line, when the growth rate is equal to zero and \( T \) is the growth temperature. The model is fitted to the square root of the growth rate because this transformation linearises the relationship between temperature and growth rate and also homogenises the variance in the growth rates, thereby improving the reliability of model fitting. This model was found to be a specific case of the Bělehrádek relationship (Bělehrádek, 1930; Ross, 1993b).

A modification to this model was presented by Ratkowsky et al. (1983) to cover the whole spectrum of temperatures supporting growth and allows description of growth rates above the optimum and up to the maximum temperature supporting growth (\( T_{\text{max}} \)). This equation is

\[
\sqrt{\mu_{\text{max}}} = b(T - T_{\text{min}}) \left\{ 1 - \exp \left[ c(T - T_{\text{max}}) \right] \right\}
\]  

(2.6)

where \( \mu_{\text{max}}, T, b \) and \( T_{\text{min}} \) have the same definitions as in Eqn. 2.5 and \( c \) is a second regression coefficient and \( T_{\text{max}} \) is the theoretical maximum temperature for growth, analogous to \( T_{\text{min}} \). This model was further modified by Zwietering et al. (1991) to prevent growth being predicted at temperatures above \( T_{\text{max}} \). These modifications are

\[
\mu_{\text{max}} = [b(T - T_{\text{min}})]^2 \times \left\{ 1 - \exp \left[ c(T - T_{\text{max}}) \right] \right\}
\]  

(2.7)

where all parameters have the same meaning as in Eqn. 2.6. These models can be further extended to include the influence of other environmental factors (Chandler and McMeekin, 1989; McMeekin et al., 1987; Presser et al., 1997) and have become part of the cardinal parameter family of models (Ross et al., 2000; Rosso et al., 1995).

**Arrhenius-Type** The development of the Arrhenius-type model for predictive microbiology is presented by Ratkowsky et al. (1991). The Schoolfield et al. (1981) model, which is the most common parameterisation of these models in predictive microbiology, as presented by Duh and
Schaffner (1993), is:

\[
\mu = \frac{\rho(25) \frac{T}{298} \exp \left[ \frac{H_A}{R} \left( \frac{1}{298} - \frac{1}{T} \right) \right]}{1 + \exp \left[ \frac{H_L}{R} \left( \frac{1}{T_{1/2L}} - \frac{1}{T} \right) \right] + \exp \left[ \frac{H_H}{R} \left( \frac{1}{T_{1/2H}} - \frac{1}{T} \right) \right]} \tag{2.8}
\]

where \( \mu \) is the growth rate; \( \rho(25) \) is the growth rate at 25 °C; \( T \) is the temperature (K); \( H_A \) is enthalpy of activation of the reaction that is catalysed by the growth limiting enzyme (cal per mol); \( R \) is the universal gas constant (1.987 cal mol\(^{-1} \) K\(^{-1} \)); \( H_L \) & \( H_H \) are the enthalpies of low and high-temperature inactivation of growth; and \( T_{1/2L} \) & \( T_{1/2H} \) are the temperatures at which the growth rate is half of the maximum rate because of high or low-temperature effects of the conformation, and hence catalytic efficiency of the growth rate limiting enzyme.

Zwietering et al. (1991) noted that if all six parameters of this model need to be fitted to the available data, large confidence intervals can result unless a very large data set is used. This problem was observed in fitting this model to 38 observations and is due to the over-parameterisation of Eqn. 2.8 compared to the Bělehrádek-type models. Zwietering et al. (1991) found that Eqn. 2.7 produced the smallest residual sum of squares compared to Eqn. 2.8, which had more parameters to describe the data.

### 2.2.2.2. Lag Phase Duration

Many of the models that have been developed for predicting the growth rate, or generation time (time required for the bacteria population to double), as a function of environmental conditions can be applied to model the lag phase duration using analogous formulae e.g. by the substitution of \( \mu = 1/\lambda \) (Duh and Schaffner, 1993). Some models have also been created specifically to describe lag phase durations. For example, the hyperbolic family of models have been developed to specifically describe the lag phase duration. These models are

\[
\ln(\lambda) = \frac{p}{T - q} \tag{2.9}
\]
2. Methods

\[ \lambda = \left[ \frac{p}{T - q} \right]^m \]  

(2.10)

where Eqn. 2.9 was the first hyperbolic model proposed for predicting lag time by Zwietering et al. (1991) and Eqn. 2.10 was the model proposed by Oscar (2002). The model by Oscar (2002) was shown to provide the best fit to the data tested, though a thorough comparison to a variety of data sets was not presented. It is not clear if the addition of the extra parameter in Eqn. 2.10 could lead to over-parameterisation, though this is less likely to be of concern in this context as many of the lag phase models based on the inverse of the growth rate have four or more parameters. Modelling the lag phase by Eqn. 2.10 appears to offer the best approach to modelling the lag phase duration based on the analysis of Oscar (2002).

2.2.2.3. Maximum Population Density

The only model identified in the public domain which describes the change in maximum population density as a function of temperature was presented by Zwietering et al. (1994), with notational changes by Oscar (2005) as

\[ N_{\text{max}} = a \frac{(T - T_{\text{min}2})(T - T_{\text{max}2})}{(T - T_{\text{submin}})(T - T_{\text{supmax}})} \]  

(2.11)

where \( N_{\text{max}} \) is the maximum population density (log_{10} CFU/unit); \( T_{\text{min}2} \) and \( T_{\text{max}2} \) are the theoretical temperatures (°C) at which the maximum population density is predicted to be 0 log_{10} CFU/unit (i.e. the \( T \)-intercepts of the curve on a log scale); \( T_{\text{submin}} \) is a temperature smaller than \( T_{\text{min}2} \) and \( T_{\text{supmax}} \) is a temperature greater than \( T_{\text{max}2} \).

2.2.3. Inactivation Models

Predictive modelling of thermal inactivation of microorganisms in foodstuffs is relatively straightforward in products where contamination is surface limited but is more difficult for products
where the contamination is internalised. While modelling inactivation of pathogens on the surface of foodstuffs requires knowledge of the rate of pathogen inactivation as a function of temperature and other environmental conditions, modelling pathogens located inside foodstuffs requires knowledge of inactivation rates and thermal heat transfer rates in the specific food. In this section, approaches that have been used to model the inactivation of pathogens in relevant, internally-contaminated, foodstuffs is discussed including burger patties, rolled roasts and non-intact tenderised and injected meats.

**2.2.3.1. Models Based on D- and z- Values**

Many published studies have considered the thermal inactivation of pathogens in foods. These experiments are typically conducted in water baths, where the temperature can be strictly controlled and samples removed at regular intervals. Results are usually reported in terms of $D$-values and $z$-values. The $D$-value is the time required for a 90% reduction in the concentration of the pathogen under a specific set of conditions of temperature and other environmental factors and the $z$-value is the temperature change required for a one $\log_{10}$ change in the $D$-value. Examples of experiments providing $D$- and $z$-values for *Salmonella* inactivation in pork include Juneja et al. (2000), Juneja et al. (2001b), Murphy et al. (2004) and Osaili et al. (2007).

$D$- and $z$-values can be used directly to estimate the inactivation of pathogens on foodstuffs with surface contamination only, but cannot be applied directly to meats with internalised pathogens. To accurately model pathogen inactivation in these products, it would be necessary to first estimate the temperature as a function of time of each part of the food during the cooking process to determine temperatures to which pathogens are exposed. One approach to this problem used by VLA/DTU/RIVM (2011) is to use the heat conduction equation (Weisstein, 2016) to determine the temperature of all parts of a pork burger patty during the entirety of the cooking process. The heat conduction equation is formulated to use information about the patty composition to accurately model the heat transfer into the patty. Once the patty temperature at all times is known, pathogen survival can be determined. The difficulty in using this approach is its computational
2. Methods

complexity, requiring a large amount of computing resources and many numeric properties of the meat and the meat/air boundary.

2.2.3.2. Cooking Method Validation Models

Another method of modelling the inactivation of pathogens in foods is to undertake a challenge study, i.e., to intentionally contaminate the food of interest with the pathogen of interest. The food is then cooked to a range of endpoint temperatures under controlled conditions and the pathogen enumerated using established standard methods. Predictive models have been developed using this method, finding that the \( \log_{10} \) concentration of pathogen could be related to the internal endpoint temperature using linear models. Juneja et al. (1997) derived from novel experiments such a model for *E. coli* O157:H7 in beef burgers and Smith et al. (2013) extended this method to describe non-intact beef contaminated with *E. coli* O157:H7. This method has the advantage of the data being able to be described by a simple relationship between the pathogen concentration and the internal endpoint temperature. The downside of this method is that the predictive model developed is only valid between the range of temperatures specifically studied, with modifications to the model required to prevent rapid growth predictions under extrapolation to low temperatures instead of inactivation. The model is also only valid for the preparation and cooking method used in the experiment.

2.2.3.3. Inactivation Model Conclusions

Inactivation models based on \( D \) and \( z \)-values are difficult to implement and integrate into two-dimensional risk assessment models: inactivation models based on cooking validation experiments are simpler to integrate. Models based on cooking experiments require only the internal endpoint temperature to estimate the pathogen reduction and internal endpoint temperatures can be obtained from surveys, e.g. EcoSure (2008), or estimated from consumer preference surveys. Inactivation models based on \( D \) and \( z \)-values require a larger number of data inputs, including cooking time,
cooking surface temperature (e.g. frying pan or grill) and properties of the meat itself, including its density, specific heat capacity, thermal conductivity and thermal diffusivity (VLA/DTU/RIVM, 2011). For this reason, cooking validation models were chosen to describe *Salmonella* inactivation in pork.

### 2.2.4. Growth and Inactivation Data

The results of experiments conducted into the growth and inactivation of microorganisms are collated into the ComBase database (http://www.combase.cc/), which currently contains over 50,000 observations. Data collated and stored in the database are converted to standardised units to allow for comparison between experiments. The database also includes metadata on the experiments conducted. Analysis of those data reveal or reinforce microbial patterns in response to different experimental conditions, e.g. the effects of temperature, water activity, pH, irradiation, modified atmosphere or vacuum packaging, use of acids, salt, serovar and strain differences.

### 2.3. Dose-Response Models

Dose-response models describe the relationship between ingestion of a quantity of a hazard and the probability of infection and/or illness. They are used in quantitative risk assessments to relate contamination levels of a hazard in a food to the mean and range of risk to consumers from consumption of that food product. Many factors make the generation of microbial dose-response models problematic, as described in the following two sections. Feeding study and outbreak data have been used to infer and describe these relationships, but typically involve large uncertainties due to the nature of the available data. Factors that must be considered in the use of dose-response models in risk assessments are outlined by Ross and McMeekin (2003) and include the various forms of dose-response formulae used to describe dose-response relationships for various foodborne pathogens. Below, the main dose-response models for salmonellosis are described, including the data they are based on and their limitations.
2. Methods

2.3.1. Feeding Studies

Feeding studies are the most direct way of quantifying the dose-response relationship for pathogens but there are clearly ethical issues that limit this approach. Feeding studies require ‘volunteers’ to be given varying doses of the pathogen and for reasons described earlier, most studies are over 60 years old. Additionally, many facets of their methodology have been questioned, in particular, whether the ‘volunteer’ group are representative of the general population or any sub-population of particular interest or susceptibility. Typically, volunteers were adult males, either as parts of prison populations or soldiers, but occasionally students.

Feeding studies on the dose-response relationship for *Salmonella* spp. are summarised by FAO/WHO (2002). The dose of *Salmonella* that was administered in those studies were cultured under laboratory conditions and, as such, may not behave the same, or have the same virulence as wild *Salmonella* strains in normally prepared food. Further, some ‘volunteers’ were given a second, larger dose of the pathogen if no response to the first dose was observed. This makes it difficult to know if the cumulative effect of the two doses influenced the probability of infection, or if the second, higher, dose alone caused the reaction. Doses administered by McCullough and Eisele (1951a,b,c,d) to volunteers were delivered in glasses of eggnog directly after the noon meal. While the increased gastric acid after the noon meal may have reduced *Salmonella* infectivity, the high fat content could also have provided a protective effect against the gastric acid, negating its effect on infectivity (FAO/WHO, 2002).

These studies also did not include lower doses to evaluate responses to reduced dosages. Thus, little information was gathered about the probability of infection at low doses, making dose-response models based on those feeding studies potentially unreliable at low doses. Also, the serovars of *Salmonella* used in those studies did not reflect those currently causing foodborne illness in Australia (see Table 1.2). Two *Salmonella* dose-response models have been derived from the existing feeding trial data.

1. Teunis et al. (1999) developed a dose-response model for *Salmonella* by fitting a beta-
2.3. Dose-Response Models

Poisson model to the results of McCullough and Eisele (1951c) for a S. Meleagridis feeding trial. This model distinguishes between the probability of illness and infection and allows estimates of both to be made based on a quantity of Salmonella ingested. The model for the probability of illness is

\[
p_{\text{ill}} = p_{\text{inf}} \times p_{\text{ill|inf}} = _1 F_1 (\alpha, \alpha + \beta, -D) \times [1 - (1 + \eta D)^{-r}] \approx 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \times [1 - (1 + \eta D)^{-r}] \tag{2.12}
\]

where \( p_{\text{ill}} \) is the probability of illness, \( p_{\text{inf}} \) is the probability of infection, \( p_{\text{ill|inf}} \) is the probability of illness given infection, \( D \) is the dose of Salmonellae ingested, \( _1 F_1 \) is the confluent hypergeometric function of the first kind, and \( \alpha, \beta, \eta \) and \( r \) are parameters of the model. When the model was fitted to the data, the following parameter estimates were found

\[
\begin{align*}
\alpha &= 0.89 \\
\beta &= 4.4 \times 10^5 \\
\eta &= 1.0 \times 10^{-16} \\
r &= 3.4 \times 10^8.
\end{align*}
\]

A limitation of this model for risk assessments is the lack of low doses (the minimum dose used was \( 1.58 \times 10^5 \) Salmonellae) in the original data, requiring that the model is extrapolated to estimate the probability of illness for low doses of Salmonella. Another limitation is that the model is only based on one serovar of Salmonella; other serovars of Salmonella may have different dose-response relationships.

2. Fazil (1996) also developed a dose-response model for Salmonella based on feeding trial data and a summary of that dose-response relationship is provided by FAO/WHO (2002).
The model includes data from multiple *Salmonella* serovars including *S. Meleagridis*, *S. Anatum*, *S. Newport*, *S. Bareilly* and *S. Derby*. A beta-Poisson dose-response model was used to describe that relationship, with the form of the model

\[ P_{\text{ill}} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha} \]

where the parameters have the same meaning as in Eqn. 2.12. The model parameters were estimated as

\[ \alpha = 0.3126 \]
\[ \beta = 2885. \]

Similar to the model of Teunis et al. (1999), no doses less than $10^4$ CFU of *Salmonella* were administered, requiring extrapolation of the model to infer the dose-response relationship at these lower doses. The original dose-response model was generated with data from subjects that were given multiple doses of *Salmonella*. Modifications to the original dose-response model were made by FAO/WHO (2002) to account for these multiple doses. Results from subjects who were administered multiple doses were removed from the data, leaving only doses administered to naive subjects in the data, and the model refitted to the ‘cleaned’ data, with fitted parameter values of $\alpha = 0.4047$ and $\beta = 5587$. Uncertainty in the parameter estimates for the original and modified models was estimated using a bootstrapping approach. A comparison of the two dose-response models and the data to which these models were fitted is provided by FAO/WHO (2002), showing that the effect of cleaning the data of subjects receiving multiple doses was small compared to other dose-response models (see Figure 2.4).
2.3. Dose-Response Models

2.3.2. Outbreak Studies

Outbreak data have also been used to characterise the dose-response relationship for Salmonella. As part of outbreak investigations, data is collected concerning the number of people who consumed the contaminated food item, the number of people who became sick and, where possible from recovered food, the dose of Salmonella in the food. Even when all of this information is available for an outbreak, there are normally large uncertainties in the data. Firstly, it is not simple to estimate the number of people who consumed the contaminated food item; multiple dishes can contain the same contaminated ingredients while some servings of the contaminated dish may not actually be contaminated. It is also difficult to determine how many people actually become ill during an outbreak, for reasons such as those outlined in Section 1.1.1. Finally, determining the dose consumed is difficult. Epidemiology can take a long time to identify an outbreak, during which, the original dish was likely consumed or disposed of, leaving no leftover food to test. If a sample of the dish can be located, Salmonella growth or inactivation may have occurred since consumption, unless the dish is refrigerated or frozen, confounding estimates of the dose consumed. In 1997, Japan issued a directive that requires restaurants and caterers to keep food samples in a freezer at below -20 °C for two weeks after service of that food, so that samples are available if an outbreak occurs (Kasuga et al., 2004). In the event of an outbreak, enumeration of frozen samples reduces the uncertainty in the dose estimate and Japanese data have been used to enhance Salmonella dose-response models.

Two dose-response models have been presented in the literature based on data from outbreak investigations:

1. Teunis et al. (2010) presented a dose-response model based on outbreak data that separates the probability of illness from the probability of infection. The form of the dose-response model used is

\[ P_{\text{ill}} = p_{\text{inf}} \times p_{\text{ill|inf}} = \left(1 - 2 F_1(\alpha, r, \alpha + \beta, -D/r) \right) \times (1 - (1 + \eta D)^{-\rho}) \] (2.13)
2. Methods

where \( P_{\text{ill}}, P_{\text{inf}}, P_{\text{inf|ill}}, \alpha, \beta, \eta, \rho \) and \( D \) have the same meanings as described in Eqn. 2.12, \( _2F_1 \) is the hypergeometric function of the second kind and \( r \) is the dispersion factor. This data includes outbreak data used by FAO/WHO (2002) but is augmented by data described by Kasuga et al. (2004). *Salmonella* serovars represented in the outbreak data include *S. Enteritidis*, *S. Typhimurium S. Heidelberg*, *S. Infantis*, *S. Napoli*, *S. Bareilly*, *S. Oranienburg*, *S. Schwarzengrund*, *S. Zanzibar* and *S. Anatum*. Fitted estimates of the model parameters are:

\[
\begin{align*}
\alpha &= 8.53 \times 10^{-3} \\
\beta &= 3.14 \times 10^{0} \\
\rho &= 8.23 \times 10^{0} \\
\eta &= 6.90 \times 10^{1}.
\end{align*}
\]

This model predicts illness at lower doses than either of the feeding trial models presented above. The ID\(_{50}\) values (number of *Salmonella* ingested to result in a 50% probability of illness) for this model is 36 *Salmonellae* and the probability of illness from consuming a dose of \( 10^4 \) *Salmonellae* is greater than 0.95, making this model the most conservative of the models presented. This model also contains large uncertainties, with the 95% predictive interval around the ID\(_{50}\) value 0.69 to \( 1.26 \times 10^7 \) *Salmonellae*. This large uncertainty range makes it difficult to have confidence in estimates produced by this model. This model is difficult to use in risk assessments because not all implementations of the \( _2F_1 \) hypergeometric function are stable in the range required by the dose-response model. Nonetheless, this model has been used in risk assessments (Guillier et al., 2013).

2. FAO/WHO (2002) developed a beta-Poisson dose-response model based on outbreak data. This model only estimates the probability of illness and does not estimate the probability
Table 2.1.: Parameter estimates for the dose-response model presented by FAO/WHO (2002), including uncertainty estimates.

<table>
<thead>
<tr>
<th></th>
<th>α</th>
<th>β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected Value</td>
<td>0.1324</td>
<td>51.45</td>
</tr>
<tr>
<td>Lower Bound</td>
<td>0.0763</td>
<td>38.49</td>
</tr>
<tr>
<td>2.5th Percentile</td>
<td>0.0940</td>
<td>43.75</td>
</tr>
<tr>
<td>97.5th Percentile</td>
<td>0.1817</td>
<td>56.39</td>
</tr>
<tr>
<td>Upper Bound</td>
<td>0.2274</td>
<td>57.96</td>
</tr>
</tbody>
</table>

of infection. The form of the model is

$$P_{ill} = 1 - \left(1 + \frac{D}{\beta}\right)^{-\alpha}. \tag{2.14}$$

Uncertainty was associated with the dose of *Salmonella* ingested and the number of people who became ill, thus, uncertainty distributions were assigned to the dose and attack rate for each outbreak. Bootstrapping of the uncertainty distributions was used to generate 5000 parameter estimates. Parameter estimates and distributions for the model arising from this bootstrapping method are presented in Table 2.1. The fitting procedure took into account the size of the outbreak with smaller outbreaks given a lower weighting than larger outbreaks. Using the expected values for $\alpha$ and $\beta$ given in Table 2.1 and a dose of $10^4$ *Salmonella* organisms in the beta-Poisson model, the probability of illness estimate is 0.503. This model has been used previously in risk assessments (Guillier et al., 2013; VLA/DTU/RIVM, 2011) and is presented graphically in Figure 2.2.

### 2.3.3. Surrogate Organisms

Dose-response models have also been developed using surrogate organisms, assuming that the surrogate organism has a similar dose-response relationship to the organism of interest. USDA-FSIS (1998) developed a beta-Poisson dose-response model for *Salmonella* based on feeding trial data from experiments conducted using *Shigella dysenteriae*. This surrogate was used to
Figure 2.2.: Dose-response model for *Salmonella* spp. of FAO/WHO (2002). The darker grey represents the 95% confidence interval for the model uncertainty and the lighter band represents the maximum and minimum bands on the model uncertainty.
2.3. Dose-Response Models

Figure 2.3: Dose-response model for *Salmonella* spp. of USDA-FSIS (1998). The grey bands represent the 95% confidence interval on the model uncertainty.

describe *Salmonella* outbreaks that occurred with low *Salmonella* doses. The resulting model only provides an estimate of the probability of *illness* and does not separate the probability of *infection*. The form of the model used and the parameter definitions are the same as Eqn. 2.14 and when fitted to the data the parameters were estimated as

\[
\alpha = 0.2767
\]

\[
\beta \sim N_{0,60}(21.159, 20).
\]

The normal distribution was used to describe the uncertainty in the estimate of \(\beta\) and was truncated at zero and 60. This dose-response model is presented graphically in Figure 2.3. This model has large uncertainty in the probability of illness estimate at low doses.
2. Methods

2.3.4. Combination Models

One unpublished model by Health Canada used a Weibull dose-response model to describe both feeding trial and outbreak data. Many of the details of this model are presented in FAO/WHO (2002), including details of the implementation and model parameters. FAO/WHO (2002) noted that while this model estimated the probability well at low *Salmonella* doses, it underestimates the probability of illness at higher doses. As this model is not published in its entirety, there are certain details about the model that are unknown, making the use of this model problematic in risk assessment modelling.

2.3.5. Dose-Response Conclusion

Many dose-response models have been developed for foodborne salmonellosis. Some of these models include uncertainty estimates or have provided methods for including uncertainty in the implemented model. A comparison of all dose-response models described above is presented graphically in Figure 2.4, with the exception of the model by Teunis et al. (2010), which could not be implemented in R due to problems implementing the \( {}_2F_1 \) hypergeometric function. Large differences between most models are apparent. Of the models presented here, the highest probability of illness at low doses was estimated by the Teunis et al. (2010) modelling approach, though this could not be confirmed, and the lowest probability of illness at low doses is estimated by the model of Teunis et al. (1999). The differences between these models are such that at some *Salmonella* doses, the Teunis et al. (1999) model estimates a probability of illness close to zero while the model of USDA-FSIS (1998) estimates a probability close to one, highlighting the large differences that exist between these models.

The FAO/WHO (2002) model was considered to provide the most appropriate model for risk assessment purposes and is adopted in the remainder of this thesis for risk assessment modelling and calculations. It was shown by FAO/WHO (2002) to accurately describe the range of salmonellosis outbreaks that have occurred and estimates of the probability of illness from a simulated
2.3. Dose-Response Models

Figure 2.4.: Comparison of dose-response relationships for *Salmonella*. Where uncertainty estimates of the parameter estimates are provided, the mean value was used for that parameter. The model of Teunis et al. (2010) is not included as it could not be reproduced in R.

dose can be readily calculated.
3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers

3.1. Abstract

Predictive models, to estimate the reduction in *E. coli* O157:H7 concentration in beef burgers, have been developed to inform risk management decisions; no analogous model exists for *Salmonella* spp. in pork burgers. In this study, “Extra Lean” and “Regular” fat pork minces were inoculated with *Salmonella* spp. (S. 4,[5],12:i:-, S. Senftenberg and S. Typhimurium) and formed into pork burger patties. Patties were cooked on an electric skillet (to imitate home cooking) to one of seven internal temperatures (46, 49, 52, 55, 58, 61, 64 °C) and *Salmonella* enumerated. A generalised linear logistic regression model was used to develop a predictive model for the *Salmonella* concentration based on the internal endpoint temperature. It was estimated that in pork mince with a fat content of 6.1%, *Salmonella* survival will be decreased by \(-0.2407 \log_{10} \text{CFU/g}\) for a 1 °C increase in internal endpoint temperature, with a 5-log_{10} reduction in *Salmonella* concentration estimated to occur when the geometric centre temperature reaches 63 °C. The fat content influenced the rate of *Salmonella* inactivation \((p = 0.043)\), with *Salmonella* survival increasing as fat content increased, though this effect became negligible as the temperature approached 62 °C. Fat content increased the time required for patties to achieve a specified internal temperature \((p = 0.0106 \text{ and } 0.0309 \text{ for linear and quadratic terms respectively})\), indicating that reduced fat pork mince may reduce the risk of salmonellosis from consumption of
3.2. Introduction

Preliminary research into the feasibility of conducting a quantitative risk assessment for *Salmonella* in Australian pork burgers uncovered that inactivation kinetics of *Salmonella* spp. in pork burger patties are a data gap in the current literature. While an inactivation model for *E. coli* O157:H7 in beef burger patties (Juneja et al., 1997) has been published, an analogous model could not be found for *Salmonella* spp. in pork burger patties. This data gap needed to be filled to allow a quantitative risk assessment model to be constructed. The predictive model that was developed from this research became part of the stochastic model presented in Chapter 5.

The work presented in this Chapter has been published as:


The following is a translation of that manuscript into the format of this thesis, with no change to the original, published, text or data. Supplemental materials provided on the publisher’s website are provided in Appendix A.

*Salmonella* is a major cause of foodborne illness worldwide. In 2011, there were 95,548 confirmed cases of salmonellosis in the European Union, exceeded in reported cases only by campylobacteriosis (EFSA, 2013). *Salmonella* spp. are responsible for the largest number of deaths from foodborne pathogens in the US, despite the relatively low death rate of 0.5% (Scallan et al., 2011). Similarly, in Australia in 2014, approximately 16,000 cases of salmonellosis were reported, an
incidence exceeded only by campylobacteriosis (Department of Health Australia, 2015). The true number of cases of salmonellosis caused by foodborne contamination in Australia, circa 2010, was estimated at 39,600 (Kirk et al., 2014b), resulting in an estimated 3,500 cases of irritable bowel syndrome and 3,250 cases of reactive arthritis (Ford et al., 2014). Common symptoms of salmonellosis include nausea, vomiting, abdominal cramps, diarrhoea, fever and headaches. Those at greatest risk of serious complications are the elderly, young and immuno-compromised (FDA, 2012). The identification of *Salmonella* spp. as the cause of illness can be difficult due to the onset of symptoms occurring 6 to 72 hours after exposure (FDA, 2012). Some *Salmonella* serovars are of greater public health interest as they are more frequently identified as the cause of salmonellosis outbreaks. *Salmonella Typhimurium* accounts for the largest proportion of salmonellosis cases in Australia: 23.5% of outbreaks were attributed to this serovar in 2010 (OzFoodNet Working Group, 2012a).

*Salmonella 4,[5],12:i:-*, an emerging strain of public health interest, has risen to prominence in the last decade. The isolation rate, and number of human infections, from this strain is increasing in the European Union (EFSA, 2013) and United States of America (CDC, 2013). This strain has also caused foodborne illness in Australia (OzFoodNet Working Group, 2012b). *S. Senftenberg* is also of interest because of reports of its unusually high heat resistance (Jay et al., 2003).

Source attribution studies for *Salmonella* spp. have identified pork products as causing a large proportion of salmonellosis cases (Hald et al., 2004; Mughini-Gras et al., 2014; Mullner et al., 2009). While no source attribution studies have been conducted for salmonellosis in Australia, pork products have been associated with salmonellosis outbreaks (Delpech et al., 1998; OzFoodNet Working Group, 2010, 2012a).

Pork burgers are currently being promoted to Australian consumers as a serving suggestion for pork mince, which has a relatively low prevalence (1.4%) of *Salmonella* (Hamilton et al., 2011.). Beef burgers have an increased risk of *E. coli* O157:H7 survival after cooking compared with beef steaks (Smith et al., 2013). Similarly, salmonellosis risk from pork products in the European Union was greater from pork burgers compared with pork cuts in two of four member
3.2. Introduction

states examined (VLA/DTU/RIVM, 2011). The inactivation model in that risk assessment used $D$-values (time required for a 1-log$_{10}$ reduction in Salmonella at a constant temperature) and $z$-values (temperature required for a 1-log$_{10}$ reduction in the $D$-value) for beef mince, not pork mince. The fat content of pork, beef, chicken and turkey mince has been shown to have an effect on Salmonella inactivation (Juneja et al., 2001a; Juneja et al., 2000; Smith et al., 2001) with increased fat content leading to increased Salmonella survival. This effect has not been quantified in pork burger patties. The risk of salmonellosis can also be influenced by the colour of the cooked burger patty if used as an indicator of the “doneness”. Colour is a poor indicator of “doneness”, with burgers cooked to 66 °C appearing as brown as burgers cooked to 71 °C (Hague et al., 1994). Factors linked to increased pinkness in cooked burgers include pH (Trout, 1989) and pigment concentration (Mendenhall, 1989). Unlike intact cuts of pork, where microbial contamination is limited to the surfaces of the food, burger patties are comminuted, with microbial pathogens potentially internalised. Heat from cooking surfaces needs to be transferred from the outer surfaces to the centre of the patty for thermal inactivation to occur. Juneja et al. (1997) presented a simple mathematical model for the reduction of E. coli O157:H7 during cooking of beef burgers, but no data exist for Salmonella thermal inactivation in pork burgers. An inactivation model for Salmonella reduction in pork burger patties cooked to mimic home cooking practices would provide information that can be used to assess food safety risk and, potentially, offer insights for food safety management. The aims of this study were to i) quantify the reduction in Salmonella caused by cooking pork burgers to various endpoint temperatures; ii) determine whether there are serovar differences in reduction due to cooking and iii) assess the influence of fat content on the rate of thermal inactivation of Salmonella in pork burgers.
3. Materials and Methods

3.3. Salmonella Strains

Three *Salmonella* serovars, of public health interest (see Section 3.2), were chosen for this experiment; *S.* 4,[5],12:i:-, *S.* Typhimurium and *S.* Senftenberg. All serovars used in this study were previously isolated from porcine sources, serotyped, and stored long-term in snap freeze medium (Oxoid, TM0171) at –80 °C. Prior to the experiment, isolates were removed from frozen storage, streaked onto nutrient agar slopes (Oxoid, TM0085) and stored at room temperature to provide working cultures for the duration of all experiments. Prior to the experiments, the viability of each strain was verified by streaking the cultures of each serovar onto nutrient agar (Oxoid, PP2036) and incubating at 37 °C for 18 ± 2 hours. A single colony was picked off and inoculated into 100ml of Tryptic Soy Broth (TSB, Bacto, Catalogue Number 211825) and incubated at 37 °C for 18 ± 2 hours. Cultures were centrifuged (3667g, 4 °C, 15 minutes) and rinsed twice with peptone saline solution (PSS, 0.1% trypicase, 0.85% NaCl, wt/vol) before being re-suspended in 5ml of PSS to minimise the change in moisture of the mince upon inoculation, and thereby, water activity and texture of the mince.

3.3.2. Mince

Each batch of burgers required two packages of retail pork mince, which were purchased from supermarkets of the same chain in 500g modified atmosphere packages (MAP). Pork mince was purchased with either a “Regular” fat level (nominally 17%, stated on packaging) or “Extra Lean” fat level (nominally 5%, stated on packaging), with the actual fat content of the mince determined analytically (see Section 3.3.6). The pork mince packages required for each week’s experiments were purchased at the start of that week. Pork mince was transported to the laboratory by car, but without refrigeration. The transport time was up to 45 minutes at ambient temperature in the range of 19 °C to 29 °C. The use-by date and other information from the product labels were
Table 3.1.: Sequence that each fat level and serovar combination was cooked. Nominal fat levels are the descriptions on the mince packages.

<table>
<thead>
<tr>
<th>Batch</th>
<th>Week</th>
<th>Nominal Fat Level</th>
<th>Serovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Regular</td>
<td>S. 4,[5],12:i:-</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>Extra Lean</td>
<td>S. Senftenberg</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>Regular</td>
<td>S. Senftenberg</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>Extra Lean</td>
<td>S. 4,[5],12:i:-</td>
</tr>
<tr>
<td>E</td>
<td>2</td>
<td>Regular</td>
<td>S. Senftenberg</td>
</tr>
<tr>
<td>F</td>
<td>2</td>
<td>Regular</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>G</td>
<td>3</td>
<td>Extra Lean</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>H</td>
<td>3</td>
<td>Extra Lean</td>
<td>S. Senftenberg</td>
</tr>
<tr>
<td>I</td>
<td>3</td>
<td>Extra Lean</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>J</td>
<td>3</td>
<td>Regular</td>
<td>S. Senftenberg</td>
</tr>
<tr>
<td>K</td>
<td>4</td>
<td>Regular</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>L</td>
<td>4</td>
<td>Extra Lean</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>M</td>
<td>4</td>
<td>Regular</td>
<td>S. 4,[5],12:i:-</td>
</tr>
<tr>
<td>N</td>
<td>4</td>
<td>Regular</td>
<td>S. 4,[5],12:i:-</td>
</tr>
<tr>
<td>O</td>
<td>5</td>
<td>Extra Lean</td>
<td>S. 4,[5],12:i:-</td>
</tr>
<tr>
<td>P</td>
<td>5</td>
<td>Extra Lean</td>
<td>S. 4,[5],12:i:-</td>
</tr>
<tr>
<td>Q</td>
<td>5</td>
<td>Regular</td>
<td>S. Typhimurium</td>
</tr>
<tr>
<td>R</td>
<td>5</td>
<td>Extra Lean</td>
<td>S. Senftenberg</td>
</tr>
</tbody>
</table>

recorded and the packages placed into a refrigerator at 4 ± 2 °C. The number of days until the expiration of the product “use-by” period ranged from 2 to 5 days with four batches having 2, 3 or 4 days and six batches 5 days remaining.

This study consisted of 18 batches (labelled A to R) of 8 burger patties with 3 Salmonella serovars, 2 fat levels and 3 replicates of each fat-serovar combination (see Table 3.1).

3.3.3. Patty Preparation

Pork mince was added to the bowl of a food mixer (Kambrook KSM500 Powermix Planetary Bench Mixer), and the leaf beater attached. The mixer was run for 1 minute at the slowest speed setting to thoroughly mix the product. A 30g sub-sample of the mince was collected for fat level determination and stored at –80 °C until analysis. Another 30g sub-sample of the mince was taken for pH determination. The 5ml re-suspension of Salmonella was poured onto the mince.
and the mixer was run at the lowest speed setting for five minutes to ensure that the *Salmonellae* were homogeneously dispersed throughout the mince.

Mince was formed into patties using a circular mould of 8cm diameter and 2cm thickness. For each patty, 100g of contaminated mince (determined by weighing) was pressed into the mould using a metal spoon. Each formed patty was placed inside a plastic, sealable container lined with baking paper and refrigerated overnight. The refrigeration served to “firm up” the patties after mixing and to condition the *Salmonella* cells to their environment, thus simulating contaminated pork mince purchased at retail.

### 3.3.4. Cooking of Pork Burger Patties

Patties were cooked to one of seven nominal target temperatures (45, 48, 51, 54, 57, 60, 63 °C, chosen to cover a realistic range of endpoint cooking temperatures at which some *Salmonella* survivors would still be able to be enumerated) measured by a type K thermocouple attached to a thermocouple thermometer (Model Number TFC-307P, OneTemp, Adelaide, Australia). Cooking times were expected to vary between burger patties cooked to the same internal endpoint temperature. For each batch of patties, the order of cooking and assigned endpoint temperature were randomised and recorded. An electric skillet (Kambrook KEF90 Essentials Skillet Frypan) was pre-heated to temperature setting 8, with the skillet chosen to mimic home cooking practices. Patties were removed from the refrigerator and stored at ambient temperature (around 22–25 °C) until the seven patties had been cooked. For each patty, vegetable oil (2.5ml) was added to the skillet and then one patty was placed into it. The thermocouple was positioned approximately at the centre of the patty and inserted midway through the thickness of the patty. This placement was judged ‘by eye’, looking initially from above the patty, and then from the side, because the dimensions of the patty changed during cooking. Thus, it was not possible to easily develop a device that would consistently and reliably position the thermocouple into the geometric centre of each patty. The patty was turned every 90 seconds, removing the thermocouple before turning
and reinserting immediately afterwards. The temperature of the patty before it was turned was recorded. Cooking and turning continued until the internal temperature displayed by the thermometer reached the internal temperature required. Once the required internal temperature was achieved, the cooking time was recorded, the patty removed from the heat, placed in a sample bag and left to rest for three minutes (determined by stopwatch) after which time the bag was sealed. The sample bag was placed under ice to prevent further *Salmonella* inactivation from the residual heat. Patties were then placed in a refrigerator until all patties for that batch had been cooked, after which *Salmonella* were enumerated in all the cooked patties. One randomly selected patty from each batch was left uncooked and used to determine the initial *Salmonella* concentration for each batch of patties. The complete experiment involved 144 patties, 126 cooked and 18 raw. After all 126 patties were cooked, the thermometer used in the experiment was calibrated against a reference thermometer certified by the National Association of Testing Authorities, Australia. Cooking temperatures were adjusted for the statistical analyses based on the results of the calibration.

### 3.3.5. *Salmonella* Enumeration

A sample weighing $32 \pm 3$g was taken from the centre of each patty using a sterile tube to punch out a sub-sample. A 1:10 homogenate of the sample was prepared in PSS, with stomaching for 60 seconds (BagMixer® 400P, Interscience). Serial dilutions were made in peptone saline solution (Micromedia PTY LTD, Moe Australia, Catalogue Number 4070) from the homogenate and 100µL of these dilutions were spread onto Xylose Lysine Deoxycholate (XLD, Micromedia PTY LTD, Moe Australia, Catalogue Number 1355) agar plates. For cooking temperatures where the lower limit of detection (<100 CFU/g) was insufficient to enumerate low levels of *Salmonellae*, 250µL of the homogenate was plated onto four XLD plates to reduce the detection limit to 10 CFU/g. XLD plates were incubated at 37 °C for 22 ± 2 hours and typical *Salmonella* colonies were counted. For each plate counted, the dilution factor of the plate was recorded to allow estimation of the *Salmonella* concentration for each cooked patty (in CFU/g).
3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers

3.3.6. Fat Level Analysis

Once all batches had been prepared, samples were sent to a commercial analytical laboratory (Waite Analytical Services, Urrbrae, South Australia) for assessment of total fat content (as percentage). Samples were thawed at room temperature and the mince prepared by breaking the sample into smaller fragments on a clean glass plate. A 1g sub-sample was taken and placed into a 12mL ground glass tube and 2ml of 0.9% saline (wt/vol, Baxter Steripour) added to the tube and the sample vortexed. The mince was homogenised using a Homogeniser (T25 Ultraturrax, IKA). Once homogenised, 3mL of methanol (with 0.005% BHA) was added and the sample vortexed again and allowed to stand for five minutes. Chloroform (6mL) was added, and the tube capped and shaken. Tubes were then centrifuged for 10 minutes at 1559×g (Megafuge 1.0, Heraeus Sepatech). The lower organic phase (chloroform) was decanted using a long Pasteur pipette and transferred to a pre-weighed 20mL glass scintillation vial. A vacuum concentrator was used to evaporate to dryness. The scintillation vial with contents was weighed and the difference in weight calculated to estimate the fat content of the original sample.

3.3.7. pH Testing

Mince samples for pH determination were stored for up to one day after purchase at 4 ± 1 °C. The pH meter (Eutech CyberScan pH 510, Eutech Instruments) was rinsed with deionised water, calibrated against pH 7.00, 4.01 and 10.01 solutions, in order, rinsing between solutions. The probe was inserted directly into the mince sample and the reading was recorded. The probe was cleaned between each sample using ethanol and then deionised water to remove pork mince adhering to the probe.

3.3.8. Statistical Analysis

To describe the reduction in *Salmonella* concentration based on an internal endpoint cooking temperature, the model of Wadley (1949) was used, with modifications as described below.
Salmonella counts in the \( d \)th 10-fold dilution \((d = -1, \ldots, -6)\) were assumed to follow a negative binomial distribution with mean \( \mu \), variance \( \mu + \mu^2/\theta \) and dispersion parameter \( \theta \), where \( \mu \times 10^{-d} \) follows a logistic curve as the endpoint temperature \( T \) changes. This curve, given by

\[
\mu \times 10^{-d} = \frac{e^\nu}{1 + \exp\left[-(\alpha + \beta T)\right]} \tag{3.1}
\]

takes its highest value, \( e^\nu \), at low temperatures, when the Salmonella concentration is at its highest level, and then progressively decreases as the endpoint temperature increases. The value of \( \beta \) in this formulation is negative, corresponding to the decrease in colony counts with increasing endpoint temperature. The parameters \((\alpha, \beta)\) are related in that the value \(-\alpha/\beta\) represents the endpoint temperature at which the Salmonella concentration is reduced by 50%. Because each batch is independently sampled, the values of \( \nu \) should vary between batches and it is anticipated that the values of \( \alpha \) and \( \beta \) may vary according to serovar and/or fat content. For each batch and endpoint temperature, a suitable dilution factor is chosen such that the Salmonella concentration can be determined accurately. Generally, the dilution factor was near \(-6\) for the uncooked batches where the Salmonella concentration in cooked patties was very high, while some samples at higher endpoint temperatures provided zero colony counts even when the dilution factor was \(-1\), the least dilute agar plates. This model rescales the logistic regression model from \((0, 1)\) to \((0, e^\nu)\), where \( e^\nu \) estimates the concentration of Salmonella in the uncooked patties, with the responses expected to be counts. The basic form of this three-parameter model is:

\[
Y \sim \text{NB}(\theta, \mu), \quad \text{E}(Y) = \mu, \quad \text{Var}(Y) = \mu + \mu^2/\theta
\]

\[
\mu = \frac{\exp(\nu)10^d}{1 + \exp\left[-(\alpha + \beta T)\right]} \tag{3.2}
\]

where NB denotes the negative binomial distribution and

\( Y \) is the vector of colonies counted on XLD agar plates associated with each burger patty;

\( d \) is the vector of dilution factors (between \(-1\) and \(-6\)) corresponding to each XLD agar plate.
3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers

counted;

\( \mu \) is the vector of expected *Salmonella* colony counts on XLD agar plates at dilution \( d \) and endpoint temperatures \( T \);

\( \theta \) is the dispersion parameter of the negative binomial model;

\( T \) is the vector of corrected temperatures in °C;

\( e^\nu \) is the estimated concentration of *Salmonella* in the uncooked pork patties (CFU/g); and

\( \alpha \) and \( \beta \) represent the intercept and slope of the “linear” component of the model on the logit scale.

The negative binomial distribution used in this model has been defined with probability distribution function

\[
    f_Y(y; \theta, \mu) = \frac{\Gamma(\theta + y)}{\Gamma(\theta) y!} \left( \frac{\mu}{\mu + \theta} \right)^y \left( \frac{\theta}{\mu + \theta} \right)^\theta \quad y = 0, 1, 2, \ldots
\]

(3.3)

where the standard parameters used in the negative binomial distribution have been re-parameterised such that, \( r = \theta \) and \( p = \frac{\theta}{\mu + \theta} \). The parameterisation used here is more appropriate and is suitable for cases where the variation in counts is greater than that seen in a Poisson distribution. This parameterisation of the negative binomial distribution is commonly used in regression modelling (Venables and Ripley, 2002).

Such a model can be applied in a number of ways. The basic model above (Eqn. 3.2) assumes that a single three-parameter model applies to the whole data set, while the full model would consist of applying this three-parameter model separately to each of the 18 batches of pork burgers. The form of the model used for this analysis was

\[
    \ln(\mu) = Z \nu + 10^\theta - \ln(1 + \exp[-(X_1 \alpha + X_2 \beta)])
\]

(3.4)

where the columns of \( Z \) are (0,1) indicator variables identifying the 18 batches of burger patties, the columns of \( X_1 \) contain indicator variables for the serovars, fat content (as percentage values)
3.3. Materials and Methods

and their interactions, and the columns of $X_2$ are the columns of $X_1$ multiplied by $T$, the vector of temperatures. Eqn. 3.4 is an extension of Eqn. 3.2, though the natural logarithm has been taken. Instead of taking the $\log_{10}$ of the *Salmonella* concentrations, we have chosen to model colony counts on each XLD agar plate. An offset was used to incorporate the dilution factors into the model to scale the predictive model to the *Salmonella* concentration estimated in each burger patty (CFU/g). Dilution factors and colony counts have been used previously in statistical analysis of microbial data in various applications (Commeau et al., 2012; Duarte et al., 2015; Gonzales-Barron et al., 2010b). Due to over-dispersion in the colony counts, the Poisson distribution could not be used. The quasi-Poisson model (the Poisson regression model where the dispersion parameter, $\phi$, is treated as an unknown parameter, see Venables and Ripley (2002, pp. 208-210)) and a negative binomial model were compared for their ability to predict the nominal XLD plate counts in each patty while dealing with the over-dispersion. The negative binomial model was the only model in which the regression assumptions were valid based on diagnostic plots, and hence this model was retained. For burger patties where no *Salmonella* colonies were present on the plate for the lowest dilution factor, burger patty plate counts were entered as ‘0’, with the dilution factor as the lowest dilution factor used. For the analysis, the raw patties were assigned a “cooking temperature” of 4 °C, a temperature at which no thermal inactivation should occur. The “percentage fat” values determined for each batch of mince were used. This model is not in the form of any standard Generalised Linear Model (GLM). Accordingly, an iterative process was developed using a Taylor series approximation of the model. Details of this process can be found in Section 3.A. The model was solved in the statistical programming language R (R Core Team, 2014) using the definition of the negative binomial regression model provided by Venables and Ripley (2002) in the “MASS” package and the “glm2” package (Marschner, 2014) due to convergence issues with the standard GLM fitting routine.

The interpretation of the model parameters in the linear component is that

$$p(T) = \frac{1}{1 + \exp[-(\alpha + \beta T)]}$$

(3.5)
represents the proportion, \( p \), by which the \( \text{Salmonella} \) concentration has been reduced at temperature \( T \) and \( \mu = \exp(\nu)10^d p(T) \). This interpretation can be used to estimate the minimum temperature required to achieve any nominal reduction in the \( \text{Salmonella} \) concentration. For example, the \( LD_{90} \), representing a 99% reduction, is achieved when \( p(T) = 0.01 \), at which point \( \exp[-(\alpha + \beta T)] = 99 \). Solving this, and similar formulae, provides the temperatures required to achieve 50%, 99% and 99.999% reduction in the \( \text{Salmonella} \) concentration as:

\[
LD_{50} = -\frac{\alpha}{\beta}, \quad LD_{90} = -\frac{\alpha + 4.595}{\beta}, \quad LD_{99.999} = -\frac{\alpha + 11.5129}{\beta}. \quad (3.6)
\]

A mixed effects model was used to analyse the temperature profiles of the patties during cooking. The temperature measurements, taken every 90 seconds, and the times required for each patty to reach the target internal temperature were combined. These data were analysed as a split-split-plot design with multiple patties in each batch and multiple observations for each patty. The fixed effects included main effects for the cooking time (linear and quadratic terms), cooking order, fat content (as continuous percentage values, as determined by testing) and serovar. Interaction terms were included for fat content and temperature (linear and quadratic terms) as well as the interaction of cooking order and temperature (linear term only). The random effects included random intercepts, slopes and quadratic terms for cooking time. The model was implemented using the “lme” function of the “nlme” R package (Pinheiro et al., 2014). Standard regression diagnostic plots were used to verify model assumptions.

### 3.4. Results

#### 3.4.1. Fat Content, pH and Temperature Calibration

Overall, the actual fat content of mince was lower than indicated on the retail labels. For packages marked as containing 5% fat, the mean fat content was 3.04%, standard deviation (SD) 0.51%, minimum 2.51% and maximum 4.08%. For the packages marked as containing 17% fat, the
3.4. Results

![Figure 3.1](image_url)

**Figure 3.1:** *Salmonella* concentration after cooking (log$_{10}$ CFU/g) versus the final internal cooking temperature. Individual regression fits are presented for mince with a fat content of 2.99% and 12.35% fat. These two values represent the mean of the two distinct groups of fat content observed, those with less than 4% fat ($n = 12$) and those with greater than 10% fat ($n = 6$).

Mean fat content was 9.19%, SD 5.00%, minimum 2.76% and maximum 14.64%. The fat content in three batches of mince marked as containing 17% fat were more consistent with the lower fat mince packages, explaining the higher SD for the packages marked as containing 17% fat. As a result, subsequent analysis used the actual estimated fat content values as percentages instead of the nominal values given on the pork mince packages. The mean fat content across all batches of mince was 6.11%. The pH of the mince ranged from 5.58 to 6.07 with a mean of 5.79. The experimental thermometer read 1.0 °C lower than the reference thermometer between 23.2 °C and 53.8 °C (reference thermometer temperatures). To correct for this, all internal endpoint temperatures for the cooked burgers were increased by 1 °C in the analysis, resulting in a corrected cooking temperature range of 46–64 °C.
### 3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers

Table 3.2: *Salmonella* concentrations in raw and cooked patties with associated cooking times required to achieve the target internal endpoint temperature. Values given are the mean and standard deviation for each group of concentrations (log_{10} CFU/g) and times (minutes). Temperatures have been corrected based on the thermometer calibration results.

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Endpoint Temperature</th>
<th>“Extra Lean” Fat</th>
<th>“Regular” Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time</td>
<td>Concentration</td>
<td>Time</td>
</tr>
<tr>
<td>S. 4, [5], 12:i:-</td>
<td>raw</td>
<td>7.24 ± 0.42</td>
<td>7.42 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>5.86 ± 1.36</td>
<td>6.29 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>6.81 ± 0.19</td>
<td>5.42 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>7.83 ± 0.58</td>
<td>4.11 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>7.72 ± 0.43</td>
<td>3.91 ± 1.25</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>8.46 ± 0.37</td>
<td>3.13 ± 0.85</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>9.35 ± 0.65</td>
<td>1.55 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>10.98 ± 0.54</td>
<td>2.61 ± 0.65</td>
</tr>
<tr>
<td>S. Senftenberg</td>
<td>raw</td>
<td>7.35 ± 0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>6.33 ± 0.30</td>
<td>6.24 ± 0.33</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>7.04 ± 0.15</td>
<td>5.23 ± 0.45</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>7.33 ± 1.61</td>
<td>4.86 ± 1.09</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>7.57 ± 0.59</td>
<td>3.03 ± 1.68</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>8.33 ± 0.38</td>
<td>2.99 ± 1.08</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>9.13 ± 1.02</td>
<td>2.38 ± 0.78</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>9.56 ± 0.22</td>
<td>1.66 ± 1.41</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>raw</td>
<td>7.64 ± 0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>46</td>
<td>5.93 ± 1.28</td>
<td>6.35 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>6.83 ± 0.78</td>
<td>5.50 ± 0.69</td>
</tr>
<tr>
<td></td>
<td>52</td>
<td>7.37 ± 0.87</td>
<td>5.01 ± 1.34</td>
</tr>
<tr>
<td></td>
<td>55</td>
<td>7.36 ± 1.03</td>
<td>3.29 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>58</td>
<td>8.23 ± 0.76</td>
<td>3.12 ± 0.42</td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>9.46 ± 0.67</td>
<td>2.75 ± 0.75</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>10.56 ± 1.15</td>
<td>3.35 ± 0.82</td>
</tr>
</tbody>
</table>
### 3.4. Results

**Figure 3.2.** *Salmonella* concentration after cooking (log$_{10}$ CFU/g) versus the final internal cooking temperature. The *Salmonella* concentrations at 4 °C represent the uncooked patties. For each *Salmonella* serovar, the regression model estimated is presented for the mean fat level (6.11%) accompanied by the notional concentrations estimated for each serovar.

**Table 3.3.** Analysis of deviance table for the inactivation of *Salmonella* in pork burgers.

<table>
<thead>
<tr>
<th></th>
<th>Df</th>
<th>Deviance</th>
<th>Resid. Df</th>
<th>Resid. Dev.</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NULL</td>
<td>142</td>
<td>873.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>1</td>
<td>252.85</td>
<td>141</td>
<td>621.11</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>359.02</td>
<td>140</td>
<td>262.09</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Batch</td>
<td>17</td>
<td>81.21</td>
<td>123</td>
<td>180.88</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Serovar</td>
<td>2</td>
<td>0.31</td>
<td>121</td>
<td>180.57</td>
<td>0.86</td>
</tr>
<tr>
<td>Fat</td>
<td>1</td>
<td>0.53</td>
<td>120</td>
<td>180.04</td>
<td>0.47</td>
</tr>
<tr>
<td>Temperature × Serovar</td>
<td>2</td>
<td>4.55</td>
<td>118</td>
<td>175.49</td>
<td>0.10</td>
</tr>
<tr>
<td>Temperature × Fat</td>
<td>1</td>
<td>3.28</td>
<td>117</td>
<td>172.21</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Table 3.4: Regression table for the inactivation model. All coefficients given in this table are in ln CFU/g.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>Std. Error</th>
<th>z value</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\nu$ parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw Intercept</td>
<td>17.15</td>
<td>0.71</td>
<td>24.16</td>
<td>&lt;0.0001</td>
<td>(16.40, 18.15)</td>
</tr>
<tr>
<td>Batch B</td>
<td>1.61</td>
<td>0.99</td>
<td>1.63</td>
<td>0.10</td>
<td>(0.34, 2.97)</td>
</tr>
<tr>
<td>Batch C</td>
<td>0.92</td>
<td>1.00</td>
<td>0.92</td>
<td>0.36</td>
<td>(–0.53, 2.23)</td>
</tr>
<tr>
<td>Batch D</td>
<td>0.35</td>
<td>0.87</td>
<td>0.40</td>
<td>0.69</td>
<td>(–0.34, 1.82)</td>
</tr>
<tr>
<td>Batch E</td>
<td>0.55</td>
<td>1.00</td>
<td>0.55</td>
<td>0.58</td>
<td>(–0.95, 1.61)</td>
</tr>
<tr>
<td>Batch F</td>
<td>1.20</td>
<td>1.04</td>
<td>1.16</td>
<td>0.25</td>
<td>(–0.26, 3.08)</td>
</tr>
<tr>
<td>Batch G</td>
<td>0.02</td>
<td>1.05</td>
<td>–0.02</td>
<td>0.99</td>
<td>(–1.05, 1.71)</td>
</tr>
<tr>
<td>Batch H</td>
<td>–0.98</td>
<td>1.01</td>
<td>–0.97</td>
<td>0.33</td>
<td>(–2.41, 0.16)</td>
</tr>
<tr>
<td>Batch I</td>
<td>1.40</td>
<td>1.03</td>
<td>1.36</td>
<td>0.17</td>
<td>(0.27, 2.91)</td>
</tr>
<tr>
<td>Batch J</td>
<td>0.49</td>
<td>0.99</td>
<td>0.50</td>
<td>0.62</td>
<td>(0.04, 2.76)</td>
</tr>
<tr>
<td>Batch K</td>
<td>0.11</td>
<td>1.02</td>
<td>0.11</td>
<td>0.91</td>
<td>(–1.28, 1.36)</td>
</tr>
<tr>
<td>Batch L</td>
<td>1.55</td>
<td>1.01</td>
<td>1.53</td>
<td>0.13</td>
<td>(0.40, 3.40)</td>
</tr>
<tr>
<td>Batch M</td>
<td>–0.77</td>
<td>0.73</td>
<td>–1.05</td>
<td>0.30</td>
<td>(–1.82, 0.17)</td>
</tr>
<tr>
<td>Batch N</td>
<td>2.25</td>
<td>0.74</td>
<td>3.06</td>
<td>0.002</td>
<td>(1.00, 3.24)</td>
</tr>
<tr>
<td>Batch O</td>
<td>–1.04</td>
<td>0.84</td>
<td>–1.25</td>
<td>0.21</td>
<td>(–1.76, 0.40)</td>
</tr>
<tr>
<td>Batch P</td>
<td>1.26</td>
<td>0.82</td>
<td>1.53</td>
<td>0.13</td>
<td>(0.37, 2.89)</td>
</tr>
<tr>
<td>Batch Q</td>
<td>–0.37</td>
<td>0.98</td>
<td>–0.38</td>
<td>0.70</td>
<td>(–1.82, 0.65)</td>
</tr>
<tr>
<td>Batch R</td>
<td>–1.35</td>
<td>1.00</td>
<td>–1.35</td>
<td>0.18</td>
<td>(–2.77, –0.09)</td>
</tr>
<tr>
<td>$\alpha$ parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Linear” Intercept</td>
<td>19.4575</td>
<td>3.0571</td>
<td>6.37</td>
<td>&lt;0.0001</td>
<td>(14.02, 25.20)</td>
</tr>
<tr>
<td>S. Senftenberg</td>
<td>2.8915</td>
<td>3.1837</td>
<td>0.91</td>
<td>0.364</td>
<td>(–3.30, 9.06)</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>–3.5511</td>
<td>2.9943</td>
<td>–1.19</td>
<td>0.236</td>
<td>(–7.45, –0.87)</td>
</tr>
<tr>
<td>Fat Content</td>
<td>0.6163</td>
<td>0.2912</td>
<td>2.12</td>
<td>0.034</td>
<td>(0.0031, 1.20)</td>
</tr>
<tr>
<td>$\beta$ parameters</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>–0.4934</td>
<td>0.0524</td>
<td>–9.42</td>
<td>&lt;0.0001</td>
<td>(–0.59, –0.40)</td>
</tr>
<tr>
<td>Temp × S. Senftenberg</td>
<td>–0.0503</td>
<td>0.0544</td>
<td>–0.93</td>
<td>0.348</td>
<td>(–0.16, 0.06)</td>
</tr>
<tr>
<td>Temp × S. Typhimurium</td>
<td>0.0713</td>
<td>0.0511</td>
<td>1.40</td>
<td>0.163</td>
<td>(0.03, 0.14)</td>
</tr>
<tr>
<td>Temp × Fat Content</td>
<td>–0.0099</td>
<td>0.0049</td>
<td>–2.02</td>
<td>0.043</td>
<td>(–0.02, 0.0008)</td>
</tr>
</tbody>
</table>
3.4. Results

3.4.2. *Salmonella* Inactivation

Summaries of the concentrations of *Salmonella* in each patty are shown in Table 3.2. The mean initial concentration of *Salmonella* in the uncooked patties was 7.47 $\log_{10}$ CFU/g with standard deviation 0.29 $\log_{10}$ CFU/g. Reductions in the mean *Salmonella* concentration ranged from 1.02 $\log_{10}$ CFU/g at 46 °C to 4.87 $\log_{10}$ CFU/g at 64 °C corrected internal endpoint temperatures. The analysis of deviance is presented in Table 3.3 and the regression table for the 26 estimated parameters of the GLM in Table 3.4. The residual deviance was 172.21 on 117 degrees of freedom. 

P-values presented in the analysis of deviance table (Table 3.3) need to be interpreted with care as these values were calculated without re-estimating the value of $\theta$ for each variable removed (see Section 3.A). The slope of the model, when the mean fat content is 6.11% can be expressed as

$$\text{Slope}(6.11\% \text{ fat}) = \frac{-0.4934 - 0.0099 \times 6.11}{\ln(10)} = -0.2407 \log_{10} \text{CFU/g/°C}$$

with values above taken from Table 3.4. This means that for every 1 °C increase in temperature, there is a $0.2407 \log_{10}$ CFU/g decrease in *Salmonella* survival. The $LD_{99.999}$ value (the minimum final internal temperature required for a 5-$\log_{10}$ reduction in *Salmonella* concentration) when the fat content is 6.11% was estimated to be 63 °C. There were significant differences in the *Salmonella* concentrations between the batches ($p < 0.0001$), implying the importance of including batch in the model. Interactions between the temperature and fat content influenced *Salmonella* survival ($p = 0.043$). As the fat content increased, *Salmonella* survival is expected to increase, though this effect vanished as the internal endpoint temperature approached 62 °C. This effect is illustrated in Figure 3.1. The *Salmonella* serovar did not affect either the intercept ($p = 0.86$) or slope ($p = 0.10$) of the fitted logistic curve (Figure 3.2). The inclusion of cooking order (order from the plastic container in which burger patties were cooked in the skillet) in the model showed no significant effect on survival.

In order to reduce correlations between intercept and slope parameters in the linear component of the model, $T$ was replaced in the model fitting procedure by $(T - 55)$, thus the columns of $X_2$ in
3. Thermal Inactivation of Salmonella spp. in Pork Burgers

Table 3.5.: The correlation matrix for the eight parameters of the logistic regression component of the model.

<table>
<thead>
<tr>
<th></th>
<th>“Linear” Intercept</th>
<th>S. Senftenberg</th>
<th>S. Typhimurium</th>
<th>Fat content</th>
<th>Temperature</th>
<th>Temp × S. Senftenberg</th>
<th>Temp × S. Typhimurium</th>
<th>Temp × fat content</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Linear” Intercept</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Senftenberg</td>
<td>-0.71</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>-0.60</td>
<td>0.52</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat content</td>
<td>-0.73</td>
<td>0.35</td>
<td>0.17</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>-0.06</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp × S. Senftenberg</td>
<td>0.05</td>
<td>-0.08</td>
<td>-0.05</td>
<td>-0.02</td>
<td>-0.71</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temp × S. Typhimurium</td>
<td>0.05</td>
<td>-0.05</td>
<td>-0.08</td>
<td>0.00</td>
<td>-0.61</td>
<td>0.54</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Temp × fat content</td>
<td>0.05</td>
<td>-0.02</td>
<td>0.00</td>
<td>-0.12</td>
<td>-0.71</td>
<td>0.33</td>
<td>0.15</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Eqn. 3.4 were the columns of $X_1$ multiplied by $(T - 55)$, where 55 °C was the median endpoint temperature investigated. Correlations between intercepts and slopes are thereby reduced to almost zero, while correlations between the first parameter in each set of four and the other three are approximately $-1/\sqrt{2} = -0.71$. Batch effects are not included as they are essentially random effects. The correlation matrix for the eight logistic model parameters given in Table 3.4 is presented in Table 3.5.

3.4.3. Temperature Profile

Cooking times ranged from 3.9 to 12.3 minutes with a mean of 8.1 minutes and were generally longer than those reported by Juneja et al. (1997) (2.25 to 4 minutes). Temperature profiles for each burger patty cooked are presented in Figure 3.3. The interaction of cooking time and cooking order influenced internal temperature ($p < 0.0001$), with patties cooked later in each batch having higher internal temperatures. This difference diminished with increasing cooking times. The interaction of the fat content and cooking time also influenced the internal temperature of the patties, with both the linear ($p = 0.0106$) and quadratic ($p = 0.0309$) terms being statistically
3.5. Discussion

3.5.1. Effect of Fat Content

In this study, the fat content of pork mince had a significant effect on Salmonella survival, with increasing fat content correlated with longer Salmonella persistence during cooking. The effect was reduced at higher cooking temperatures. Increasing fat content was also correlated with slower heating of the patties.

Our observations on the effect of fat content are consistent with those of Juneja et al. (2000), who...
found that $D$-values inferred from data obtained early in the cooking process were longer for pork and beef mince with higher fat levels. Our results also concur with Juneja et al. (2001a) who found that lag times before inactivation during cooking increased with increasing fat levels in chicken and turkey. Smith et al. (2001) found that *Salmonella* in beef mince containing 19% fat was more heat resistant than beef mince containing 4.8% fat. While the effect of fat content hindering *Salmonella* inactivation has been observed in many foodstuffs by many authors, no literature could be identified to explain the potential mechanism, or whether different mechanisms exist for different forms of fat. Peanut butter is a product where typical fat levels (50.0% total fat) have been observed to enhance *Salmonella* survival compared to product with reduced fat (33.3% total fat) (Li et al., 2014).

One possible explanation for the effect of fat content on *Salmonella* survival is that the fat in the pork mince may hinder the transfer of heat from the outside surfaces, that were in contact with the skillet, to the patty’s geometric centre. The reason for this is not clear, though it appears that the fat in the mince does not transfer heat to the interior of the burger patty as effectively as the muscle tissues. This could be caused by the heat being required to melt the extra fat in the higher fat burgers (Houšová and Topinka, 1985) and/or the thermal conductivity of fat is lower than other components of the patty (Oroszvári et al., 2005). Increasing fat content has been linked previously with increasing cooking times (Oroszvári et al., 2005), though the opposite result has also been shown, with decreased cooking times linked to increasing fat content (Troutt et al., 1992).

### 3.5.2. Effect of Serovar

Non-significant differences between serovars in terms of *Salmonella* survival are consistent with previous research, e.g. van Asselt and Zwietering (2006), who observed “no significant differences between strains, products or laboratory media tested for most pathogens”, with the exception of *Salmonella* in chocolate. In this study, *S. Senftenberg* had the fastest rate of inactivation and
S. Typhimurium the lowest. *S. Senftenberg* 775W is particularly heat resistant (Ng et al., 1969), though this difference in heat resistance compared to other *Salmonella* serovars decreases at reduced water activities (Baird-Parker et al., 1970; Goepfert et al., 1970; Silva and Gibbs, 2012). It appears that *Salmonella* 4,[5],12:i:-, a monophasic variant of *S. Typhimurium*, has a similar heat resistance to *S. Senftenberg*. This finding is useful as little research has been conducted on *S. 4,[5],12:i:-*, an emerging *Salmonella* serovar (EFSA BIOHAZ Panel, 2010).

### 3.5.3. Temperature Profile

Differences in cooking times observed in this experiment compared to cooking times observed by Juneja et al. (1997) are possibly due to the thicker burgers used in this study. Dagerskog (1979) showed that increasing thickness in burger patties increases the cooking time required to reach a set internal temperature. The increase in internal temperature in patties cooked later in each batch at lower cooking times was possibly caused by the whole batch of patties being removed from the refrigerator at the same time, so that patties cooked later would initially be warmer. Including this effect in the model allowed its effect on the temperature increase to be standardised in the model.

### 3.5.4. Predictive Modelling

To our knowledge, this is the first time that this form of the model by Wadley (1949) has been used to analyse inactivation data. This form of inactivation model can be used in any scenario where the rate of pathogen inactivation is assumed to be log-linear decreasing with respect to time or temperature. The advantage of this is that the *Salmonella* concentrations in the raw burger patties can be incorporated into the analysis. For predictive purposes, a linear model needs to be artificially truncated at *Salmonella* concentrations greater than those observed in the raw burgers. As this model can be fitted to data without the use of non-linear methods, it allows for the investigation of covariates in a more simplified manner compared to non-linear models. This model also uses the raw XLD agar plate counts and dilution factors, instead of the calculated
3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers

count values. The use of raw data in estimating the true prevalence and concentration of foodborne organisms has become more common recently (Commeau et al., 2012; Duarte et al., 2015; Gonzales-Barron et al., 2010b).

High variability was observed in the *Salmonella* counts at the same internal endpoint temperature (see standard deviations in Table 3.2). Possible sources of variability could include the surface temperature of the skillet or the placement of the thermocouple. The surface temperature of the skillet at setting 8 was observed, using an infrared thermometer, to be highly variable, ranging from around 160 °C to 260 °C. This may be due to poor temperature control by the skillet thermostat, which may have contributed to the variability. Another potential source of variability is the placement of the thermocouple during this experiment. As previously stated, the changing dimensions of the patties during cooking meant a physical device designed to accurately place the thermocouple would not consistently locate the centre of the patty. Based on how frequently the patties were turned, the coldest part of the burger patty during cooking would be the geometric centre of the patty. While placing the thermometer “by eye” may have added to the variability in the final *Salmonella* counts observed, this approach is similar to the method that a person cooking a burger patty (either domestically or commercially) would use to place a thermometer in a patty to judge doneness. Food safety experts and regulatory bodies state that burgers should be cooked to a minimum internal endpoint temperature (USDA-FSIS state 160 °F, Van (2011)). This provides consumers and food service with a simple target to achieve as indicated on a thermometer to guarantee the safety of the burger patty, and removes variables of thickness and duration from assessment of the safety of the cooking process. A poorly positioned thermometer may lead to overestimates of the endpoint temperature reached for each patty cooked, and thereby, overestimates of the safety of the cooked burger patty.

Previous research has measured the inactivation of *E. coli* in beef burger patties (Juneja et al., 1997; Røssvoll et al., 2014) but no reports have specifically assessed inactivation of *Salmonella* in pork burger patties. Juneja et al. (1997) found that the concentration of *E. coli* O157:H7 in
beef burger patties with an initial starting concentration of 6.6 log_{10} CFU/g was given by

\[
\log_{10} \text{CFU}(E. \ coli \ O157:H7)/g = 16.69 - 0.21 \times T(\degree C)
\]  

where \( T \) is the internal temperature (converted from the original Fahrenheit), irrespective of cooking time. To allow direct comparison, a linear model fitted to our results for \textit{Salmonella} concentrations (log_{10} CFU/g) with temperature as the only predictor variable yielded the equation

\[
\log_{10} \text{CFU}(\text{Salmonella})/g = 16.88 - 0.24 \times T(\degree C) \quad (R^2 = 0.76).
\]

Both of these equations are based on different initial pathogen concentrations in the raw burger patties. To allow for a more accurate comparison between these two models, the pathogen concentrations determined from the raw patties (6.6 log_{10} CFU/g \textit{E. coli} \ O157:H7 and 7.47 log_{10} CFU/g \textit{Salmonella}) were subtracted from the estimated intercepts of Eqns. 3.7 and 3.8. The resulting equations describe the relative reduction in pathogen concentrations for each of the two target organisms. These equations are

\[
\log_{10} \text{CFU relative reduction (\textit{E. coli} O157:H7)}/g = 10.09 - 0.21 \times T(\degree C)
\]

\[
\log_{10} \text{CFU relative reduction (\textit{Salmonella})}/g = 9.41 - 0.24 \times T(\degree C)
\]

where Eqns. 3.7 and 3.9 are only valid for \( T \) between 56.1 \degree C and 74.4 \degree C (Juneja et al., 1997) and Eqns. 3.8 and 3.10 are only valid for \( T \) between 46 \degree C and 64 \degree C. The slopes of Eqns. 3.9 and 3.10 are similar, though the intercepts appear to be different. In the range of inactivation temperatures, Eqn. 3.9 estimates a higher \textit{E. coli} O157:H7 concentration compared to Eqn. 3.10. The reason for this difference could be due to the thicker patties used in our study, which would increase the time required to reach the same internal endpoint temperature (Dagerskog, 1979). It could also be due to differences in the raw \textit{Salmonella} concentration between the two studies, differences in inactivation rates between the \textit{Salmonella} serovars used in our study and
3. Thermal Inactivation of *Salmonella* spp. in Pork Burgers

*E. coli* O157:H7 strains used by Juneja et al. (1997), and/or differences in inactivation rates of *Salmonella* and *E. coli* O157:H7 in pork and beef mince, respectively. In this study, a longer cooking time was required than reported by Juneja et al. (1997) to achieve the same internal endpoint temperature in the patties which may have created large differences in the temperatures of the patty surface and geometric centre. Due to the additional time required for heat penetration in the thicker patties used in our experiments, a given temperature at the geometric centre of the patty would be expected to result in a higher surface temperature compared to Juneja et al. (1997). If this is true, as patty thickness increases, the mean reduction in foodborne pathogens would increase for the same internal cooking temperature.

Care must be taken when extrapolating past 64 °C, as it is unknown how the inactivation curves will behave. Assuming the relationship between *Salmonella* survival and temperature continues, the relationship between fat content and *Salmonella* survival will also reverse at these temperatures, so that an increase to fat content will result in a reduction in *Salmonella* survival compared to a lower fat content pork mince. These issues should not have a great impact on the use of the model for risk assessment as at these higher temperatures, any predicted reduction will result in a greater than 5-log$_{10}$ CFU/g reduction in *Salmonella* concentration.

3.5.5. Doneness and Colour

The internal colour of pork burger patties may also be a poor indicator of doneness as was reported for beef burger patties (Røssvoll et al., 2014). In this experiment, all patties cooked were observed to have varying degrees of pinkness in the centre, with patties cooked to a higher temperature appearing less pink. Hague et al. (1994) found that only 34% of the variation in beef patty colour could be explained by the cooking temperature and that burgers cooked to 66 °C could appear to be as brown as burgers cooked to 71 °C. Increased pH in beef and pork mince has been linked to increased pinkness after cooking to the same internal temperature (Trout, 1989). Differences in pinkness in beef burger patties can also be explained by the total pigment in the mince, with
Mendenhall (1989) observing an increase in pigment leading to increased redness in bull meat patties. This may also be true for pork burger patties, though no information concerning pigment of the pork mince was obtained in this experiment.

3.5.6. Practical Findings

The two observations concerning the influence of fat content may be linked: greater *Salmonella* survival after cooking may be a result of the reduced rate of temperature increase, due to the increased fat content of the mince. These findings may also be independent; the fat level in the mince may hinder *Salmonella* inactivation and may also hinder heat transfer to the geometric centre of the patty. These findings have implications for both commercial food service providers and consumers. Commercial establishments may need to validate their cooking practices for pork burger patties against variable fat contents for pork mince. By not considering the fat content, the risk of salmonellosis from consumption may be underestimated during commercial cooking process validation, and pork burgers with increased fat content sold to consumers may be undercooked. As this is a critical control point for *Salmonella* in commercial food service, small differences in *Salmonella* concentration caused by the fat content could have important effects on overall risk to consumers. Consumers at retail are offered a choice of mince with multiple fat contents, with the reduced fat content being the more expensive option. For a given cooking time and temperature, higher fat content mince used to make pork burgers could increase the risk of salmonellosis from consumption. Thus, consumers who select reduced fat pork mince for their burgers may have a reduced risk of salmonellosis.

3.6. Conclusions

The relationship between the internal cooking temperature and the reduction in *Salmonella* survivors was quantified for *Salmonella* 4,[5],12,i:-, *S*. Senftenberg and *S*. Typhimurium at varying fat levels for pork burgers that were approximately 2cm thick. The fat level of the mince
3. Thermal Inactivation of Salmonella spp. in Pork Burgers

had a significant effect on the Salmonella survival, with increased fat in the pork mince leading to increased Salmonella survivors. Using reduced fat pork mince may reduce the risk of illness from consumption at cooking temperatures lower than 62 °C. The serovar of Salmonella did not affect Salmonella survival. A logistic regression model, based on Wadley (1949) and not previously used in this context, was fitted successfully to the data and allowed inclusion of data obtained from uncooked patties in the analysis. This research will be useful in informing risk assessments conducted into the risk of salmonellosis from consumption of contaminated pork burgers.

3.A. Statistical Fitting of the model

The link function described in Section 3.3.8 is not in the form of any of the link functions recognised by the GLM routine in the R statistical package (R Core Team, 2014). The first order Taylor Series expansion of the link function was obtained at \((a_0, \beta_0)\) to reformulate the link function into the form of a log link with offset, which can be solved as a GLM. The Taylor series expanded link function is

\[
\ln(\mu) = d \ln(10) + Zv + \ln(p) \\
\approx d \ln(10) + Zv + \ln(p_0) + \frac{\partial \ln (p_0)}{\partial \alpha} (\alpha - \alpha_0) + \frac{\partial \ln (p_0)}{\partial \beta} (\beta - \beta_0).
\]

Now

\[
\frac{\partial \ln (p_0)}{\partial \alpha} = (1 - p_0)X_1 \quad \text{and} \quad \frac{\partial \ln (p_0)}{\partial \beta} = (1 - p_0)X_2.
\]

So we get

\[
\ln(\mu) = [d \ln(10) + \ln(p_0) - W_1 \alpha_0 - W_2 \beta_0] + Zv + W_1 \alpha + W_2 \beta
\]
where the term in square brackets represents the offset parameter, evaluated at the current estimates \((\alpha_0, \beta_0)\), and the parameters \((\nu, \alpha, \beta)\) are the parameters to be estimated. The columns of \(Z\) are \((0, 1)\) indicator variables identifying the 18 batches and \(W_1\) and \(W_2\) are simply \(X_1\) and \(X_2\), with each column multiplied by \((1 - p_0)\).

Initial parameter estimates for \(\alpha_0\) and \(\beta_0\) in the above link function were obtained by solving the model as a negative binomial GLM with log link function and \ler\-variables consisting of an indicator variable (0 for raw and 1 for cooked) and the cooking temperature. These parameter estimates were then used as starting values for \(\alpha_0\) and \(\beta_0\) in the offset parameter of the Taylor series expansion above in the GLM routine. Once the GLM routine had converged locally, the parameter estimates produced were used as starting values in the next iteration of the GLM routine to estimate the model parameters at the new starting values. This process was continued until the model converged globally. The convergence criterion, locally (as determined by the GLM routine) and globally, was

\[
\frac{|\text{deviance}_{\text{new}} - \text{deviance}_{\text{old}}|}{|\text{deviance}_{\text{new}}| + 0.1} < 10^{-8}.
\]
4. Growth of *Salmonella* in Moisture-Infused Pork

4.1. Abstract

Pork loin steaks were injected with a brine solution containing a cocktail of *Salmonella* serovars and incubated at 8, 15, 20, 25 or 30 °C. At appropriate times, based on expected growth rates from published predictive models, *Salmonella* levels in samples were enumerated. *Salmonella* growth was observed at temperatures above 14 °C, with distinct exponential growth and stationary phases. One-step and two-step model fitting procedures were used to estimate the growth rate and maximum population density in moisture-infused pork steaks, with the two model fits compared. Despite limited data, estimates of the change in growth rate and maximum population density with respect to temperature were made, but the two fitting procedures identified the one-step approach as superior, producing narrower confidence intervals around parameter estimates. Growth rate and maximum population density estimates were also compared with ComBase data. Growth characteristics of *Salmonella* in moisture-infused pork were similar to those in intact, non-infused pork and comparable with ComBase data, suggesting that existing models for *Salmonella* growth rate in meat and poultry could be used to estimate *Salmonella* growth in moisture-infused pork.
4.2. Introduction

During the infusion process, a brine solution is injected into pork primals to increase their weight to 10% above their standard weight to improve its sensory qualities (APL, 2009). During planning for the risk assessment of salmonellosis from consumption of moisture-infused pork, a number of data gaps were identified, including whether *Salmonella* could grow on/in infused pork and, if growth was possible, at what rate. The work described in this chapter investigates *Salmonella* growth potential in moisture-infused pork and develops a predictive model for *Salmonella* growth rate in moisture-infused pork, which is used in Chapter 6 of this thesis.

Non-intact meat products are defined as products which have been mechanically tenderised, injected, vacuum tumbled or have had proteolytic enzymes applied (USDA-FSIS, 2011). They represent a large proportion of fresh meat sold in USA each year (Muth et al., 2012) and are now being promoted in Australia. In moisture infusion, also known as moisture enhancement or brine injection, meat products are injected with a brine solution that can include water, salt, sodium phosphates, sodium/potassium lactates, flavourings and other ingredients (APL, 2015). Moisture infusion is used in some countries to offset moisture loss during cooking and has been shown to improve aroma, flavour, tenderness, juiciness and overall acceptability of cooked pork (Moore et al., 2012). During the infusion process, a brine solution is held in a reservoir called a “balance tank” and a grid of hollow needles is used to inject the brine solution into the product interior. Any excess brine not retained by the product is returned to the balance tank. Brine returning to the balance tank can be contaminated with foodborne pathogens from the product or other sources, including those belonging to the Enterobacteriaceae family (Greer et al., 2004). Contaminated recirculated brine could subsequently be injected into processed primals, with those primals becoming internally contaminated. Brine injected beef products have been linked to outbreaks of foodborne illness (Heiman et al., 2015; Laine et al., 2005), highlighting the risk posed by these manufacturing processes.

As discussed in Chapter 1, *Salmonella* is one of the largest causes of foodborne illness worldwide.
4. Growth of *Salmonella* in Moisture-Infused Pork

In USA (Scallan et al., 2011), Europe (EFSA, 2013) and Australia (Department of Health Australia, 2016), *Salmonella* is the second largest reported cause of foodborne illness, exceeded only by *Campylobacter*. Symptoms of salmonellosis can include nausea, vomiting, abdominal cramps, diarrhoea, fever and headaches and, in rare cases, death. Those at greatest risk of more serious complications and/or sequelae are the elderly, young and the immunocompromised (FDA, 2012). Many products, including pork, have been implicated in salmonellosis outbreaks in Australia (OzFoodNet Working Group, 2011, 2012a,b).

*Salmonella* typically does not grow in foods at temperatures lower than 7 °C (ICMSF, 1996) and growth can also be influenced by sodium chloride, with increasing concentrations resulting in slower *Salmonella* growth (Gibson et al., 1988). However, little information is available on the growth rate of *Salmonella* in moisture-infused pork products. While studies into changes in the population of *Salmonella* in vacuum packaged, moisture-infused pork exist (Wen and Dickson, 2012), these data cannot be easily translated to the moisture-infused pork steaks considered in this thesis because they are also sold in Australia at retail in either modified atmosphere packaging, or unpackaged in bulk, under aerobic conditions. Additionally, those studies were conducted at 4 °C and 10 °C only. The aim of this study was to determine if growth of *Salmonella* was possible in moisture-infused pork steaks under aerobic conditions at a wider range of temperatures, including temperature abuse and, if so, to determine the growth rate as a function of temperature and to assess whether existing predictive models for *Salmonella* growth can adequately describe growth in moisture-infused pork or whether specialised models are needed.

4.3. Methods

4.3.1. Preparation of Pork Steaks

Non-infused pork loin medallions were specifically ordered and purchased from a local butcher in Hobart, Tasmania, on two occasions for all experiments conducted. They were cut on request
into approximately 2.5 cm thick steaks and transported to the laboratory in an insulated box
containing ice bricks, with the travel time less than 15 minutes. On arrival at the laboratory, the
steaks were stored at 4 ± 1 °C.

For each experiment, 50ml of Zart-o-Fresh (MBL Food Service, Adelaide, Australia) brine
premix was prepared as per the manufacturer’s instructions, by adding 7.5g of brine premix to
42.5g of sterilised tap water. Brine was then refrigerated (4 ± 1 °C) for a maximum of four hours
until required for injection.

*Salmonella* cultures were prepared as a cocktail of four *Salmonella enterica* serovars, containing
*Salmonella* 4,[5],12:i:-, *S. Typhimurium* PT35, *S. Typhimurium* PT135 and *S. Infantis*. All
serovars used in this study had been isolated from porcine sources, serotyped and stored long-
term in snap freeze medium (Oxoid, TM0171) at -80 °C. As needed, isolates were removed from
the -80 °C freezer and inoculated onto a nutrient agar slope (Catalogue Number 3305, Micromedia
Pty Ltd, Moe, Australia), which was incubated for 24 hours at 37 ± 1 °C. Each isolate was then
streaked onto a nutrient agar plate and incubated at 37 ± 1 °C for 18 ± 2 hours to assess culture
purity, and then stored at 4 ± 1 °C for the duration of the experiment. For each experiment, a single
colony of each serovar was picked off and inoculated into 10mL of Brain Heart Infusion broth
(BHI, Oxoid, CM 1135), and incubated at 37 ± 1 °C for 18 ± 2 hours. Cultures were centrifuged
twice at 1667×g for 15 minutes at 4 °C (Hettich Zentrifugen EBA 12, Tuttlingen, Germany) and
re-suspended in 1ml of Buffered Peptone Water (BPW, Oxoid, CM 1049), supplemented with 1%
NaCl after each centrifugation step. Cultures were temperature-conditioned (4 °C) because pork
meat and brine are refrigerated during commercial processing.

Salt (1% NaCl) was included to condition the cells to the environment inside brine injected pork
steaks. Cultures were combined by re-suspending the first culture in 1mL of BPW containing 1%
NaCl and using that suspension to re-suspend the other cultures. From this mixed culture, 5μL
was re-suspended in 50ml of the prepared brine mixture.

Steaks were placed on a sanitised (70% ethanol:water) plastic chopping board. Each steak was
weighed to determine the volume of brine to be injected into each steak. The weight of brine injected was 10% of the weight of the steak, as per the manufacturer’s instructions. An SGE NLL-7/18 luer needle (77mm long, 18 gauge) was attached to a syringe and each steak was injected with brine 3-4 times depending on the weight of the steak. Two injections were made lengthways from one end of the steak and either one or two injections from the other end. For each brine injection, the syringe was inserted as far as possible into the steak without exiting the other side and, as the needle was slowly withdrawn, the brine was injected. The brine was apportioned approximately equally between the injection sites.

4.3.2. Incubation Conditions

Inoculated and infused steaks were incubated at nominal temperatures of 8, 15, 20, 25 and 30 °C. For each incubation temperature, two injected steaks were cut into 13 strips of approximately equal width, perpendicular to the direction of injection and placed into Nasco Whirl-Pak sterile bags (B01196WA, 150X230MM, Fort Atkinson, WI). At 15 °C, replicate steaks were also placed into vacuum bags and vacuum sealed at 96% vacuum (Technovac T60, Bergamo, Italy). From each steak, six strips were placed into the assigned incubator. From each group of steaks injected, one strip was not incubated but used immediately to enumerate the initial Salmonella concentration. The temperature of each incubator was monitored throughout the experiment, with the temperature logged at least once per hour using a combination of temperature data loggers (iButton, DS1921G, Maxim Integrated Products Inc., Dallas, TX, USA) and a temperature monitoring system (Saveris T3 D - 2-channel temperature probe, Testo, Inc, Sparta, NJ, USA).

4.3.3. Salmonella Enumeration

Pork strips were removed from incubators at pre-determined times, i.e., estimated for each incubation temperature from published predictive models for growth rates of Salmonella in meat. Each strip was suspended in 9 times the BPW without added NaCl and stomached for 5 minutes
4.3. Methods

Serial ten-fold dilutions of this suspension were prepared in BPW without added NaCl and 0.1 mL of appropriate dilutions plated on Xylose Lysine Deoxycholate agar (XLD, Oxoid, CM 0469), and incubated at 37 °C for 18 to 24 hours. Typical *Salmonella* colonies on XLD were counted and presumptive *Salmonella* concentration \( \log_{10} \text{CFU g}^{-1} \) calculated for each time-temperature combination.

### 4.3.4. Sterilised Pork Experiment

To determine if the injection procedure resulted in a uniform distribution of *Salmonella* in all pork strips cut from each injected steak and to assess the reliability of enumeration of (potentially injured) *Salmonella* from moisture-infused pork by directly plating onto the highly selective XLD agar media, an additional study was conducted. A piece of pork loin was purchased from the same retailer (see Section 4.3.1) and cut to be approximately 10 cm thick. The portion was placed in boiling water for approximately 10 seconds to surface-sterilise it with respect to vegetative bacterial cells. This was done to ensure that only *Salmonella* injected into the steaks and not any *Salmonella* on the loin at retail would be enumerated. A sanitised (70% ethanol:water) knife was used to cut away the boiled surface tissue and the remaining material cut into two steaks approximately 2.5 cm thick from the centre of the loin section to mimic the steaks used in the growth studies described above. Surface sterilised steaks were then injected with the *Salmonella* and brine cocktail and sliced as described previously. Samples were immediately processed as described above to enumerate *Salmonella* using XLD and total viable counts using Standard Plate Count agar (Oxoid, CM 0463), which were incubated at 37 °C for 18 to 24 hours.

### 4.3.5. Statistical Analysis

Two model fitting procedures were compared for their ability to describe the growth data obtained. The first, a two-step procedure, is the most commonly used method of modelling in predictive microbiology. For two-step fitting, growth curves are fitted to the concentration data from each
incubation temperature to describe the change over time. The parameter estimates of each growth curve are used as responses in the secondary model, which relate those responses to temperature or other environmental factors (Jewell, 2012). The second approach, a one-step routine (Jewell, 2012), uses non-linear modelling techniques to combine primary and secondary models and thus models the change in *Salmonella* numbers as a function of time and temperature simultaneously. The one-step approach has been reported to produce more precise coefficient estimates compared to estimates obtained by two-step fits (Jewell, 2012).

For one-step model fitting, the three-parameter growth model of Baranyi and Roberts (1994), which describes the exponential growth and stationary phases and does not include a lag phase parameter, with modifications by Baty and Delignette-Muller (2014), was used to describe the *Salmonella* concentration at time $t$. A lag phase parameter was not required as the *Salmonella* were conditioned to their new environment. This equation is:

$$ N(t; N_0, N_{\text{max}}, \mu, \lambda) = N_{\text{max}} - \log_{10}\left[1 + \left(10^{N_{\text{max}}-N_0} - 1\right) \times \exp(-\mu t)\right] $$  \hspace{1cm} (4.1)

where $N$ is the population of *Salmonella* ($\log_{10}$ CFU g$^{-1}$) at time $t$ (h); $N_0$ is the population of *Salmonella* ($\log_{10}$ CFU g$^{-1}$) at $t = 0$; $N_{\text{max}}$ is the maximum population density ($\log_{10}$ CFU g$^{-1}$) observed and $\mu$ is the maximum growth rate of *Salmonella* ($\log_{10}$ CFU g$^{-1}$ h$^{-1}$).

$N_{\text{max}}$, the maximum population density, was allowed to vary with temperature according to Eqn. 2.11. This equation was modified to describe only the sub-optimal temperature range and over-estimates the maximum population density above this temperature range. This equation is:

$$ N_{\text{max}}(T; a, T_{\text{min1}}, T_{\text{submin}}) = a \frac{T - T_{\text{min1}}}{T - T_{\text{submin}}} $$  \hspace{1cm} (4.2)

where $a$ is the regression coefficient of the equation; $T$ is the temperature at which the sample was incubated ($^\circ$C); $T_{\text{min1}}$ is the $x$-intercept, i.e. the temperature ($^\circ$C) at which the maximum population density is predicted to be 0 log$_{10}$ CFU g$^{-1}$ (i.e. 1 CFU g$^{-1}$), and $T_{\text{submin}}$ is a temperature
4.3. Methods

slightly smaller than $T_{min1}$ at which the maximum population density is predicted to be 0 CFU g$^{-1}$.

The effect of temperature on growth rate, $\mu$, was modelled according to the square of Eqn. 2.5. Eqns. 2.5, 4.1 and 4.2 were combined into Eqn. 4.3, which estimates the concentration of Salmonella at time $t$ and temperature $T$.

$$N = a \frac{T - T_{min1}}{T - T_{submin}} - \log_{10} \left[ 1 + \left( 10^{\frac{T - T_{min1}}{T_{submin}} - N_0} - 1 \right) \times \exp \left( -t \left[ b (T - T_{min2}) \right]^2 \right) \right]$$

(4.3)

This one-step model fitting process was explored in this experiment due to the small number of temperatures studied and because previous research (Jewell, 2012) demonstrated the advantages of using a one-step model fitting process over the “classical” two-step approach.

A traditional two-step model fitting process was also used, including secondary models for maximum population density (Eqn. 4.2) and growth rate (Eqn. 2.5) as a function of temperature. The goodness of fit for both the one-step and two-step procedures were compared by the root mean squared error (RMSE), which is calculated by

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (\hat{y}_i - y_i)^2}{n}}$$

(4.4)

where $y_i$ is the $i$-th observed value and $\hat{y}_i$ is the corresponding predicted value. When estimating the RMSE for the two-step fits, predictions for the growth rate and maximum population density were obtained first from Equations 2.5 and 4.2 and were used as inputs when predicting the Salmonella concentration by Eqn. 4.1.

To allow the predictions of the models generated in this study for Salmonella growth in moisture-infused pork to be compared to existing data for growth of non-infused products, growth rates and maximum population densities for Salmonella growth in pork, beef and chicken were extracted from the ComBase database (http://www.combase.cc/) and are presented in Table B.1. Experiments in modified atmospheres or acid treated products were excluded because those
treatments would be expected to inhibit Salmonella growth. For experiments where the original experimental data are provided in ComBase, the DMFit Excel add-on (Version 3.0) was used to estimate the growth rate, lag phase duration and maximum population density with each parameter included only where evident in the data. Growth rates for 53 pork experiments, 111 beef experiments and 849 poultry experiments and maximum population densities for 10 pork experiments, 13 beef experiments and 245 poultry experiments were collated, with lag phase duration estimates not collated as they were not required for comparison. Eqn. 2.7 was then fitted to the ComBase growth rate data. The full equation for maximum population density, Eqn. 2.11, was fitted to the ComBase maximum population density data.

Non-linear models for the one-step and two-step procedures as well as the ComBase fits were fitted to the data using the statistical programming language R (R Core Team, 2016) and the ‘nlsLM’ function for fitting non-linear models from the ‘minpack.lm’ R package (Elzhov et al., 2013). Standard diagnostic plots, including the quantile-quantile plot and residuals vs. fitted values plot, were used to verify non-linear regression assumptions for all models fitted using one-step and two-step procedures.

An analysis of variance was performed on the results of the experiment involving surface sterilised product to determine if there were differences in Salmonella or other organism growth, between the two steaks or between the strips from each steak.

### 4.4. Results

From the logged temperature data, the actual mean incubation temperatures were 8.9, 14.0, 20.7, 24.9 and 30.4 °C (see Table 4.1). The mean temperature recorded in each incubator was used as the incubation temperature for each experiment and for modelling.

Growth of Salmonella was observed in all moisture-infused pork steak samples (n = 42) stored at mean temperatures ≥14.0 °C (see Figure 4.1); no growth was observed in samples stored at 8.9 °C.
Table 4.1: Nominal temperatures of incubators (°C) with the number of temperatures recorded for that incubator and the mean and standard deviation of those temperatures.

<table>
<thead>
<tr>
<th>Nominal Temperature</th>
<th>Recorded Temp</th>
<th>n</th>
<th>mean</th>
<th>sd</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>1536</td>
<td>8</td>
<td>8.9</td>
<td>0.8</td>
</tr>
<tr>
<td>15</td>
<td>359</td>
<td>15</td>
<td>14.0</td>
<td>1.5</td>
</tr>
<tr>
<td>20</td>
<td>167</td>
<td>20</td>
<td>20.7</td>
<td>0.3</td>
</tr>
<tr>
<td>25</td>
<td>576</td>
<td>25</td>
<td>24.9</td>
<td>0.1</td>
</tr>
<tr>
<td>30</td>
<td>190</td>
<td>30</td>
<td>30.4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Salmonella growth was not observed in samples stored under vacuum at 14 °C. Exponential growth and stationary phases were clearly identifiable in the growth curves, but lag phases were not apparent.

After initial attempts at modelling, three anomalous observations were identified. These observations, $6.56 \log_{10} \text{CFU g}^{-1}$ after 52.6 h at 14.0 °C and $5.36 \log_{10} \text{CFU g}^{-1}$ after zero hours at both 20.7 and 30.4 °C may have been caused by operator error during Salmonella enumeration. These observations are highlighted in Figure 4.1. These three observations were deemed to be unrepresentative of cell concentrations expected at the time points at which they were observed and removed from the observed Salmonella concentration data for modelling.

The regression parameter estimates obtained from the one-step fitting procedure are presented in Table 4.2, including coefficient estimates, standard errors and confidence intervals. The residual standard error was $0.72 \log_{10} \text{CFU g}^{-1}$ on 33 degrees of freedom and the RMSE was equal to 0.66.

The secondary model parameters generated using the two-step procedure are also presented in Table 4.2. The two-step modelling process resulted in residual standard errors of 0.46, 0.52, 0.27 and 1.16 $\log_{10} \text{CFU g}^{-1}$ on 7, 7, 5 and 8 residual degrees of freedom, respectively for the primary models at 14.0, 20.7, 24.9 and 30.4 °C. For the maximum population density, the residual standard error was $0.61 \log_{10} \text{CFU g}^{-1}$ on one degree of freedom and for the growth rate, the residual standard error was $0.03 \log_{10} \text{CFU g}^{-1} \text{h}^{-1}$ on two degrees of freedom. The RMSE was equal to 0.71.
Table 4.2.: Growth model parameter estimates with associated standard errors, 95% confidence intervals (CI) for fits obtained using one-step and two-step fitting procedures. The fits obtained from the ComBase database are also provided for comparison. Asymptotic confidence intervals based on log-likelihood were calculated using the “confint2” function of the “nlstools” R package (Baty et al., 2015).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>One-step</th>
<th>Two-step</th>
<th>ComBase Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N_0^* ) (log( \text{10} ) CFU g(^{-1} ))</td>
<td>3.25</td>
<td>0.227</td>
<td>(2.79, 3.71)</td>
</tr>
<tr>
<td>( a )</td>
<td>9.11</td>
<td>2.36</td>
<td>(4.30, 13.9)</td>
</tr>
<tr>
<td>( T_{\text{min}} ) (°C)</td>
<td>8.48</td>
<td>9.85</td>
<td>(-11.6, 28.5)</td>
</tr>
<tr>
<td>( T_{\text{submin}} ) (°C)</td>
<td>6.11</td>
<td>16.0</td>
<td>(-26.3, 38.6)</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (°C)</td>
<td>50.3</td>
<td>3.92</td>
<td>(42.6, 58.0)</td>
</tr>
<tr>
<td>( T_{\text{supmax}} ) (°C)</td>
<td>53.1</td>
<td>7.37</td>
<td>(38.6, 67.6)</td>
</tr>
<tr>
<td>( b )</td>
<td>0.0264</td>
<td>0.00439</td>
<td>(0.0175, 0.0354)</td>
</tr>
<tr>
<td>( c )</td>
<td>0.0267</td>
<td>0.00274</td>
<td>(0.0148, 0.0385)</td>
</tr>
<tr>
<td>( T_{\text{min}} ) (°C)</td>
<td>5.66</td>
<td>3.09</td>
<td>(-0.615, 11.9)</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (°C)</td>
<td>48.7</td>
<td>0.180</td>
<td>(48.4, 49.1)</td>
</tr>
</tbody>
</table>

* For the two-step fit, the estimated values for \( N_0 \) were 3.00, 4.01, 3.11 and 3.73 at 14.0, 20.7, 24.9 and 30.4 °C, respectively.
4.4. Results

The parameter estimates obtained from the ComBase data are also included in Table 4.2 and the growth rate and maximum population densities estimated by both fitting procedures and the ComBase fits are presented in Figures 4.2 and 4.3 respectively. A separate model was fitted to the subset of the growth rates estimated in pork, with no significant difference observed between this model and the model estimated for all the growth rates \( p = 0.61 \).

The results of the analysis of variance performed on surface sterilised pork revealed a significant interaction effect on the colony counts between the two steaks and type of agar used \( p = 0.008 \). For counts on both growth media, colony counts obtained from one steak were, on average, 0.33 \( \log_{10} \) CFU g\(^{-1} \) higher than the other. For both steaks, colony counts obtained on standard plate count agar were, on average, 0.60 \( \log_{10} \) CFU g\(^{-1} \) higher than those obtained on the XLD agar. For both steaks, colony counts obtained on standard plate count agar \( n = 12 \) were consistently greater than those obtained on XLD agar \( n = 11 \). Counts on standard plate count agar were
4. Growth of *Salmonella* in Moisture-Infused Pork

![Graph showing growth rate fits obtained using one-step and two-step fitting procedures and data from ComBase database.](image)

**Figure 4.2.** Comparison of growth rate fits obtained using the one-step and two-step fitting procedures and the data obtained from the ComBase database.

between 0.15 and 1.02 log\(_{10}\) CFU g\(^{-1}\) greater, and were, on average, 0.47 log\(_{10}\) CFU g\(^{-1}\) higher. The colony counts observed on the two enumeration media were not significantly different between the strips cut from each steak \((p = 0.49)\). It appears that while there was variation in *Salmonella* concentration between steaks, the concentrations between the strips cut from each steak were similar.

### 4.5. Discussion

Based on the root mean squared errors, which were lower for the one-step fitting procedure, we can see that the one-step procedure produces a better fit to the data than the combination of primary and secondary models estimated by the two-step fitting procedure. We can also see in Table 4.2 that the confidence intervals around the one-step parameter estimates are narrower than those of the two-step parameter estimates. This result is in agreement with Jewell (2012), who
Figure 4.3: Comparison of the maximum population density fits obtained using the one-step and two-step fitting procedures. These fits are compared to the data obtained from the ComBase database, and associated model.
showed that the one-step procedure produced more precise coefficient estimates.

From Figure 4.2 and Table 4.2, we can see that, for growth rates, the fitted models obtained from the one-step and two-step procedures, and also from the ComBase data and model are similar. Both procedures give estimates of $T_{\text{min2}}$ that are lower than that estimated from the ComBase data, with the two-step fit lower than the one-step fit. The one-step fit predicts slower growth of *Salmonella* at temperatures $>6.03$ °C and the two-step model at temperatures $>7.67$ °C. The values of $T_{\text{min2}}$ fitted by the one-step (5.66 °C) and two-step (5.43 °C) procedures are within the range of lower temperatures at which *Salmonella* has been reported to grow (ICMSF, 1996). The growth rate model parameters ($b$ and $T_{\text{min2}}$) estimated here differ from those estimated by Møller et al. (2013) for the growth of *Salmonella* in ground pork, where $b$ was estimated as 0.02346 (converted from 0.0356 on specific growth rate scale) and $T_{\text{min2}}$ as 2.33. These values are lower than those estimated by the models presented here, though the reason for this is unclear. Based on the product specifications and brine application rate, we estimated that the water activity of the moisture-infused steaks is 0.989–0.990 which is similar to, but slightly lower than, the water activity naturally expected in meat, i.e., approximately 0.992.

The maximum population density estimates (Figure 4.3, Table 4.2), of the $x$-intercept for both the one-step ($T_{\text{min1}} = 8.48$) and two-step ($T_{\text{min1}} = 10.0$), fitting procedures were higher than the estimates derived from the ComBase data ($T_{\text{min1}} = 3.84$), though the one-step estimates were closer to the ComBase intercept than the two-step estimate. Both the one-step and two-step models estimate slightly higher maximum population density values at higher temperatures compared to the ComBase-derived model estimates. The reason is possibly the modifications made to the formula by Zwietering et al. (1994), which includes terms to decrease the estimated maximum population density as the temperature increases towards the upper temperature bound on *Salmonella* growth. However, these modifications were required because no experiments were conducted at temperatures above 30.4 °C.

These results agree with those presented by Wen and Dickson (2012), who reported the slow inactivation of *Salmonella* in moisture-infused vacuum-packed pork at 4 °C and 10 °C. In our
study, we did not observe *Salmonella* growth in moisture-infused pork steaks at temperatures <14 °C stored aerobically. *Salmonella* growth was not observed in vacuumed samples stored at 14 °C, reinforcing that *Salmonella* growth is inhibited, as expected, in vacuum packaged conditions.

This study only used one commercially prepared brine formulation, whereas multiple brine formulations are available and used commercially on pork in Australia. Different brine formulations may affect *Salmonella* growth more than others based on their composition and investigating these other formulations would be beneficial in determining if other formulations reduce the growth of *Salmonella* in pork, although initial evaluation of the likelihood of significant differences due to formulation, and application rates, could be achieved by recourse to predictive microbiology models.

Significant differences in microbial populations were observed using surface-sterilised pork portions. Differences in the *Salmonella* concentrations between steaks were observed, indicating that one steak was injected with, on average, 0.33 log<sub>10</sub> CFU g<sup>-1</sup> more *Salmonellae*. This variance in *Salmonella* concentration in the samples may also have occurred in the growth experiments and may explain some of the variability in the observed *Salmonella* concentrations in those experiments. It may also have contributed to difficulty in obtaining stable estimates of the maximum growth rate.

Differences in colony counts derived from the two media used for enumeration in the “sterile” pork experiment were also observed, with colony counts obtained on standard plate count agar consistently greater than those obtained on XLD agar. Differences in the number of organisms recovered on the two enumeration media could occur if the *Salmonellae* introduced via the brine were injured by the combined chilling and water activity stresses experienced in the meat, and were not able to grow subsequently on the highly selective and stressful XLD medium. No significant differences in colony counts observed between strips from the same steak, indicating that the injection procedure introduced *Salmonella* uniformly within each steak.
4. Growth of *Salmonella* in Moisture-Infused Pork

Growth of *Salmonella* in moisture-infused pork, with distinct exponential growth and maximum population phases, was observed at temperatures above 14 °C. Despite the limited number of samples, growth rate and maximum population density parameters were able to be estimated and suggest that the injection of brine has little effect on the growth rate of *Salmonella* in pork meat. It does not appear that an alternative growth model is required to predict the growth of *Salmonella* in infused pork products. This is perhaps as expected as the salt added to the meat via the brine solution does not decrease the meat water activity to the extent that it will significantly reduce *Salmonella* growth. In conclusion, the results suggest that growth rate and the amount of *Salmonella* in infused pork products is adequately predicted by existing data and models for *Salmonella* growth in meat.
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5. Quantitative Microbial Risk Assessment of Salmonellosis From the Consumption of Australian Pork: Minced Meat From Retail to Burgers Prepared and Consumed at Home

*Note:* Most of the material presented in this Chapter has been submitted for publication in the journal *Risk Analysis* on 12 November 2015

Gurman, PM, Ross, T and Kiermeier, A. ‘Quantitative Microbial Risk Assessment of Salmonellosis from the Consumption of Australian Pork: Minced Meat from Retail to Burgers Prepared and Consumed at Home’

5.1. Abstract

Pork burgers could be expected to have an elevated risk of salmonellosis compared to other pork products due to their comminuted nature. A stochastic risk assessment was conducted to estimate the risk of salmonellosis from Australian pork burgers and considered risk-affecting factors in the pork supply chain from retail to consumption at home. Conditions modelled included
prevalence and concentration of *Salmonella* in pork mince, time and temperature effects during retail, consumer transport and domestic storage and the effect of cooking with the probability of illness from consumption estimated based on these effects. The model used was constructed in two dimensions, to allow for the separation of variability and uncertainty in reported results. Potential changes to production practices and consumer behaviours were examined through alternative scenarios. Under current conditions in Australia, the mean risk of salmonellosis from consumption of 100g pork burgers was estimated to be $1.54 \times 10^{-8}$ per serving or one illness per 65,000,000 servings consumed. Under a scenario in which all pork mince consumed is served as pork burgers, and with conservative (i.e. worst-case) assumptions, 0.746 cases of salmonellosis per year caused by pork burgers in Australia were predicted. Despite the adoption of several conservative assumptions to fill identified data gaps, it is predicted that pork burgers have a low probability of causing salmonellosis in Australia.

### 5.2. Introduction

As discussed in Chapter 1, Australia has approximately 16,000 notified cases of salmonellosis each year (Department of Health Australia, 2016), with the true number of cases of salmonellosis, circa 2010, attributed to contaminated food estimated at 39,600 (Kirk et al., 2014a). Many food products, including pork, are believed to contribute to this.

Australian salmonellosis outbreaks have been linked to pork products (OzFoodNet Working Group, 2008, 2009, 2011, 2012b; South Australian Health, 2010). No source attribution study was identified that quantifies the food sources contributing most to total annual salmonellosis incidence in Australia. A recent source attribution study for the state of South Australia in Australia (Glass et al., 2016), however, estimated that 2.5% of sporadic cases of salmonellosis were attributable to porcine sources, behind ovine (2.9%), bovine (7.4%), chicken (34.6%) and eggs (37.1%). Conversely, international source attribution studies have concluded that pork products contaminated with *Salmonella* spp. represent large proportions of total foodborne
outbreaks. For example, in Denmark, domestic and imported pork was estimated as the largest food source of salmonellosis, representing 22.9% of laboratory-confirmed salmonellosis cases, followed by broilers (4.9%) and eggs (3%) (Anonymous, 2015). In Italy, pork was identified as the major source of human salmonellosis from 2002-2010 with 43-60% of infections attributed to pork (Mughini-Gras et al., 2014). These nations, however, have different agricultural and culinary traditions to Australia, where pork consumption is relatively low. In New Zealand, however, it might be expected that pork consumption patterns would be similar to those in Australia by virtue of the cultural history of these two nations. In New Zealand, pork was shown to be the major source of infections over a three year period (Mullner et al., 2009).

In Australia, *Salmonella* spp. appear to be the main cause of foodborne illness where pork products are identified as the cause. Of all the outbreaks of foodborne illness associated with pork or pork products in Australia between 2003 and 2012 (OzFoodNet Working Group, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2011, 2012b; South Australian Health, 2010), only one outbreak was attributed to a foodborne pathogen other than *Salmonella*. (OzFoodNet Working Group, 2007)

The Australian pork industry is currently marketing pork burgers as an alternative serving suggestion for pork mince. Based on the history of illnesses from consumption of *E. coli* O157:H7 in beef burgers (Rangel et al., 2005), pork burgers are considered a potentially higher risk product compared to whole pork cuts, due to potential redistribution of pathogens throughout the meat that can result from mincing. These products require a more thorough cooking step compared to intact pork due to the internalisation of pathogens (Van, 2011), with higher cooking temperatures or longer times needed to ensure transfer of heat into the interior of the patty. For this reason, the Australian pork industry commissioned this risk assessment to strengthen industry risk management approaches.

The risk of salmonellosis from the consumption of pork burgers in Australia was quantified by development of a two-dimensional stochastic risk assessment model including the pork supply chain from retail to consumption at home. Factors explicitly considered in the model included
5.3. Methods

A stochastic quantitative risk assessment model for salmonellosis from the consumption of pork burgers in Australia was developed. The model is consistent with Codex Alimentarius Commission guidelines for food safety microbial risk assessment (Codex Alimentarius Commission, 1999). To inform the Exposure Assessment and Hazard Characterisation components of this risk assessment, the model describes the effect of each supply chain or process step on the Salmonella contamination of pork mince and pork burgers from the retailer to consumption.

The risk assessment model was implemented in the statistical software R (R Core Team, 2016) and the mc2d package for two-dimensional stochastic modelling (Pouillot and Delignette-Muller, 2010). This R package includes tools that allow the effects of variability and uncertainty to be modelled separately, allowing for uncertainty in the risk estimate to be quantified. The model’s structure is shown schematically in Figure 5.1 and the model parameters and formulae are

prevalence and concentration of Salmonella in pork mince, time and temperature effects during retail handling and display, consumer transport, domestic storage and the effect of cooking.

The probability of salmonellosis from consumption of pork burgers in Australia was estimated based on these effects and the model used to identify factors that most influence that risk.

As noted in Chapter 3, data gaps in the literature were identified during the planning for this risk assessment. The first of these gaps was a predictive model for the inactivation of Salmonella in pork burger patties, which was determined in Chapter 3. The second data gap identified concerned the concentration of Salmonella in pork mince, which is presented in this Chapter. This was determined by using the MPN method to enumerate Salmonella on pork trim and belly strip samples. Finally, the internal endpoint temperature to which pork burgers are cooked was determined by the addition of a question on consumer preference for ‘doneness’ in separately conducted consumer sensory studies.
presented in Table 5.6. The effects of cross contamination were not included in this model, largely because these events would occur prior to the point that the prevalence and concentration inputs were modelled.

Alternative scenarios were used to assess the influence of various factors on the final risk estimate. These scenarios were used to investigate theoretical deviations from current conditions (status quo) and were named Scenario 1 to Scenario 11. These scenarios investigated changes to *Salmonella* concentration, retail storage temperatures, cooking temperatures and stochastic modelling methods. Scenarios 12 to 15 were used to estimate the expected number of illnesses based on conservative estimates of pork burger consumption in Australia.

### 5.4. Exposure Assessment

#### 5.4.1. Initial Contamination of *Salmonella*

The prevalence of *Salmonella* in Australian pork mince at retail has been investigated (Hamilton et al., 2011). Two *Salmonella* were detected among 148 tests, using enrichment, from 25 gram samples of retail pork mince, inferring a *Salmonella* prevalence in pork mince of 1.4% per 25g. Uncertainty in this estimate was incorporated into the model by allowing the prevalence to vary according to a beta distribution with parameters $\alpha = 3$ and $\beta = 149$ (Vose, 2000).

The concentration of *Salmonella* on pork belly strip excision samples ($n = 403$) and trim samples ($n = 417$) collected from pork abattoirs in Australia was investigated in a national study (D. Hamilton, 2015, South Australian Research and Development Institute, Pers. Comm.). Each sample was initially tested for the presence of *Salmonella* by enrichment of either the whole belly strip (weight: mean 170g, standard deviation 131.3g) homogenised 1:1 in peptone saline solution (PSS, 0.1% trypticase, 0.85% NaCl, wt/vol) or a 25g sample of trim homogenised 1:10 in peptone saline solution, with a 5mL aliquot of the homogenate, pre-enrichment, stored at 4 °C until the presence of *Salmonella* in that sample was known. From retained 5mL aliquots
Figure 5.1.: Flowchart showing the overall structure of the quantitative risk assessment model. Inputs to the model are depicted as trapeziums. Each stage of the supply chain modelled is depicted as a rectangle.
Table 5.1: Results from the scenario analysis. Ratio to baseline values are the ratio of the mean probability of illness for each scenario against the mean of the probability of illness for the baseline scenario.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Description</th>
<th>Reason for Scenario</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$C_{\text{mince}} \sim N(\mu = 0.22, \sigma = 0.97)$</td>
<td>Examine effect on risk of increase in $Salmonella$ concentration.</td>
</tr>
<tr>
<td>2</td>
<td>$C_{\text{mince}} \sim N(\mu = -1.78, \sigma = 0.97)$</td>
<td>Examine effect on risk of decrease in $Salmonella$ concentration.</td>
</tr>
<tr>
<td>3</td>
<td>$T_r \sim S\text{Normal}(\mu = 3, \sigma = 2.11, \xi = 0.77)$</td>
<td>Examine effect on risk of decrease in retail storage temperature.</td>
</tr>
<tr>
<td>4</td>
<td>$T_r \sim S\text{Normal}(\mu = 5, \sigma = 2.11, \xi = 0.77)$</td>
<td>Examine effect on risk of increase in retail storage temperature.</td>
</tr>
<tr>
<td>5</td>
<td>$T_r \sim S\text{Normal}(\mu = 6, \sigma = 2.11, \xi = 0.77)$</td>
<td>Examine effect on risk of increase in retail storage temperature.</td>
</tr>
<tr>
<td>6</td>
<td>$T_r \sim S\text{Normal}(\mu = 7, \sigma = 2.11, \xi = 0.77)$</td>
<td>Examine effect on risk of increase in retail storage temperature.</td>
</tr>
<tr>
<td>7</td>
<td>$T_{\text{ct}}$ constant</td>
<td>Examine effect on risk of constant consumer transport temperature in retail storage temperature.</td>
</tr>
<tr>
<td>8</td>
<td>$\Delta T_{\text{ct}}$ sampled from data</td>
<td>Examine effect on risk of sampling with replacement the temperature change during consumer transport directly from data.</td>
</tr>
<tr>
<td>9</td>
<td>$T_{\text{cook}}$ sampled from (EcoSure, 2008)</td>
<td>Examine effect on risk of replacing Australian consumer cooking temperatures with US data.</td>
</tr>
<tr>
<td>10</td>
<td>$S$. Senftenberg used for inactivation model</td>
<td>Examine effect on risk of using $S$. Senftenberg as the serovar in the inactivation model</td>
</tr>
<tr>
<td>11</td>
<td>$S$. Typhimurium used for inactivation model</td>
<td>Examine effect on risk of using $S$. Typhimurium as the serovar in the inactivation model</td>
</tr>
</tbody>
</table>
of the 22 belly strip and 7 trim samples in which Salmonella was detected, a Most Probable Number (MPN) method was used to enumerate Salmonella in the samples. Triplicate tubes of Buffered Peptone Water (Difco™, Catalogue Number 218105) were inoculated with 1.0, 0.1 and 0.01mL of the retained 1:10 suspension for pre-enrichment and incubated at 37 °C for 18–24 hrs. From each MPN tube, 33µL of culture was then spot inoculated on a Modified Semi-solid Rappaport-Vassiliadis (MSRV, LabM Limited, Lancashire UK) plate and incubated at 42 °C for 18–24 hrs. MSRV plates were examined for typical halos around colonies that formed. As appropriate, growth from the halo was plated onto the surface of a third of a plate of Xylose Lysine Deoxycholate agar (XLD, Micromedia PTY LTD, Moe Australia, Catalogue Number 1355) and incubated at 37 °C for 18–24 hours. Typical colonies on XLD agar plates were confirmed as Salmonella species using polyvalent Salmonella latex agglutination (Rapid Culture Confirmation Test, Microgen Bioproducts, Camberley, UK). The MPN of Salmonella was calculated using appropriate tables (Roberts and Greenwood, 2002). These results are given in Table 5.2.

The enumeration results were “censored”, i.e. some values were not known exactly, but were known to be either below, between or above some limits (<0.3 MPN/g for belly strip samples and <3 MPN/g for trim samples). Functions for fitting distributions to censored observations in the “fitdistrplus” R package (Delignette-Muller and Dutang, 2015) were used to fit a normal distribution to the log10 concentration data, which has been used previously in this context (Nauta et al., 2013). For samples where Salmonella was detected (by enrichment) that yielded no growth

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**Table 5.2.:** Concentrations of Salmonella (MPN/g) estimated on samples where Salmonella was detected. The limit of quantification was 0.3 MPN/g for the belly strip excision samples and 3 MPN/g for the trim samples.

<table>
<thead>
<tr>
<th>Salmonella Concentration (MPN/g)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown</td>
<td>1</td>
</tr>
<tr>
<td>&lt;0.3</td>
<td>18</td>
</tr>
<tr>
<td>&lt;3</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
</tr>
</tbody>
</table>

in the MPN method, the concentration of *Salmonella* was assumed to be between the limit of detection (LOD = 1/w, where w is the weight of the sample tested, i.e. 0.04 CFU/g for trim samples and variable for belly strip samples) and the limit of quantification of the MPN method (<0.3 MPN/g for belly strip and <3 MPN/g for trim) and was recorded as interval censored data. The mean of the normal distribution describing the *Salmonella* concentration, $C_{\text{mince}}$, was estimated as $-0.79 \log_{10} \text{CFU/g}$ and standard deviation $0.66 \log_{10} \text{CFU/g}$. Verification was performed using visual examination of diagnostics plots, because no statistical tests exist to verify the goodness of fit for censored distributions. Uncertainty in the maximum likelihood estimates of the mean and standard deviation was included in the model by generating non-parametric bootstrap samples using the “bootdistcens” function in the “fitdistrplus” R package (Delignette-Muller and Dutang, 2015), with the number of samples equal to the length of the uncertainty dimension in the model. These bootstrap samples were assigned as the mean and standard deviation for the concentration distribution in the uncertainty dimension of the two-dimensional model. The effect of changes to the *Salmonella* concentration were evaluated in two hypothetical scenarios (‘1’ and ‘2’) by adding ±1 to the mean, resulting in mean concentrations of 0.22 and -1.78 log$_{10}$ CFU/g, respectively, for the two scenarios.

The prevalence and concentration values were used to estimate the number of *Salmonellae* per serving. The prevalence was adjusted from an estimate based on a 25g laboratory sample to a 100g serving size. This adjustment was made by

\[ P_{\text{serve}} = 1 - (1 - P_{\text{mince}})^4 \]

where $P_{\text{serve}}$ and $P_{\text{mince}}$ are the prevalences in a pork burger patty serving and a lab sample, respectively. The exponent, four, is the ratio of the serving size weight ($W_{\text{serve}} = 100g$) and the weight of the laboratory sample tested (25g). The number of *Salmonella* in each contaminated pork burger serving was based on a Zero-Truncated Poisson distribution (ZTP) by
5.4. Exposure Assessment

\[ N_{\text{serve}} \sim ZTP \left( \lambda = 10^{C_{\text{mince}} \times W_{\text{serve}}} \right) \]

where \( \lambda \) is the mean of the regular Poisson distribution before truncation, with \( C_{\text{mince}} \) and \( W_{\text{serve}} \) as previously defined. The truncated distribution was used as this distribution required each contaminated serving to contain at least one Salmonella organism. The mean of the resulting distribution is slightly larger than \( \lambda \), though this difference is small because only approximately 3% of servings were truncated by this method.

5.4.2. Growth Model for Salmonella

To model Salmonella growth in pork, predictive models for exponential growth rate and maximum population density derived from ComBase data in Chapter 4 were used. The exponential growth rate, \( GR \), was modelled by Eqn. 2.7 and the maximum population density, \( MPD \), was modelled by Eqn. 2.11, with parameter estimates for both equations taken from the “ComBase Fit” column in Table 4.2. The lag phase was not modelled in this risk assessment. A lag phase would be expected to reduce the expected growth of Salmonella on pork products. By omitting the lag phase, the risk estimate would be higher and, therefore, more conservative. MPD values were converted from \( \log_{10} \) CFU per gram to CFU per serve to estimate the total number of Salmonella per serving (\( MP_{\text{serve}} \)).

5.4.2.1. Growth Implementation

The growth of Salmonella during retail storage and domestic storage was calculated by

\[ N_r = \min \left[ 10^{\log_{10}(N_{\text{serve}}) + GR(T = T_r) \times t_r}, MP_{\text{serve}}(T = T_r) \right] \]  \hspace{1cm} (5.1)  
\[ N_{ds} = \min \left[ 10^{\log_{10}(N_{ct}) + GR(T = T_{ds}) \times t_{ds}}, MP_{\text{serve}}(T = T_{ds}) \right] \]  \hspace{1cm} (5.2)
respectively, where $N_{serve}$, $N_r$, $N_{ct}$ and $N_{ds}$ are the populations of Salmonella per serving before and after retail, after consumer transport and domestic storage, respectively; $T_r$ and $T_{ds}$ are the temperatures during the retail storage and domestic storage, $t_n$ is the duration of the current model step and $GR$, $MP_{serve}$ and $W_{serve}$ are as previously defined. If the number of Salmonella in the previous model step was greater than the maximum population estimated for the current model step, then no change was made to the number of Salmonella. The methods used for describing growth during consumer transport are described in Section 5.4.4.

In this risk assessment model, the effect of competing microbiota has not been modelled. The effect of competition would likely be to cause a reduction in the growth potential for Salmonella in pork mince. By not considering the effects of competing microbiota, a further level of conservatism has been added to the model predictions.

5.4.3. Retail Storage

Pork mince is expected to be refrigerated at retail until it is purchased by the consumer. If the temperature control is not adequate Salmonella may grow, with the time and temperature of storage dictating how much growth will occur.

No empirical data are available that describe the storage duration of meat on retail shelves in Australia or overseas. Previous risk assessments for E. coli O157:H7 in beef burgers have assumed an exponential distribution for the storage time of beef mince at retail in the United States (2001, USDA-FSIS.), Australian beef mince sold in the US (Kiermeier et al., 2015) and Canada (truncated at a maximum of 10 days) (Smith et al., 2013). Uncertainty was incorporated into those risk assessments by modelling the rate parameter of the exponential distribution as being uniformly distributed between 0.5 and 1.5 days. Without evidence to the contrary, this risk assessment used the same approach to describe the storage time of pork mince at retail in Australia, truncated at 10 days.

The modelled temperature of pork mince at retail was based on survey results of poultry product
in Australian supermarkets during summer (Pointon et al., 2009). Pork products are often stored in the same cabinets as poultry products in Australia, i.e., poultry retail display temperatures were considered to be a credible surrogate. Negative skewness in the data was observed ($S = -0.44$), such that the data were poorly described by a regular Normal distribution. Other distributions are either positively skewed or require positive values only. A Skew Normal distribution, as defined in the “fGarch” R package (Wuertz et al., 2013), was fitted to the data, with the mean estimated as 3.40 °C, the standard deviation 2.11 °C, and the skewness parameter 0.77 °C. The fit of the distribution to the data was verified using a quantile-quantile plot. Uncertainty in the maximum likelihood estimates of the mean, standard deviation and skewness parameters was included in the model by generating non-parametric bootstrap samples. The influence of the mean retail temperature on the overall risk estimate was examined in Scenarios 3 to 6, by changing the mean to 3, 5, 6 or 7 °C, respectively.

For each serving, a storage duration and temperature were randomly sampled from their distributions. The temperature of the product was assumed to be constant throughout the storage time. The growth rate model was used to determine the increase in *Salmonella* levels during the storage period and this was added to the initial *Salmonella* population of that serving.

### 5.4.4 Consumer Transport

Little quantitative data describing the range of transport times or temperatures for pork in Australia is readily accessible. These factors have been investigated in the United States (EcoSure, 2008) by tracking fresh meat products ($n = 916$) during consumer transport from retail to the home and those data were used as surrogates to describe transport times and temperature changes for pork mince in Australia. For the transport time, a gamma distribution was fitted to the range of transport durations with $\alpha = 7.40$ and $\beta = 6.32$, resulting in a mean transport duration of 1.17 hours. The fit of the gamma distribution was verified using the quantile-quantile plot and the Kolmogorov-Smirnov test ($p = 0.36$). For the change in product temperature during transport,
a gamma distribution was fitted to the range of observed temperature increases (converted to degrees Celsius) with $\alpha = 1.78$ and $\beta = 0.55$, resulting in a mean temperature change of 3.24 °C. As this distribution was not a good fit to the data at the upper tail of the distribution, the effect of sampling the temperature change during consumer transport directly from the data instead of using a gamma distribution was investigated in Scenario 8. Both transport duration and temperature increase are strictly positive and as such, gamma and exponential distributions were compared for their ability to fit the data, with the gamma distribution providing a better fit to the data. For both of these distributions, uncertainty in the estimates of the $\alpha$ and $\beta$ parameters was included in the model by generating non-parametric bootstrap samples. The correlation between the transport time and temperature change during transport was estimated as 0.11. This correlation was found to be significant ($p < 0.001$) and was incorporated into the model using the method of Iman and Conover (1982) as implemented in the “cornode” function of the “mc2d” R package (Pouillot and Delignette-Muller, 2010).

The temperature profile for consumer transport was assumed to be linear. As the growth model requires constant temperature over the period being evaluated, two different methods were investigated as scenarios to model growth. The method used in the baseline scenario divides consumer transport into 100 subintervals of equal duration. The total transport time ($t_{ct}$) and change in transport temperature ($\Delta T_{ct}$) were divided by 100 to determine the duration of each interval ($\delta t_{ct}$) and the temperature change per interval ($\delta T_{ct}$). For subinterval $i$, Salmonella growth was estimated by

$$N_{ct,i} = \min \left\{ 10^{\log_{10}(N_{ct,i-1}) + GR[T = T_r + i \times \delta T_{ct}] \times i \times \delta t_{ct}}, MP_{serve}[T = T_r + i \times \delta t_{ct}] \right\}. \quad (5.3)$$

The population of Salmonella estimated in the last interval was labelled $N_{ct}$. This method is, in effect, the Riemann sum of the right endpoints of each interval (Stewart, 2005).

The second method used to estimate growth during consumer transport is simpler, and more conservative, and assumed that the temperature of the product throughout the transport period
5.4. Exposure Assessment

is equal to $T_r + \Delta T_{ct}$, the product temperature at the end of consumer transport. Growth during consumer transport was estimated by

$$N_{ct} = \min \left[ 10^{\log_{10}(N_r) + \text{GR}(T = T_r + \Delta T_{ct}) \times t_{ct}}, MP_{serve}(T = T_r + \Delta T_{ct}) \right].$$  \hspace{1cm} (5.4)

The effect of this method on the final risk estimate is examined in Scenario 8.

5.4.5. Domestic Storage

In the model, it was assumed that pork mince is refrigerated in the consumer’s home until cooked, but no data describing how long Australians store pork mince in their refrigerator before cooking could be found. Instead, the results of a survey of New Zealand consumers (Gilbert et al., 2007) were used to describe the storage of pork mince in Australia. Consumers ($n = 293$) were asked to choose from one of four durations of holding, with the following distribution of times reported: 0 to 2 days: 216; 2.5 to 4 days: 62; 4.5 to 7 days: 14 and 7 to 14 days: 1. An exponential distribution was fitted to these data to describe the storage time for pork mince in domestic fridges, with the Exponential distribution chosen based on distributions used to describe domestic storage durations previously (Kiermeier et al. 2015; Smith et al. 2013; USDA-FSIS, 2001). The “fitdistrplus” package (Delignette-Muller and Dutang, 2015) was used to fit the distribution to these interval censored responses. The rate parameter was estimated as 1.53 days and the fit of this distribution assessed by examination of diagnostic plots. This distribution gives a more conservative estimate of home storage time for product than those used in previous risk assessments (Kiermeier et al. 2015; Smith et al. 2013; USDA-FSIS, 2001).

Uncertainty in the maximum likelihood estimate of the rate parameter was included in the model by generating non-parametric bootstrap samples using the “bootdistcens” function in the “fitdistrplus” R package (Delignette-Muller and Dutang, 2015), with the number of samples equal to the length of the uncertainty dimension in the model. These bootstrap samples were assigned as the values of the rate parameter in the uncertainty dimension of the two-dimensional model.
The temperature of domestic refrigerators has been investigated in Australia (NSW Food Authority, 2009). The New South Wales Food Authority recorded the temperature of 57 domestic refrigerators at ten minute intervals over a normal weekend (i.e. midnight Friday to midnight Sunday) and six further refrigerators during a special event period (Easter or New Years Eve) that lasted several days. Raw temperature data recorded during the survey for each refrigerator were provided by the New South Wales Food Authority. High temperatures were evident in the data, likely caused by opening of the refrigerator door and adding food items (possibly hot) or the thermometer sitting outside the refrigerator for part of the survey period. Accordingly, “data cleaning” was performed to remove these anomalously high temperatures where either i) temperatures greater than 15 °C were recorded, or ii) the participant recorded on their survey form that they opened the refrigerator door. Cleaning was performed assuming that short term changes in temperature would have little effect on the contents as items inside would buffer each other against sudden temperature changes and individual items would require extended periods to warm. In these cases, temperatures were removed that were greater than the maximum temperature observed during the rest of the survey period. The mean of all cleaned temperatures was calculated for each refrigerator. It was assumed that this distribution is representative of temperatures of refrigerators in Australia and was described by a normal distribution with mean 3.35 °C and standard deviation 1.94 °C. The normal distribution was chosen based on the shape of the histogram of the temperatures. The fit of this distribution was verified by a quantile-quantile plot and the Kolmogorov-Smirnov test \((p = 0.9912)\). Uncertainty in the maximum likelihood estimates of the mean and standard deviation was included in the model by generating non-parametric bootstrap samples.

It was assumed that the temperature of the product in the refrigerator remained constant during storage and the mean temperature of the refrigerator was the temperature of the product in the refrigerator. Using these assumptions, the growth of *Salmonella* for each pork mince serving was calculated using Eqn. 5.1 and the duration and temperature of pork mince during domestic refrigeration were randomly sampled from their distributions.
5.4.6. Inactivation in Pork Burgers

The reduction in *Salmonella* due to cooking was estimated in the model from the internal endpoint cooking temperature of the burger patty.

There are no data on the internal temperature to which Australian consumers cook pork burgers. To overcome this data gap, consumers who answered an advertisement seeking participants for sensory studies, were asked to complete a paper questionnaire, before tasting pork products prepared for them. Consumers were asked to rate their preference for “doneness” of either fresh pork (*n* = 1199) or for pork burgers (*n* = 1200). Consumers were asked to choose from four levels of “doneness”: “medium rare, pink”, “medium, hint of pink”, “medium/well done, white” or “well done”. De Santos et al. (2007) gives internal cooking temperatures required to attain each degree of “doneness” in pork. These values were used, to convert the survey categories to cooking temperatures, with the medium rare temperature the average of the medium and rare temperatures reported (De Santos et al., 2007). The survey results and the conversion from “doneness” descriptions to internal temperatures are shown in Table 5.3. To incorporate these temperatures into the model, random samples were drawn from the four internal temperatures, weighted by the consumer preference percentages. Variability in internal cooking temperatures was included by adding a random draw from a standard normal variate to each cooking temperature. The effect of a shift in Australian consumer preference towards less thoroughly cooked pork burgers was investigated in Scenario 9. In this scenario, internal endpoint cooking temperatures for ground beef by US consumers (EcoSure, 2008) were sampled with replacement to assign each burger a cooking temperature.

The model presented in Chapter 3 was used to estimate the survival of *Salmonella* from cooking pork burgers based only on the internal endpoint temperature. Other models require cooking time and modelling of heat transfer in the pork patty (Juneja et al., 2000; Murphy et al., 2004). The model presented in Chapter 3 provides separate parameterisations for *S. 4,[5],12:i:-*, *S. Senftenberg* and *S. Typhimurium*. The model for *S. 4,[5],12:i:-* was chosen for use initially because it is a
Table 5.3: Consumer preference for pork products.

<table>
<thead>
<tr>
<th>“Doneness”</th>
<th>Internal Temperature (°C)</th>
<th>Consumer Preference, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Pork</td>
<td>Pork Burgers</td>
</tr>
<tr>
<td>medium rare, pink</td>
<td>63</td>
<td>67 (5.6)</td>
</tr>
<tr>
<td>medium, hint of pink</td>
<td>71</td>
<td>415 (34.6)</td>
</tr>
<tr>
<td>medium/well done, white</td>
<td>74</td>
<td>463 (38.6)</td>
</tr>
<tr>
<td>well done</td>
<td>77</td>
<td>254 (21.2)</td>
</tr>
<tr>
<td>do not eat</td>
<td>Not an option</td>
<td>70 (-)</td>
</tr>
</tbody>
</table>

serovar of current public health interest, with increasing isolation rates in the European Union (EFSA, 2013) and United States of America (CDC, 2013). The effect of using the other two Salmonella serovars described in the inactivation model was also investigated (Scenarios 10 and 11). The reduction due to cooking for S. 4,[5],12:i:-, was estimated from

\[
N_{\text{cook}} = \exp \left[ \ln \left( N_{ds} \right) - \ln \left( 1 + z \right) \right]
\]

where

\[
z = \exp \left\{ - \left[ 19.46 + 2.89s_{\text{senft}} - 3.55s_{\text{typh}} + 0.62F_{\text{mince}} - \left( 0.49 + 0.050s_{\text{senft}} + 0.071s_{\text{typh}} + 0.0099f \right) T_{\text{cook}} \right] \right\}.
\]

For each burger, \( T_{\text{cook}} \) is the internal endpoint temperature, \( s_{\text{senft}} \) is the indicator variable for S. Senftenberg, \( s_{\text{typh}} \) is the indicator for S. Typhimurium and \( F_{\text{mince}} \) is the fat percentage of the raw mince. When \( s_{\text{senft}} = 0 \) and \( s_{\text{typh}} = 0 \), the model estimates the Salmonella survival for S. 4,[5],12:i:-. (Chapter 3) observed that the fat content of pork mince significantly affected inactivation during cooking. For Australian pork mince, the mean fat percentage was estimated to be 9.4g per 100g, i.e. 9.4% (Sinclair et al., 2010) and the minimum and maximum fat values were 2.51% and 14.64%, respectively (Chapter 3). Variability in the fat percentage was modelled by describing the fat percentage as a triangular distribution with mode 9.4, minimum 2.51, and maximum 14.64.
5.5. Hazard Characterisation

The hazard characterisation step describes the likelihood that consumption of *Salmonella* contaminated pork burgers will result in human salmonellosis. Based on the number of viable *Salmonella* that remain in each burger at the point of consumption, the probability of illness given contamination of the burger with *Salmonella* was calculated. It is assumed here that burgers are approximately 100g in weight, 8cm in diameter and 2cm thick based on the inactivation model presented in Chapter 3. The mean probability of illness estimates the mean risk of salmonellosis per serving.

5.5.1. Dose-Response Models

Several dose-response relationships have been devised that relate the number of *Salmonella* organisms to a probability of illness from consumption. For this risk assessment, the dose-response model for salmonellosis by FAO/WHO, (2002) was used, which estimates the probability of illness given the number of *Salmonellae* consumed in contaminated servings ($P_{\text{ill|cont}}$) and does not estimate the severity of illness. This dose-response model, generated from outbreak data, was shown to be a better fit to the data compared to previously generated models (FAO/WHO, 2002) and has been used previously in risk assessments of salmonellosis (Guillier et al., 2013). The dose-response model by Teunis et al. (2010) published subsequently, provides conservative estimates of the dose-response relationship, with an ID$_{50}$ value of 36 *Salmonellae*, with a 95% predictive interval of ($0.69 - 1.26 \times 10^7$) and was deemed too conservative and uncertain compared to previously published dose-response relationships. Uncertainty in the dose-response model parameters was included by allowing them to vary according to a triangular distribution (Guillier et al., 2013). For $\alpha$, the minimum was 0.0763, the mode 0.1324 and the maximum 0.2274. For $\beta$, the minimum was 38.49, the mode 51.45 and the maximum 57.96.

The probability of illness ($p_{\text{ill}}$) over all servings, including uncontaminated servings, was estimated from the prevalence per serving ($P_{\text{serve}}$) and the probability of illness given contamination
by multiplying these two numbers. While this method reduces the number of iterations required to estimate the mean probability of illness, this method distorts the meaning of the probability of illness for each serving, with the probability of illness now not reflecting the true probability of illness for that serving, but instead an estimate weighted by the *Salmonella* prevalence.

To allow the results of this risk assessment to be related to the number of servings of pork burgers consumed in Australia per year, two hypothetical scenarios were considered (Scenarios 12 and 13) which overestimate the number of servings of pork burgers consumed. As no data were available to estimate the number of pork burger servings consumed per year, these scenarios provide upper bounds on national consumption. Scenario 12 assumed that all pork mince sold in Australia per year is consumed as pork burgers. Retail sales data for pork mince in Australia during the 52 week period ending 27 December 2014 showed 4.85 million kilograms of pork mince sold, equating to 48.52 million servings of pork burgers (assuming 100g servings). Scenario 13 assumed that all fresh pork products are cooked as pork burgers in Australia. Sales data for the same 52 week period for pork chops, cutlets, steaks, fillets, ribs, rashers, roasts and mince, indicated 42.79 million kilograms of pork consumed which, if consumed as pork burgers, would equate to 427.89 million servings. The same calculations used in Scenarios 12 and 13 were combined with the probability of illness estimate obtained in Scenario 9 to estimate the expected number of salmonellosis cases expected if Australian pork burger patties are cooked to the same temperatures observed in the United States (Scenarios 14 and 15 respectively).

The risk assessment model was run for 250,000 contaminated iterations, with 500 contaminated iterations in the variability dimension and 500 contaminated iterations in the uncertainty dimension. These values were chosen based on the convergence of the running means and confidence intervals estimated. Latin hypercube sampling was used, where possible, to reduce the number of simulations required for convergence. The mean probability of illness was calculated for each of the 500 iterations in the variability dimension, resulting in 500 calculated means and the mean of these estimates used to estimate the mean probability of illness. The 95% credible interval on the
5.6. Risk Characterisation

5.6.1. Risk Estimate

The model predicts that the mean probability of symptomatic salmonellosis (mean of the calculated means) from the consumption of pork burgers is $1.54 \times 10^{-8}$, i.e., it would be expected that for every 65,000,000 servings consumed, there would be around one case of salmonellosis. The 95% credibility interval on the uncertainty is $(7.2 \times 10^{-10}, 4.96 \times 10^{-8})$.

The change in the mean Salmonella concentration during retail storage is estimated as $0.0030 \log_{10}$ CFU/serve and is $0.0850$, $0.3481$, $0.7859$ and $1.3 \log_{10}$ CFU/serve at the 99%, 99.9%, 99.99% and 99.999% quantiles of the variability, respectively. The change in mean Salmonella concentration during transport to the consumer’s home is $0.00205 \log_{10}$ CFU/serve and is $0.0456$, $0.126$, $0.249$ and $0.372 \log_{10}$ CFU/serve at the 99%, 99.9%, 99.99% and 99.999% quantiles of the variability, respectively. The change in mean Salmonella concentration during domestic refrigeration is $0.0059 \log_{10}$ CFU/serve and is $0.155$, $0.662$, $1.55$ and $2.62 \log_{10}$ CFU/serve at the 99%, 99.9%, 99.99% and 99.999% quantiles of the variability, respectively.

5.6.2. Sensitivity Analysis

Sensitivity analysis was performed by calculating the Spearman rank correlations between the inputs and the probability of illness ($p_{ill}$). These correlations are presented in Figure 5.2, with the 95% credible intervals around the uncertainty. The three inputs that have the largest effect on the probability are the cooking temperature, $T_{cook}$ ($\rho = -0.731$), the concentration of Salmonella in pork mince, $C_{mince}$ ($\rho = 0.609$) and the fat content of pork mince, $F_{mince}$ ($\rho = -0.128$).

The effect of the input uncertainties on the uncertainty in the final risk estimate was also examined and results are presented in Figure 5.3. The largest sources of uncertainty are the concentration of
5. Quantitative Microbial Risk Assessment of Salmonellosis From the Consumption of Australian Pork: Minced Meat From Retail to Burgers Prepared and Consumed at Home

Figure 5.2.: Tornado plot of the Spearman rank correlations between inputs of the model and the probability of illness in the variability dimension. For each input, the mean correlation, with 95% credible intervals are given.

Salmonella in pork mince, $C_{\text{mince}}$, with $\rho = 0.663$ at the 97.5% quantile of the variability, followed by the prevalence of Salmonella in pork mince, $P_{\text{mince}}$, with $\rho = 0.569$ and the $\alpha$ parameter of the dose-response model ($\rho = 0.205$).

5.6.3. Scenario Analysis

To allow for comparisons between scenarios, the random seed of the random number generator was set to the same number before simulating each scenario. The mean risk estimates for Scenarios 1 to 11 are presented in Table 5.4. The baseline risk estimate is also included for comparison, with the relative difference between each scenario and the baseline calculated. The scenario that had the largest relative effect on the risk estimate was the use of the US cooking temperature data instead of Australian cooking temperature data. The other scenario that had a large effect on the risk was increasing the mean Salmonella concentration.

The expected number of illnesses per year from pork burger consumption if all pork mince sold in Australia is consumed as pork burgers (Scenario 12) would be 0.746, with a 95% uncertainty credible interval of (0.0349, 2.41). The expected number of illnesses per year from pork burger consumption if all fresh pork products are consumed as pork burgers (Scenario 13) would be
5.6. Risk Characterisation

Figure 5.3.: Tornado plot of the Spearman rank correlations between the inputs of the model and the probability of illness in the uncertainty dimension. For nodes with variability and uncertainty, correlations were calculated for the mean, standard deviation and 97.5% quantiles of the variability.
Table 5.4.: Results from the scenario analysis. Ratio to baseline values are the ratio of the mean probability of illness for each scenario against the mean of probability of illness for the baseline scenario.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Description</th>
<th>Mean $P_{ill}$</th>
<th>Ratio to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Model</td>
<td></td>
<td>$1.54 \times 10^{-8}$</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>$C_{mince} \sim N(\mu = 0.22, \sigma = 0.97)$</td>
<td>$8.95 \times 10^{-8}$</td>
<td>5.82</td>
</tr>
<tr>
<td>2</td>
<td>$C_{mince} \sim N(\mu = -1.78, \sigma = 0.97)$</td>
<td>$4.84 \times 10^{-9}$</td>
<td>0.315</td>
</tr>
<tr>
<td>3</td>
<td>$T_r \sim S\text{Normal}(\mu = 3, \sigma = 2.11, \xi = 0.77)$</td>
<td>$1.53 \times 10^{-8}$</td>
<td>0.994</td>
</tr>
<tr>
<td>4</td>
<td>$T_r \sim S\text{Normal}(\mu = 5, \sigma = 2.11, \xi = 0.77)$</td>
<td>$1.9 \times 10^{-8}$</td>
<td>1.24</td>
</tr>
<tr>
<td>5</td>
<td>$T_r \sim S\text{Normal}(\mu = 6, \sigma = 2.11, \xi = 0.77)$</td>
<td>$4.14 \times 10^{-8}$</td>
<td>2.7</td>
</tr>
<tr>
<td>6</td>
<td>$T_r \sim S\text{Normal}(\mu = 7, \sigma = 2.11, \xi = 0.77)$</td>
<td>$1.91 \times 10^{-7}$</td>
<td>12.4</td>
</tr>
<tr>
<td>7</td>
<td>$T_{ef}$ constant</td>
<td>$1.56 \times 10^{-8}$</td>
<td>1.01</td>
</tr>
<tr>
<td>8</td>
<td>$\Delta T_{ef}$ sampled from data</td>
<td>$1.53 \times 10^{-8}$</td>
<td>0.996</td>
</tr>
<tr>
<td>9</td>
<td>$T_{cook}$ sampled from (EcoSure, 2008)</td>
<td>$1.73 \times 10^{-4}$</td>
<td>11,300</td>
</tr>
<tr>
<td>10</td>
<td>$S$. Senftenberg used for inactivation model</td>
<td>$8.64 \times 10^{-9}$</td>
<td>0.562</td>
</tr>
<tr>
<td>11</td>
<td>$S$. Typhimurium used for inactivation model</td>
<td>$6.38 \times 10^{-8}$</td>
<td>4.15</td>
</tr>
</tbody>
</table>

6.58, with a 95% uncertainty credible interval of (0.308–21.2). Based on the probability of illness estimated if all Australian pork burgers are cooked to the same temperatures observed in the United States (Scenario 9), the predicted number of illnesses assuming all pork mince was consumed as pork burgers is 8,410, with a 95% uncertainty credible interval of (1,400 – 23,700), while the predicted number of illnesses assuming that all fresh pork is consumed as pork burgers (Scenario 15) is 74,100, with a 95% uncertainty credible interval of (12,300 – 209,000).

5.7. Discussion

The mean probability of illness estimate for salmonellosis from pork burgers presented here is lower than those estimated by VLA/DTU/RIVM, (2011) for four (unspecified) member states of the European Union for the same pathogen-serving combination (see Table 5.5). It was not possible to use the risk assessment model of VLA/DTU/RIVM, (2011) to estimate the risk of salmonellosis from pork burger consumption in Australia because Australian data, analogous to country-specific data used in the EU model, could not be obtained. To overcome this limitation
Table 5.5: Comparison of risk estimate to VLA/DTU/RIVM (2011) results for pork burgers. MS stands for member state of the European Union.

<table>
<thead>
<tr>
<th>Location</th>
<th>Probability of Illness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>$1.54 \times 10^{-8}$</td>
</tr>
<tr>
<td>MS1</td>
<td>$8.84 \times 10^{-7}$</td>
</tr>
<tr>
<td>MS2</td>
<td>$2.24 \times 10^{-5}$</td>
</tr>
<tr>
<td>MS3</td>
<td>$2.32 \times 10^{-7}$</td>
</tr>
<tr>
<td>MS4</td>
<td>$2.58 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

The model presented here uses data that were available for Australia and different methods to incorporate these data into the risk assessment model. In some cases, surrogate data from other countries were used to fill data gaps. These sources were used only where they were expected to be similar to or more conservative than Australian conditions or practices.

The risk estimates presented here are low relative to those of VLA/DTU/RIVM, (2011) due to a combination of factors. Firstly, the *Salmonella* prevalence and concentration is low on Australian pork mince and little growth appears to occur during retail display, consumer transport and consumer storage (see Section 5.6.1). Australian consumers also claim to prefer pork products cooked to a higher degree of “doneness” than US consumers, with an estimated 72.2% of pork burgers cooked to temperatures around or exceeding 74 °C. This, coupled with the inactivation model estimating a 5-log$_{10}$ reduction in *Salmonella* concentration occurring at 63 °C (see Chapter 3), results in a large predicted cooking effect on burgers that have low levels of *Salmonella* contamination before cooking.

Retail storage is the final point at which product can be controlled and monitored by government regulation. The retail temperature distribution ($T_r$) predicts that 16% of retail product will be stored at temperatures in which *Salmonella* growth is predicted to occur (assuming constant temperature during storage). While empirical data were available for the storage temperature at retail, the storage duration was not known. Estimates based on expert opinion have been used in this, and previous risk assessments to estimate this duration, with large uncertainty ranges included in their implementation. Scenarios 3 to 6 investigated the effect of changes to the
mean product temperature at retail. Increases in the mean product temperature resulted in an increased risk of salmonellosis to consumers, though the relative increase in risk was far greater at higher temperatures (6 °C to 7 °C) compared to lower temperatures (3 °C to 5 °C). Any increase in the mean retail temperature would require careful consideration by risk managers as any cost savings due to reduced power usage may be outweighed by a higher risk to consumers. While retail temperatures above 5 °C are not allowed under Australian law (FSANZ, 2014), scenarios above this temperature were included to investigate the effect of loss of temperature control. Consideration of the effect of “use-by” dates are not included in this model because their specification varies greatly depending on the type of retailer. Note that in Australia, “use-by” date refers to the time beyond which the product should not be consumed, based on the potential for food safety risk. Only pre-packaged product is required to display “use-by” or “best before” dates in Australia. Product which exceeds “use-by” at retail cannot be sold in Australia, though product that exceeds its nominal “use-by” date while in the possession of the consumer may still be consumed. “Use-by” dates would reduce the likelihood that product with a high *Salmonella* concentration would be consumed. If “use-by” dates were included in the model, they would be expected to decrease the estimated risk of salmonellosis, although the size of this reduction could not be estimated without data on consumer behaviour about product beyond its use-by date.

Assuming a constant maximum temperature during consumer transport does not greatly influence the final risk estimate as shown in Scenario 7, with little difference observed between this scenario and the baseline. Further, little difference was estimated between Scenario 8, in which the change in temperature during transport was directly sampled from the data, and the baseline model.

*Salmonella* growth during domestic storage caused the largest increase in the mean concentration of *Salmonella* compared to the retail and consumer transport sections of the model. For the domestic storage of pork mince, the storage temperature distribution \(T_{ds}\) and the *Salmonella* growth model predict that *Salmonella* growth (assuming constant storage temperature) will occur in 13.6% of product. This means that the data suggest that slightly less product is stored at temperatures supporting *Salmonella* growth during domestic storage than during retail storage.
The mean storage time at retail was predicted to be shorter (one day) than domestic storage (1.5 days). Therefore, while the proportion of product exposed to growth temperatures is greater at retail, products are stored longer on average in domestic storage.

The cooking temperatures used in this analysis were derived from consumer preference data for “doneness” of pork. Ideally, this data would be obtained from a survey of final internal temperatures reached during cooking of pork burgers by consumers. This estimate does not give any information about the proportion of burgers cooked to extremes (either very rare or very well done). Consumer preference for pork burgers presented in Table 5.3 decreased as the “doneness” decreased. Based on this, the proportion of burgers cooked to less than “medium rare” would likely be small. It is also difficult to convert the “doneness” descriptors to temperatures because “doneness” is a subjective assessment. The results from Scenario 9, which investigated the effects of a reduction in cooking temperatures similar to those reported in USA for beef burgers, present a mean risk estimate that is 11,300 times higher than the mean baseline risk estimate. The results for this scenario need to be interpreted with care because the temperatures reported by Ecosure (2008) are for beef burgers, not pork burgers. The cooking temperatures reported for pork (De Santos et al., 2007) are higher than those reported for beef (Jackson et al., 1996) for the equivalent degree of “doneness.” This implies that pork requires a higher internal cooking temperature to reach the same visual degree of “doneness” compared to beef. In Scenario 9, the temperature to which Australian consumers claim to cook pork burgers are shown to be relatively high and contribute greatly to the low risk of illness observed. A shift in consumer preference to pork burgers that are more “rare” would be expected to greatly increase the risk of salmonellosis to Australian consumers from consuming pork burgers.

Other dose-response models, of varying levels of complexity, have been proposed and are based on either feeding trial data, outbreak data or surrogate data. The most conservative dose-response model published (Teunis et al., 2010), predicts a probability of illness in excess of 0.95 for a Salmonella dose of 10,000 organisms. The least conservative dose-response model (Oscar, 2004), which was based on feeding trial data for Salmonella Pullorum, predicts a probability of illness of
5. Quantitative Microbial Risk Assessment of Salmonellosis From the Consumption of Australian Pork: Minced Meat From Retail to Burgers Prepared and Consumed at Home

0 for a dose of 10,000 Salmonellae. Due to these differences in dose-response models, the choice of dose-response model can have a large effect on the final risk estimate. For this risk assessment, we used the FAO/WHO Salmonella dose-response model (FAO/WHO, 2002) which was based on Salmonella outbreak data. Based on the fit of the model to the available data, this model appears to best describe the known observed outbreaks.

At present, there have been no reported outbreaks of salmonellosis from pork burger consumption in Australia. This provides further evidence that the risk estimates presented here are a credible reflection of current practices and conditions in Australia. Pork burgers do not appear likely to contribute to outbreaks of salmonellosis in Australia under current conditions.

5.8. Conclusion

The estimated risk of salmonellosis from pork burgers is low, even after a series of conservative assumptions were included in variations on the risk assessment. This is largely due to low prevalence and concentration of Salmonella in Australian produced pork, good refrigeration of pork and, particularly, Australian consumer preference for “well-done” pork burgers. If marketing is successful in increasing consumption of pork burgers, it appears unlikely that this will cause outbreaks of salmonellosis. A ten-fold increase in sales of pork mince and consumption of pork burgers would not be expected to lead to a large increase in the number of cases of salmonellosis.

5.A. Model Parameters
Table 5.6.: Parameters used in the stochastic model. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Model</td>
<td>( b )</td>
<td>Coefficient 1 of Ratkowsky equation</td>
<td>C</td>
<td>0.0304</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Growth Model</td>
<td>( c )</td>
<td>Coefficient 2 of Ratkowsky equation</td>
<td>C</td>
<td>0.105</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Growth Model</td>
<td>( T_{\text{min1}} )</td>
<td>Minimum temperature at which growth of <em>Salmonella</em> is estimated to occur</td>
<td>C</td>
<td>5.71</td>
<td>°C</td>
</tr>
<tr>
<td>Growth Model</td>
<td>( T_{\text{max1}} )</td>
<td>Maximum temperature at which growth of <em>Salmonella</em> is estimated to occur</td>
<td>C</td>
<td>48.72</td>
<td>°C</td>
</tr>
<tr>
<td>Growth Model</td>
<td>( \text{GR}^a )</td>
<td>Maximum growth rate ( \left[ \frac{b(T - T_{\text{min1}})^2}{1 - \exp \left[ c(T - T_{\text{max1}}) \right]} \right] )</td>
<td>VU</td>
<td>( \log_{10} )</td>
<td>CFU g (^{-1}) h (^{-1})</td>
</tr>
</tbody>
</table>

*Continued on next page*
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<th>Description</th>
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<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Model</td>
<td>$a$</td>
<td>Coefficient of maximum population density equation</td>
<td>C</td>
<td>11.83</td>
<td></td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{\text{min2}}$</td>
<td>Lower temperature at which the MPD is estimated to be $0 \log_{10}$ CFU/g</td>
<td>C</td>
<td>3.84</td>
<td>°C</td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{\text{submin}}$</td>
<td>Temperature less than $T_{\text{min2}}$</td>
<td>C</td>
<td>-2.54</td>
<td>°C</td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{\text{max2}}$</td>
<td>Upper temperature at which the MPD is estimated to be $0 \log_{10}$ CFU/g</td>
<td>C</td>
<td>50.31</td>
<td>°C</td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{\text{supmax}}$</td>
<td>Temperature greater than $T_{\text{max2}}$</td>
<td>C</td>
<td>53.12</td>
<td>°C</td>
</tr>
</tbody>
</table>

Continued on next page
Table 5.6: Parameters used in the stochastic model. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Model</td>
<td>MPD</td>
<td>Maximum population density function with respect to temperature</td>
<td>VU</td>
<td>( a \frac{(T - T_{min2})(T - T_{max2})}{(T - T_{submin})(T - T_{supmax})} )</td>
<td>( \log_{10} CFU/g )</td>
</tr>
<tr>
<td>Growth Model</td>
<td>( MP_{serve} )</td>
<td>Maximum population density per serve</td>
<td>VU</td>
<td>( 10^{MPD(T)} \times W_{serve} )</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>( W_{serve} )</td>
<td>The weight of a serving</td>
<td>C</td>
<td>100</td>
<td>g</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>( P_{mince} )</td>
<td>Prevalence of ( Salmonella ) in pork mince</td>
<td>U</td>
<td>Beta(( \alpha = 3, \beta = 147 ))</td>
<td>Probability</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>( P_{serve} )</td>
<td>The prevalence of ( Salmonella ) adjusted for the serving size</td>
<td>U</td>
<td>( 1 - (1 - P_{mince})^4 )</td>
<td>Probability</td>
</tr>
</tbody>
</table>

Continued on next page
Table 5.6: Parameters used in the stochastic model. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Conditions</td>
<td>$C_{\text{mince}}$</td>
<td>Concentration of <em>Salmonella</em> in pork mince</td>
<td>VU*</td>
<td>Normal($\mu = -0.79, \sigma = 0.66$)</td>
<td>log$_{10}$ CFU/g</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>$N_{\text{serve}}$</td>
<td>The number of <em>Salmonella</em> per contaminated serving</td>
<td>VU</td>
<td>ZTP($\lambda = 10^{C_{\text{mince}} \times W_{\text{serve}}}$)</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Retail</td>
<td>$\theta_{tr}$</td>
<td>Uncertainty in the mean duration of product storage at retail</td>
<td>U</td>
<td>Unif(0.5, 1.5)</td>
<td>days</td>
</tr>
<tr>
<td>Retail</td>
<td>$t_{rb}$</td>
<td>Time that product remains on retail shelf</td>
<td>VU</td>
<td>Exp($\theta = \theta_{tr}$) $\times$ 24</td>
<td>h</td>
</tr>
<tr>
<td>Retail</td>
<td>$T_r$</td>
<td>Temperature of product on retail shelf</td>
<td>VU*</td>
<td>SNormal($\mu = 3.41, \sigma = 2.11, \xi = 0.77$)</td>
<td>°C</td>
</tr>
<tr>
<td>Retail</td>
<td>$N_r$</td>
<td>Population of <em>Salmonella</em> in serving after growth at retail</td>
<td>VU</td>
<td>min[10$^{\log_{10}(N_{\text{serve}}) + G(T = T_r) \times t_r}$, $MP_{\text{serve}}(T = T_r)$]</td>
<td>CFU/serve</td>
</tr>
</tbody>
</table>

*Continued on next page*
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<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumer Transport</td>
<td>$t_{ct}$</td>
<td>Time taken for transport from retail to home by consumer</td>
<td>VU*</td>
<td>Gamma($\alpha = 7.37, \beta = 6.30$)</td>
<td>h</td>
</tr>
<tr>
<td>Consumer Transport</td>
<td>$\Delta T_{ct}$</td>
<td>Change in temperature during consumer transport</td>
<td>VU*</td>
<td>Gamma($\alpha = 1.77, \beta = 0.55$)</td>
<td>°C</td>
</tr>
<tr>
<td>Consumer Transport</td>
<td>$T_{ct}$</td>
<td>Temperature of pork mince after consumer transport</td>
<td>VU</td>
<td>$T_r + \Delta T_{ct}$</td>
<td>°C</td>
</tr>
<tr>
<td>Consumer Transport</td>
<td>$N_{ct}$</td>
<td>Population of <em>Salmonella</em> in mince after consumer transport</td>
<td>VU</td>
<td>See text for description</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Domestic Storage</td>
<td>$t_{ds}$</td>
<td>Time that product is in domestic storage</td>
<td>VU*</td>
<td>Exp($\theta = 1.53 \times 24$)</td>
<td>h</td>
</tr>
<tr>
<td>Domestic Storage</td>
<td>$T_{ds}$</td>
<td>Temperature of domestic storage</td>
<td>V</td>
<td>Normal($\mu = 3.35, \sigma = 1.94$)</td>
<td>°C</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 5.6: Parameters used in the stochastic model. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic Storage</td>
<td>$N_{ds}$</td>
<td>Population of <em>Salmonella</em> in mince after domestic storage</td>
<td><strong>UV</strong></td>
<td>$\min \left[ 10^{\log_{10}(N_{ct}) + GR(T = T_{ds}) \times z_{ds}}, MP_{serve}(T = T_{ds}) \right]$</td>
<td>CFU/g</td>
</tr>
<tr>
<td>Cooking</td>
<td>$T_{int}$</td>
<td>The internal temperature for each degree of doneness</td>
<td><strong>C</strong></td>
<td>${63, 71, 74, 77}$</td>
<td>°C</td>
</tr>
<tr>
<td>Cooking</td>
<td>done$_{weights}$</td>
<td>The percentage of consumers who prefer each degree of doneness</td>
<td><strong>C</strong></td>
<td>${4.7, 23.1, 39.1, 33.1}$</td>
<td>%</td>
</tr>
<tr>
<td>Cooking</td>
<td>$T_{cook}$</td>
<td>Final internal temperature each serving is cooked to</td>
<td><strong>V</strong></td>
<td>$\text{Normal}(\mu \sim \text{EmpericalD}(T_{int}, \text{done$_{weights}$}), \sigma = 1)$</td>
<td>°C</td>
</tr>
<tr>
<td>Cooking</td>
<td>$F_{mince}$</td>
<td>The fat percentage in the raw mince</td>
<td><strong>V</strong></td>
<td>$\text{Triangular}(\min = 0.025, \text{mode} = 0.094, \max = 0.146)$</td>
<td>%</td>
</tr>
</tbody>
</table>

*Continued on next page*
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<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking</td>
<td>$N_{cook}$</td>
<td>Population of <em>Salmonella</em> survivors after cooking</td>
<td>V</td>
<td>$\exp [\ln (N_{ds}) - \ln (1 + z)]$</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Dose-response</td>
<td>$\alpha$</td>
<td>Parameter 1 of the Beta Poisson dose response model</td>
<td>U</td>
<td>Triangular(min = 0.0763, mode = 0.1324, max = 0.2274)</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Dose-response</td>
<td>$\beta$</td>
<td>Parameter 2 of the Beta Poisson dose response model</td>
<td>U</td>
<td>Triangular(min = 38.49, mode = 51.45, max = 57.96)</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Dose-response</td>
<td>$p_{ill</td>
<td>cont}$</td>
<td>The probability of illness for salmonellosis given contamination</td>
<td>VU</td>
<td>$1 - \left(1 + \frac{N_{cook}}{\beta}\right)^{-\alpha}$</td>
</tr>
<tr>
<td>Dose-response</td>
<td>$p_{ill}$</td>
<td>Probability of illness including uncontaminated servings</td>
<td>VU</td>
<td>$P_{ill</td>
<td>cont} \times P_{serve}$</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 5.6: Parameters used in the stochastic model. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
</table>

* Uncertainty in this parameter was included using non-parametric bootstrap sampling. Parameter values given in value column are those produced by maximum likelihood estimation.

a No growth occurs at temperatures less than $T_{\text{min}}$ or above $T_{\text{max}}$.

b Truncated to a maximum of 10 days.
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6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

6.1. Abstract

While moisture-infused pork may have improved sensory qualities compared to intact pork, the microbial risks associated are poorly understood. During the injection of brine into these products, there is the potential for either the needles or the brine to transfer pathogens to the interior of the product. To better understand the effect of this process, a quantitative risk assessment model was constructed, describing the effect of the infusion process on the prevalence and concentration of Salmonella on/in infused pork loins and then pork steaks during retail, consumer transport, domestic storage and cooking before the risk of illness from consumption was estimated. Under current conditions in Australia, the mean risk of salmonellosis from consumption of 100g pork steaks assuming approximately 7.5% of steaks are moisture-infused, was estimated at $4.12 \times 10^{-8}$ per meal or one illness per 24,300,000 servings consumed. Based on this, moisture-infused pork in Australia is currently considered to pose a low risk of salmonellosis, due in part to relatively low consumption, and the tendency of Australian consumers to cook pork to high internal temperatures.
6.2. Introduction

Needle/blade tenderisation or brine injection is used to improve the sensory qualities of meat products. These treatments, however, may allow foodborne pathogens to be transferred to the interior of the product, and needle tenderisation leaves no visible signs of alteration. Similar to minced meat products (see Chapter 5), if these products are cooked as though intact, there is a potential increased risk compared to intact pork steaks. Moisture infusion injects pork products with a brine solution to enhance flavour, juiciness and overall acceptability of the cooked product (Moore et al., 2012) and is being applied to pork products in Australia for two reasons. Australian pork has been selectively bred over the long-term for pigs with decreased back-fat, resulting in reduced intramuscular fat and a drier cooked product. Further, Australians prefer fresh pork cooked to at least “medium/well-done” (Channon et al., 2013a) further reducing sensory acceptance of low fat products. As noted in previous Chapters, circa 2010 there were estimated to be 4.1 million cases of gastroenteritis yearly in Australia, of which salmonellosis is the second largest cause, with 39,600 cases per annum estimated (Kirk et al., 2014a). Symptoms of non-typhoidal salmonellosis can include nausea, vomiting, abdominal cramps, diarrhoea, fever and headaches. Circa 2010, an estimated 15 deaths were caused by salmonellosis (0.04% of estimated salmonellosis cases) and was estimated the joint-leading cause of death from foodborne illness (Kirk et al., 2014a). Brine injected beef products in USA have been linked to outbreaks of foodborne illness (Laine et al., 2005), though no outbreaks involving brine injected products in Australia were found in the literature.

The moisture infusion or brine injection process can be considered in two parts. In the tenderisation process, needles pierce the surface of the primals as they pass through the machine, potentially leading to two modes of pathogen transfer, named horizontal/lateral transfer and vertical transfer (Wen and Dickson, 2013). Horizontal/lateral describes pathogens transferred from the surface of one contaminated primal to subsequently tenderised primals via the needles or blades. Vertical transfer describes surface contamination of primals being transferred into the
interior of mechanically tenderised products via the needles or blades of the tenderiser. Both modes of transfer have been observed in beef (Huang and Sheen, 2011; Johns et al., 2011; Luchansky et al., 2008) and pork (Wen and Dickson, 2013) contaminated with E. coli. The injection of brine into the deep tissues of the product allows pathogens to be internalised in the meat i.e., vertical transfer via the brine, not the needles. Any brine that is injected into the product, but not retained by the product, returns to the balance tank and counts of Enterobacteriaceae have been shown to increase during production (Greer et al., 2004). With increasing contamination of the balance tank, the potential for contaminated brine to be injected into primals increases.

Previous risk assessments of non-intact meats, while finding an increased risk associated with these products, have not fully considered all production and supply processes that may influence risk. Smith et al. (2013) found an increased risk of illness associated with non-intact beef, though they only considered the potential transfers associated with the needles while discounting the effect of the recirculating brines. Miller et al. (2008) assessed the risk of salmonellosis from consumption of intact and non-intact pork, basing the Salmonella concentration estimates on retail survey data of intact and non-intact pork chops. Non-intact pork had a higher Salmonella prevalence compared to intact pork though the risk estimate at each endpoint cooking temperature modelled was similar for non-intact and intact pork. These risk assessments were also conducted in USA and Canada and some country-specific data required to use these models were not available for Australia. As these products have been estimated to present increased risk to consumer health compared to intact pork, and analogous products have caused outbreaks (Laine et al., 2005), a science-based risk assessment was undertaken to evaluate the risk posed by this value-added product to support risk management decisions and strategies.

The aims of this risk assessment were to i) estimate the risk of salmonellosis to Australian consumers from the consumption of pork steaks that are either infused or intact, based on current moisture infusion prevalences at retail, ii) compare to the risk from only intact or infused pork steaks and iii) identify supply chain steps that influence the risk to consumers from pork steaks, with particular emphasis on the influence of the process on Salmonella prevalence and
6.3. Methods

A stochastic quantitative risk assessment model for salmonellosis from consumption of moisture-infused pork steaks cut from pork loins was constructed consistent with Codex Alimentarius Commission guidelines for food microbial safety risk assessment (Codex Alimentarius Commission, 1999). The model presented in this Chapter was implemented in the statistical programming language R (R Core Team, 2016), using the mc2d package (Pouillot and Delignette-Muller, 2010). The predicted risk associated with a number of alternative scenarios was also investigated.

No data describing the prevalence or concentration of *Salmonella* on pork steaks, either intact or infused, in Australia was available. To fill this data gap, the infusion process was modelled stochastically as a module that describes the transfers of *Salmonella* between pork loins, the needles of the infusion machine and the brine tank of the infusion machine during the infusion process. The outputs of the infusion module were prevalence and concentration estimates for intact and infused steaks and were used as inputs into a second module describing the *Salmonella* concentration changes during retail, consumer transport, consumer storage and cooking. Here, we define a module as a self-contained stochastic model, relying only on the parameter estimates generated from the previous module. The second module describes the changes to *Salmonella* prevalence and concentration. The final output of the second module is the estimated mean risk of illness from consumption of a serving of cooked pork. Retail, consumer transport and domestic storage for pork burgers in Australia were modelled previously (Chapter 5), and, where appropriate, the temperature and duration distributions described in that work were used.

At various steps in the model, uncertainty in the maximum likelihood estimates of distribution parameters was included in the model by generating non-parametric bootstrap samples using the “bootdistcens” function in the “fitdistrplus” R package (Delignette-Muller and Dutang, 2015), with the number of samples equal to the length of the uncertainty dimension in the model. These
6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

Table 6.1.: Scenarios explored to better understand relative risk, and most influential contributing factors, for salmonellosis from moisture-infused pork steaks in Australia.

<table>
<thead>
<tr>
<th>Scenario 'Number'</th>
<th>Scenario Description</th>
<th>Reason for Scenario</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>25L maximum balance tank capacity</td>
<td>Examine effect on risk of decreased balance tank capacity.</td>
</tr>
<tr>
<td>b</td>
<td>75L maximum balance tank capacity</td>
<td>Examine effect on risk of increased balance tank capacity.</td>
</tr>
<tr>
<td>c</td>
<td>100L maximum balance tank capacity</td>
<td>Examine effect on risk of increased balance tank capacity.</td>
</tr>
<tr>
<td>1</td>
<td>All steaks as intact</td>
<td>Examine effect on risk of ceasing moisture-infusion on pork steaks sold nationally.</td>
</tr>
<tr>
<td>2</td>
<td>All steaks as moisture-infused</td>
<td>Examine effect on risk of performing moisture-infusion on all pork steaks sold nationally.</td>
</tr>
<tr>
<td>3</td>
<td>Cooking temperatures as described by EcoSure (2008)</td>
<td>Examine effect on risk of decreased endpoint cooking temperatures in Australia to temperatures similar to US consumers.</td>
</tr>
<tr>
<td>4</td>
<td>Cooking temperatures for pork burgers</td>
<td>Examine the effect on risk of cooking pork steaks as similar to pork burgers, i.e., as if they were non-intact.</td>
</tr>
<tr>
<td>5</td>
<td>All pork fillet steaks sold in Australia consumed as pork steaks</td>
<td>Estimate the total number of illnesses expected in Australia per year from pork steak consumption.</td>
</tr>
</tbody>
</table>

Bootstrap samples were assigned as parameter estimates of that distribution in the uncertainty dimension of the two-dimensional model.

A number of ‘alternative’ scenarios were examined, which were included to examine the effect of changes to current production practices and consumption behaviours, and are outlined in Table 6.1. Scenarios labelled alphabetically examined the effect the scenario had on Salmonella prevalence and concentration while numbered scenarios examined the effect on salmonellosis risk from pork steak consumption.
6.3. Methods

6.3.1. Infusion Module

6.3.1.1. Weight and Length of Loin

The weight of whole pork loins generally ranges between 3.5 and 5.5kg (Anonymous, 2013). Based on this, the loin weight, \( w_{\text{loin}} \), was described by a Normal distribution with mean 4.5kg and standard deviation 0.51kg, such that 95% of pork loins are modelled to weigh between 3.5 and 5.5 kg. Similarly, a Normal distribution was used to describe loin length, \( l_{\text{loin}} \), with mean 52.5cm and standard deviation 1.28cm, such that 95% of loins are between 50 and 55cm (Anonymous, 2013).

6.3.1.2. Surface Area of Loin

The surface area of a loin was determined based on the density of a pork loin and the simplifying assumption that a pork loin is approximately cylindrical. The volume of a pork loin was thus expressed as \( V = \frac{w_{\text{loin}}}{\rho} \), where \( \rho \) is the density, estimated at 0.93 g/cm\(^3\), which is based on the density of “Pork, medium, with bone, raw” (FAO/INFOODS, 2012). The volume of a cylinder can also be expressed by its dimensions as \( V = \pi r_{\text{loin}}^2 l_{\text{loin}} \), where \( r_{\text{loin}} \) is the radius of a loin and, \( l_{\text{loin}} \), the length of the loin. Equating these two expressions for \( V \) and solving for \( r \) gives \( r_{\text{loin}} = \sqrt{\frac{w_{\text{loin}}}{\rho \pi}} \). Hence, the surface area is given by

\[
S A_{\text{loin}} = 2\pi r_{\text{loin}} [l_{\text{loin}} + r_{\text{loin}}] = \frac{2}{\rho l_{\text{loin}}} \left( \frac{\pi \rho l_{\text{loin}}^3 w_{\text{loin}} + w_{\text{loin}}}{l_{\text{loin}}} \right).
\]

6.3.1.3. Prevalence of Salmonella per Loin

The prevalence of *Salmonella* on pork shoulders was used as a surrogate for the prevalence on pork loins. This assumption was made because both pork cuts are processed on the same equipment and surfaces, and in the same way. Swabs of approximately 100cm\(^2\) were taken from
shields from the general conveyor belt just prior to packing or from various slicers as they returned them to the belt or tubs. From 435 shoulder swabs, four shoulders had detectable levels of *Salmonella* (D. Hamilton, 2015, South Australian Research and Development Institute, Pers. Comm.), resulting in a prevalence estimate of 0.0092. Uncertainty in the true prevalence was incorporated by allowing it to vary according to a Beta distribution, with $\alpha = 5$ and $\beta = 432$ (Vose, 2000). The prevalence per loin was calculated as

$$P_{primal} = 1 - (1 - P_{shoulder})^{SA_{loin}/100}.$$ 

Each simulated loin was determined to be either contaminated with *Salmonella*, $z_{loin} = 1$ or not contaminated with *Salmonella*, $z_{loin} = 0$, based on random draws from a Bernoulli distribution with $p = P_{loin}$

### 6.3.1.4. Concentration of *Salmonella* per Loin

Few data are available describing the *Salmonella* concentration in/on Australian pork products. A Normal distribution was used to describe the concentration of *Salmonella* on contaminated pork belly strip and trim samples (see Section 5.4.1), with mean $-0.79 \log_{10} \text{CFU/g}$ and standard deviation $0.66 \log_{10} \text{CFU/g}$. In the absence of specific data, this estimate was used to describe the *Salmonella* concentration on pork loins, $C_{loin}$. Uncertainty in the mean and standard deviation was incorporated using bootstrapping as described in the introduction to Section 6.3.

### 6.3.1.5. *Salmonellae* per Primal

The number of *Salmonella* on the exterior of contaminated loins, $N_{ext|cont}$, was assumed to follow a Poisson distribution with $\lambda = 10^{C_{loin} \times SA_{loin}}$, which was truncated at ‘1’ so that the surface of each contaminated primal contained at least one *Salmonella* organism. The number of *Salmonella* on the exterior of all loins, contaminated and uncontaminated, $N_{ext}$, was then determined by
6.3. Methods

multiplying $N_{\text{ext}}$ by $z_{\text{loin}}$.

6.3.1.6. Brine Injection per Primal

The target volume of brine to be injected into each loin is 10% of the weight of the loin, with limits of 8% to 15% (APL, 2009; Moore et al., 2012). The volume of brine required to raise the weight of each primal by 10% was calculated based on the weight of each primal, using the simplifying assumption that the brine has a density of 1 g/ml. The volume of brine required for each primal was $V_{\text{inj}} = W_{\text{primal}} \times 0.1$ and the total volume of brine required to inject all primals was the sum of the individual volumes of brine injected.

Commercial brine injection machines have two tanks to hold the brine, the balance tank, from which brine is injected, with excess brine returning to a balance tank and a storage tank, used to refill the balance tank and where the brine is mixed. From visual inspection, it was estimated that the balance tank holds 50L of brine (G. Holds, 2015, South Australian Research and Development Institute, Pers. Comm.). The effect of changes in the balance tank maximum volume on the prevalence and concentration of Salmonella on infused pork steaks were examined in Scenarios a, b and c. We assume that the storage tank has no effective maximum capacity because this tank is refilled if empty and is separate from the injection system.

6.3.1.7. Contamination Locations

To determine the number of Salmonellae on each primal after infusion, the influence of the injection process was considered to comprise of four components:

i. the surface/external contamination on a primal,

ii. existing interior contamination of the primal,

iii. contamination on the needles and

iv. contamination of the brine.
6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

The injection process was then modelled assuming that pork loins are injected individually (thus, direct cross contamination caused by contact of primals was not considered) and that all loins in the variability dimension are injected one after the other in a theoretical production shift. If we consider the \(i\)-th primal in the variability dimensions and the \(j\)-th primal in the uncertainty dimension, then the four modelled contamination sites (external, internal, needles and brine) are combined as

\[
N_{ij} = \begin{bmatrix}
N_{\text{ext}} \\
N_{\text{int}} \\
N_{\text{needles}} \\
N_{\text{brine}}
\end{bmatrix}^T
\]

where \(N_{\text{int}}\) is the number of *Salmonella* inside the primal \(ij\), \(N_{\text{needles}}\) is the number of *Salmonella* on the needles of the injection machine before primal \(ij\) and \(N_{\text{brine}}\) is the number of *Salmonella* in the brine injected into primal \(ij\). The transpose, \(T\), of the vector, \(N\), was taken to allow for matrix multiplication by the transfer matrix. It was assumed that \(N_{\text{int}}\), \(N_{\text{needles}}\) and \(N_{\text{brine}}\) were all equal to zero for all primals before the start of a shift.

6.3.1.8. Transfer Matrix

To describe the transfers of pathogens between the four aspects of the injection process, a matrix of transfer coefficients was constructed assuming that *Salmonella* on/in the exterior (\(E\)), interior (\(I\)), needles (\(N\)) or brine (\(B\)) can either be transferred to any of the other three sites, or remain at their current position. This matrix was constructed to incorporate uncertainty in the coefficient estimates by allowing these coefficients to vary for different primals (i.e. different values of \(j\)) in the uncertainty dimension. We use the notation \(T_{EI}\) to describe the percentage of the total *Salmonella* on the exterior of the loin (source) that are transferred to the interior of the product during injection (destination) and analogous notation for the other fifteen potential ‘transfers’. Transfer coefficients are constrained between 0 and 1, the upper bound ensuring that *Salmonella*
are not ‘created’ during the infusion process. If we assume that *Salmonella* can only be transferred to the four sites described here, then the sum of the four transfers with the same source must equal one. Negative transfers coefficients are not allowed as this effect is captured in the transfer coefficient with reversed source and destination, i.e., a negative value for $T_{BE}$ is captured by $T_{EB}$. The four “transfers”, $T_{EE}, T_{II}, T_{NN}, T_{BB}$, where the source and destination are the same, represent the proportion of *Salmonellae* that are not transferred to another site by the infusion process and are the complement of the sum of the other three transfers for that source, i.e. $T_{EE} = 1 - (T_{EI} + T_{EN} + T_{EB})$. As no internal contamination exists for primals before injection, we can set $T_{II} = 1$ and $T_{IE} = T_{IN} = T_{IB} = 0$. Based on this, a matrix of transfer coefficients between zero and one was defined such that:

$$
T = \begin{bmatrix}
T_{EE} & T_{EI} & T_{EN} & T_{EB} \\
0 & 1 & 0 & 0 \\
T_{NE} & T_{NI} & T_{NN} & T_{NB} \\
T_{BE} & T_{BI} & T_{BN} & T_{BB}
\end{bmatrix}
$$

The other transfer coefficients were then estimated from the published literature or expert opinion, as described below:

- The transfer of *Salmonellae* from the exterior of the primal to the interior, $T_{EI}$, was estimated from the data of Luchansky et al. (2008). This study found that the percentage transfer of *E. coli* O157:H7 from the exterior to the interior of blade tenderised beef primals ranged between 37% and 55% at varying *E. coli* O157:H7 concentrations with a mean *E. coli* O157:H7 transfer of 45.5%. From this, a Triangular distribution was used to describe this coefficient, with minimum 0.37, mode 0.455 and maximum 0.55.

- The transfer of *Salmonellae* from the exterior of the primal to the needles, $T_{EN}$, was estimated from Smid et al. (2012), who investigated the transfer of *Salmonella* via knives on pork. We use here the transfer ratios estimated by the Bayesian model which were
6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

estimated by a beta distribution $\alpha = 1.8$ and $\beta = 8.89$, resulting in a mean transfer ratio of 17%.

- The transfer of *Salmonellae* from the exterior of the primal to the brine, $T_{EB}$, was estimated from data of Spescha et al. (2006). That study investigated changes in Total Viable Counts (TVCs) on pig carcases throughout the slaughter process and provides mean TVCs for before and after carcase washing. Based on those means, $T_{EB}$ was modelled by a Triangular distribution with mode 0.257, minimum -0.20 and maximum 0.532, which are the minimum, mean and maximum of the mean TVC changes. This distribution was truncated at zero to ensure that the coefficient values lie in the accepted range.

- The transfer of *Salmonellae* during cutting of pork was investigated by Smid et al. (2012), who used a Bayesian model to estimate the parameters of a beta distribution, with $\alpha = 7.43$ and $\beta = 11.62$, resulting in a mean transfer ratio of 39%. This distribution estimates the total transfer, which would be divided between the exterior and interior of the pork loin by the needles. If we assume that half the transfer is to the surface of the loin and half is to the interior then $T_{NE} \sim \text{Beta}(\alpha=7.43,\beta=11.62)/2$ and $T_{NI} \sim \text{Beta}(\alpha=7.43,\beta=11.62)/2$.

- The transfer of *Salmonella* from the needles to the returning brine, $T_{NB}$, was assumed to be 0.05 because only a small volume of returning brine would come in contact with the needles.

- The transfer of *Salmonella* from the brine to the exterior of the primal, $T_{BE}$, was assumed to be 0.05 because only brine that is not retained by a loin can contaminate the exterior.

- The transfer of *Salmonella* from the brine to the interior of the primal, $T_{BI}$, was assumed to be 0.90 because most, but not all brine, and therefore, *Salmonella* is retained by the primal.

- The transfer of *Salmonella* from the brine to the needles, $T_{BN}$, was assumed to be 0.05 because only a small proportion of the brine will come in contact with the surface of the needles.

To evaluate the effect that changes to these transfer coefficients have on the estimated prevalence
and concentration of *Salmonella*, each transfer coefficient was set to either its mean or median and ±5, 10, 15 or 20% were added to each transfer coefficient, where appropriate. The prevalence of *Salmonella* per 100g steak and concentration as $\log_{10} \text{CFU/g}$ were then monitored over the same number of iterations as the baseline model (see Section 6.4).

To balance the transfer coefficients, it was assumed that the pathogen transfers occur in a specific order. For “external” as the source of contamination, it was assumed that transfer to the interior would occur first, followed by the needles and brine, with the remaining *Salmonella* remaining on the exterior. Based on this order of transfers, the exterior to interior remains $T_{EI}$, with the proportion remaining on the exterior after this transfer $1 - T_{EI}$, the exterior to the needles is $(1 - T_{EI})T_{EN}$, with the proportion remaining $(1 - T_{EI})(1 - T_{EN})$, the exterior to the brine is $(1 - T_{EI})(1 - T_{EN})T_{EB}$, and the proportion remaining on the exterior $(1 - T_{EI})(1 - T_{EN})(1 - T_{EB})$.

Applying this to the needles and brine as sources of *Salmonellae* results in adjusted transfer matrix coefficients of

\[
\begin{align*}
T_{EI}^* &= T_{EI} \\
T_{EN}^* &= (1 - T_{EI})T_{EN} \\
T_{EB}^* &= (1 - T_{EI})(1 - T_{EN})T_{EB} \\
T_{EE}^* &= (1 - T_{EI})(1 - T_{EN})(1 - T_{EB}) \\
T_{NE}^* &= T_{NE} \\
T_{NI}^* &= (1 - T_{NE})T_{NI} \\
T_{NN}^* &= (1 - T_{NE})(1 - T_{NI})(1 - T_{NB}) \\
T_{NB}^* &= (1 - T_{NE})(1 - T_{NI})T_{NB} \\
T_{BE}^* &= (1 - T_{NI})(1 - T_{NB})T_{BE} \\
T_{BI}^* &= T_{BI}
\end{align*}
\]
A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

\[ T_{BN}^* = (1 - T_{BI})T_{BN} \]
\[ T_{BE}^* = (1 - T_{BI})(1 - T_{BN})(1 - T_{BE}) \]

and an adjusted transfer matrix of

\[
T^* = \begin{bmatrix}
T_{EE}^* & T_{EI}^* & T_{EN}^* & T_{EB}^* \\
0 & 1 & 0 & 0 \\
T_{NE}^* & T_{NI}^* & T_{NN}^* & T_{NB}^* \\
T_{BE}^* & T_{BI}^* & T_{BN}^* & T_{BE}^* \\
\end{bmatrix}
\]

6.3.1.9. Processing

Based on the above conditions, the injection process was modelled for each loin. In each iteration of the simulation, before each loin was “injected”, the modelled volume of brine in the balance tank was checked to verify that the volume of brine was greater than one litre. In practice, the minimum volume of brine in the balance tank is variable and is controlled subjectively by the operator of the equipment. If the modelled volume is below this value, the balance tank is modelled to be refilled to its maximum value of 50 litres and that volume of brine subtracted from the main tank.

The number of Salmonella injected into each primal was calculated by assuming that the portion of brine injected into each primal is a partition of the total brine in the balance tank. Based on the formula of Nauta (2005), the number of Salmonella in the brine injected, \( N_{brine} \), is distributed as a Binomial distribution with \( n = N_{balance} \), the number of Salmonellae in the balance tank and \( p = V_{inj}/V_{balance} \), where \( V_{brine} \) is the volume of brine to be injected into the primal and \( V_{balance} \) is the total brine in the balance tank. In the model, brine and Salmonella injected into each loin are subtracted from the volume modelled to be in the brine tank \( (V_{balance} = V_{balance} - V_{brine}, N_{balance} = N_{balance} - N_{i,j,k}) \) The transfer of Salmonella was modelled by multiplying the four Salmonella contamination sites considered by the transfer matrix
and the predicted *Salmonella* remaining in the brine after injection were simulated to be added back into the brine tank ($N_{\text{balance}} = N_{\text{balance}} + N_{i,j,4}$). *Salmonella* remaining on the needles after the infusion of primal $i$ were modelled to be carried over to the next primal, $i + 1$.

While suspension in brine causes slow *Salmonella* inactivation (Wen and Dickson, 2013), this was not modelled because the rate of inactivation is very slow, the production shift duration was unknown, and the brine is replaced after each shift at the latest. This assumption results in a more conservative risk estimate.

### 6.3.1.10. Post Processing

The prevalence and concentration of *Salmonella* on intact and infused pork loins were estimated based on the modelled weight and surface area of the loin and also the proportion of those quantities that were ‘transferred’ with the steak cut from the loin as follows. If each serving $W_{\text{serve}} = 100$ g, then the thickness of a steak, $t_{\text{serve}}$ is $W_{\text{serve}}/(r_{\text{loin}}^2 \rho \pi)$ and the surface area of the steak before slicing, $S A_{\text{serve}}$ is $2 \pi r_{\text{loin}} t_{\text{loin}}$.

Under the assumption of an equal probability of distribution of *Salmonella* between steaks cut from the same loin, the prevalence of *Salmonella* on a steak cut from a loin that is either intact or moisture-infused was calculated based on the formula of Nauta (2005) as

$$P_i = 1 - \left(1 - \frac{S A_{\text{serve}}}{S A_{\text{loin}}} N_{\text{loin}}\right)^{N_{\text{loin}}}$$

$$P_{mi} = P_{\text{ext}} + P_{\text{int}} - P_{\text{ext}} P_{\text{int}}$$

where
In estimating the *Salmonella* prevalence for the infused steaks, it was assumed that steaks can be externally contaminated, internally contaminated or both externally and internally contaminated. The mean probability of contamination was calculated for each uncertainty iteration and a beta distribution was fitted to those mean probabilities.

The concentration of *Salmonella* in moisture-infused pork steaks was then determined. For each uncertainty iteration, the *Salmonella* concentration (log$_{10}$ CFU/g) was calculated from loins containing one or more *Salmonella* organisms by dividing the predicted *Salmonella* counts on each loin by their weights. Means and standard deviations were then calculated for each iteration in the uncertainty dimension, assuming that the concentration is normally distributed.

These prevalence and concentration distributions are used as inputs to the supply and consumption module.

### 6.3.2. Supply and Consumption Module

The supply and consumption module describes the changes in *Salmonella* prevalence and concentration during retail storage and display, consumer transport, domestic storage and cooking, with the probability of illness per serving estimated based on the number of *Salmonella* consumed.

#### 6.3.2.1. Initial Steak Conditions

No data were available on the proportion of pork steaks that are moisture-infused in Australia, thus, industry expert opinion was used (H. Channon, 2015, Australian Pork Limited, Pers. Comm.), resulting in an estimate of 5–10% of pork steaks in Australia being moisture-infused.
This proportion, \( \text{prop}_{mi} \), was modelled using a triangular distribution, with minimum 0.05, mode 0.075 and maximum 0.10. Two scenarios (1 and 2) were also investigated to determine the effect that changes to this proportion have on the mean risk of illness. In Scenario 1, all pork steaks were assumed to be intact (\( \text{prop}_{mi} = 0 \)) and in Scenario 2, all pork steaks were assumed to be infused (\( \text{prop}_{mi} = 1 \)). These scenarios cover the extremes of moisture infusion uptake by industry. Steaks were then modelled to be either moisture-infused or intact, \( i_{smi} \), based on random draws from a Bernoulli distribution, with \( p = \text{prop}_{mi} \).

Prevalence and concentration of \textit{Salmonella} on intact and infused 100g pork steaks \((P_i, P_{mi}, C_i, C_{mi})\) were calculated based on the distributions and values defined in the infusion module. The proportion of \textit{Salmonella} on/in pork steaks (infused and intact) was calculated, with the overall prevalence estimated as \( P_{\text{all}} = [P_{mi} \times \text{prop}_{mi}] + [P_i(1 - \text{prop}_{mi})] \), by weighting the prevalence of \textit{Salmonella} for each type of steak by the proportion of that type of steak in the supply chain. Concentrations were then converted to the population of \textit{Salmonella} in each steak \((N_i \sim \text{ZTP}(\lambda = 10^{C_i}), N_{mi} \sim \text{ZTP}(\lambda = 10^{C_{mi}}))\) and each steak was assigned a \textit{Salmonella} population based on the value of \( I_{mi} \); if \( I_{mi} = 0 \), then \( N_{\text{all}} \) was assigned the value of \( N_i \) and if \( I_{mi} = 1 \), \( N_{\text{all}} \) was assigned the value of \( N_{mi} \).

### 6.3.2.2. Growth Model

The influence of temperature on growth rate and maximum population density of \textit{Salmonella} in moisture-infused pork steaks has been investigated in Chapter 4 and those relationships are used here.

The growth rate of \textit{Salmonella} was estimated using Eqn. 2.5 and the maximum population density estimated using Eqn. 2.11, with the parameter estimates presented in the “One-step” column of Table 4.2. No data were identified that reliably describe the lag phase duration of \textit{Salmonella} on pork meats. Thus, lag phase duration is not considered in the modelling. Lag phases decrease the overall growth of \textit{Salmonella}, however, and by omitting the lag phase the final risk estimate
will be higher, i.e. conservative. Maximum population density values were converted from $\log_{10}$ CFU per gram to CFU per serve to estimate the total number of *Salmonella* per serving ($MP_{\text{serve}}$). *Salmonella* growth was then estimated for retail storage, consumer transport and domestic storage.

### 6.3.2.3. Retail and Consumer Transport

Statistical distributions for storage duration and temperature at retail were modelled as described in Section 5.4.3 and consumer transport duration and temperature increase were modelled as described in Section 5.4.4.

### 6.3.2.4. Domestic Storage

The consumer storage duration was based on the survey results of Gilbert et al. (2006) for storage of fresh, raw meats in refrigerators in New Zealand. From the 306 respondents, 222 stated that they store raw meats for 0–2 days, 68 for 2.5–4 days, 15 for 4.5–7 days and one for 7–14 days. As the durations were positive and the responses were weighted towards shorter durations, an exponential distribution was fitted to the interval censored responses using the “fitdistrplus” R package. The rate parameter was estimated as 1.56 days. The fit of the distribution was assessed by examination of diagnostic plots because no goodness-of-fit tests exist for distributions fitted to censored data. Temperature during consumer storage was modelled as described in Section 5.4.5.

### 6.3.2.5. Inactivation

No published models for *Salmonella* inactivation in moisture-infused and intact pork steaks were identified. The closest surrogates for these relationships are the linear models of Smith et al. (2013) for inactivation of *E. coli* O157:H7 in intact and brine injected beef steaks. In using these models to describe the inactivation of *Salmonella* in pork steaks, it was assumed that any differences between organism and meats were small and that product type (infused vs. intact) was more critical to pathogen survival. This assumption is validated in Appendix
6.3. Methods

6.A, with no significant differences in inactivation rates found between *Salmonella* in pork and *E. coli* O157:H7 in beef. These models were truncated to allow only positive values so that only *Salmonella* inactivation is predicted and not growth. Thus, *Salmonella* survival at temperature $T_{\text{cook}}$ is

$$N_{\text{cook}} = 10^{N_{\text{ds}} - \text{cook}_{\text{red}}}$$

where the reduction in *Salmonella* concentration is estimated as

$$\text{cook}_{\text{red}} = \begin{cases} \max(-1.24 + 0.091T_{\text{cook}}, 0) & s_{\text{mi}} = 0 \\ \max(-1.72 + 0.070T_{\text{cook}}, 0) & s_{\text{mi}} = 1 \end{cases}.$$  

The internal endpoint temperature to which pork steaks are cooked was based on survey results for consumer preference of “fresh pork”, which were converted to internal temperatures using the observations in Chapter 5. The resulting statistical distribution is

$$T_{\text{cook}} \sim \text{Normal}(\mu \sim \text{EmpericalD}(T_{\text{int}}, \text{done weights}), \sigma = 1)$$

where $T_{\text{int}} = \{63, 71, 74, 77\}$ and done weights = $\{5.6, 34.6, 38.6, 21.2\}$ and the “EmpericalD” distribution means that the $T_{\text{int}}$ values are sampled with replacement, weighted by the proportions in done weights. The effect that changes to these endpoint cooking temperatures have towards those typical of USA consumers is evaluated in Scenario 3, where each serving was assigned a cooking temperature through sampling by replacement the cooking temperature for pork products of EcoSure (2008). In Scenario 4, the effect of cooking pork steaks similar to pork burgers is examined. Internal endpoint temperatures for “fresh pork” were replaced with endpoint temperatures for “pork burgers” (see Chapter 5).
6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

6.3.2.6. Consumption

The probability of illness given contamination of each pork steak was calculated based on the number of *Salmonellae* in each contaminated serving. The dose-response model used here is the same model described in Section 5.5.1 and the prevalence and probability of illness were also combined as described Section 5.5.1.

Consumption data was then used to estimate the expected number of illnesses in Australia annually. Retail sales data for pork fillet steaks in Australia during the 52 week period ending 27 December 2014 showed 5.2 million kilograms of pork steaks sold, equating to 52.3 million servings of pork steaks (assuming 100g servings). This data does not differentiate if steaks were moisture-infused or intact but represented the total steaks sold per year.

6.4. Results

The model was run for 12,500,000 iterations of the infusion module (5,000 iterations in the variability dimension and 2,500 iterations in the uncertainty dimension) and 2,500,000,000 iterations of the supply chain and consumption module (50,000 iterations in the variability dimension and 50,000 iterations in the uncertainty dimension). These values were chosen based on the convergence of the running means for each module. For each parameter, means were calculated for each iteration in the variability dimension, with 95% credible intervals on the uncertainty calculated based on the 2.5% and 97.5% quantiles of these means.

6.4.1. Prevalence and Concentration in Infused Pork Loins

The results of the infusion module estimated the mean prevalence of *Salmonella* on 100g intact pork steaks (probability that 100g steak is contaminated) as 0.18 (95% credible interval 0.06, 0.34) and on 100g infused pork loin steaks as 0.45 (95% credible interval 0.16, 0.76). A beta distribution was fitted to these proportions with the parameters on the intact steak distribution.
estimated as $\alpha = 5.00$ and $\beta = 22.52$, and the parameters on the moisture-infused distribution estimated as $\alpha = 3.72$ and $\beta = 4.61$.

For the concentration of *Salmonella* on/in intact steaks, the mean of the normal distribution was estimated as $-1.14 \log_{10} \text{CFU/g}$ (95% credible interval $-1.33 \log_{10} \text{CFU/g}, -0.87 \log_{10} \text{CFU/g}$) and the standard deviation $0.61 \log_{10} \text{CFU/g}$ (95% credible interval $0.05 \log_{10} \text{CFU/g}, 0.88 \log_{10} \text{CFU/g}$). For the concentration of *Salmonella* on/in infused steaks, the mean of the normal distribution was estimated as $-2.10 \log_{10} \text{CFU/g}$ (95% credible interval $-2.79 \log_{10} \text{CFU/g}, -1.43 \log_{10} \text{CFU/g}$) and the standard deviation $0.69$ (95% credible interval $0.58, 0.81$).

### 6.4.2. Risk Estimates

The model predicts that the mean probability of salmonellosis (mean of all observations across the variability and uncertainty dimensions) from the consumption of infused and intact pork steaks at current infusion rates is $4.12 \times 10^{-8}$, with a 95% credibility interval on the uncertainty of $(9.85 \times 10^{-9}, 7.75 \times 10^{-8})$. It is estimated that for every $2.43 \times 10^7$ servings consumed, there would be one case of salmonellosis.

Based on sales data for pork steaks in Australia, approximately 2.16 illnesses annually are predicted from consumption of pork steaks in Australia (95% credible interval 0.52, 4.06).

### 6.4.3. Growth Observations

The change in the mean *Salmonella* concentration during retail storage is estimated as $0.0006 \log_{10} \text{CFU/serve}$ and is $0.0000, 0.2252, 0.6139$ and $1.0175$ at the 99%, 99.9%, 99.99% and 99.999% quantiles of the variability, respectively. The change in mean *Salmonella* concentration during transport to the consumer’s home is $0.0001 \log_{10} \text{CFU/serve}$ and is $0.0000, 0.0025, 0.2579$ and $0.3234 \log_{10} \text{CFU/serve}$ at the 99%, 99.9%, 99.99% and 99.999% quantiles of the variability, respectively. The change in mean *Salmonella* concentration during domestic refrigeration is $0.0021 \log_{10} \text{CFU/serve}$ and is $0.0251, 0.4828, 1.2400$ and $2.1037 \log_{10} \text{CFU/serve}$ at the 99%,
99.9%, 99.99% and 99.999% quantiles of the variability. This means that the smallest amount of *Salmonella* growth occurs during domestic refrigeration and the largest during retail storage and display.

### 6.4.4. Sensitivity Analysis

Sensitivity analysis was performed by calculating the Spearman rank correlations between the inputs and the probability of illness. These correlations are presented in Figure 6.1, with the 95% credible intervals around the uncertainty. The inputs that have the largest effect on the probability are the cooking temperature, $T_{\text{cook}}$ ($\rho = -0.805$) and the *Salmonella* concentration on/in intact steaks, $C_i$ ($\rho = 0.0911$).

The effect of uncertainties in the inputs on the uncertainty in the final risk estimate was also examined and results presented in Figure 6.2. The largest sources of uncertainty were the prevalence of *Salmonella* on/in intact steaks, $P_i$ ($\rho = 0.708$), the $\alpha$ parameter of the dose-response model ($\rho = 0.458$), and the proportion of steaks that are moisture-infused, $\text{prop}_{mi}$ ($\rho = 0.269$).

Spider plots (Figures 6.3 and 6.4) demonstrate the effect of changes in the transfer parameters on the prevalence and concentration of *Salmonella* in infused pork.

### 6.4.5. Scenario Analysis

The results of the scenario investigations are presented in Tables 6.2 and 6.3, which presents the mean probability of illness for each scenario and the ratio of the mean probabilities of illness for the scenario investigated and the baseline scenario.

### 6.5. Discussion

The risk of salmonellosis from moisture-infused and intact pork steaks estimated from the modelling presented here is lower than the risk estimated for pork chops in four nations in the
Figure 6.1.: Tornado plot of the Spearman rank correlations between inputs of the model and the probability of illness in the variability dimension. For each input, the mean correlation, with 95% credible intervals are given.

Table 6.2.: Results from the scenario analysis for scenarios denoted by letters. Ratio to baseline values are the ratio of the scenario with the baseline for the prevalence and concentration estimates.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Description</th>
<th>Salmonella Prevalence Mean per 100g steak</th>
<th>Ratio to baseline</th>
<th>Salmonella concentration Mean (log10 CFU/g)</th>
<th>Ratio to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.446</td>
<td></td>
<td>-2.101</td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>25L maximum balance tank capacity</td>
<td>0.441</td>
<td>99.06</td>
<td>-2.129</td>
<td>101.29</td>
</tr>
<tr>
<td>b</td>
<td>75L maximum balance tank capacity</td>
<td>0.456</td>
<td>102.39</td>
<td>-2.098</td>
<td>99.85</td>
</tr>
<tr>
<td>c</td>
<td>100L maximum balance tank capacity</td>
<td>0.459</td>
<td>102.99</td>
<td>-2.093</td>
<td>99.58</td>
</tr>
</tbody>
</table>

152
6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

![Tornado plot of the Spearman rank correlations between the inputs of the model and the probability of illness in the uncertainty dimension. For nodes with variability and uncertainty, correlations were calculated of the mean, standard deviation, 75% and 97.5% quantiles of the variability.](image)

**Figure 6.2.**
6.5. Discussion

![Transfer Coefficients](image)

**Figure 6.3.** Spider plot of the effect of changes to the transfer coefficients on the *Salmonella* prevalence.

![Transfer Coefficients](image)

**Figure 6.4.** Spider plot of the effect of changes to the transfer coefficients on the *Salmonella* concentration per gram.
Table 6.3: Results from the scenario analysis for numbered scenarios. Ratio to baseline values are the ratio of the mean probability of illness for each scenario against the mean of the probability of illness for the baseline scenario.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Description</th>
<th>Mean $P_{ill}$</th>
<th>Ratio to baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Baseline Model</td>
<td>$4.12 \times 10^{-8}$</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>All steaks as moisture-infused</td>
<td>$8.19 \times 10^{-7}$</td>
<td>19.88</td>
</tr>
<tr>
<td>3</td>
<td>Cooking temperatures as described by EcoSure (2008)</td>
<td>$1.19 \times 10^{-6}$</td>
<td>28.96</td>
</tr>
<tr>
<td>4</td>
<td>Cooking temperatures for pork burgers</td>
<td>$3.67 \times 10^{-8}$</td>
<td>0.89</td>
</tr>
</tbody>
</table>

European Union (VLA/DTU/RIVM, 2011). In that risk assessment, the mean probability of salmonellosis per serving was estimated at $7.65 \times 10^{-7}$, $1.86 \times 10^{-5}$, $3.88 \times 10^{-7}$ and $2.55 \times 10^{-6}$ for the four each member states.

In the risk assessment presented in this chapter, the mean risk estimates from the baseline scenario and Scenario 1 (all pork steaks as intact) were lower than those estimated for the European Union member states. Only in Scenario 2, where all steaks were assumed to be moisture-infused, was the risk estimated similar to the European Union member states. It should be noted that while acknowledging that pork chops are being compared to pork steaks, this comparison seems reasonable as these products would be handled similarly during processing, during the supply chain and at the consumer’s home. These estimated risks can also be compared to those from Australian pork burgers. The mean estimated probability of illness of $1.54 \times 10^{-8}$ per meal (see Section 5.6.1) is slightly higher than that estimated here for the probability of illness from the consumption of moisture-infused and intact pork steaks. This seems credible as pork trim is a pork product that is normally more contaminated than other pork products (D. Hamilton, 2015, South Australian Research and Development Institute, Pers. Comm.).

This risk assessment has provided valuable information on the risk posed by a relatively new pork product in Australia. It suggests that the risk posed by moisture-infused pork is greater than that posed by regular pork products. In Scenario 2, the effect of increasing moisture-infusion to cover all pork steaks consumed in Australia was examined, with this change resulting in a
mean risk of salmonellosis 19.88 times higher than under the current infusion prevalence. The moisture infusion proportion had the third largest correlation in the uncertainty sensitivity analysis (see Figure 6.2). This increase would have a large impact on the mean risk and may require interventions to minimise the impact of increasing moisture infusion prevalence. One potential intervention to reduce risk is for consumers to cook moisture-infused pork as they would pork burgers. This intervention was explored in Scenario 4, where the mean probability of illness was reduced by 10.89% through changes in cooking practice. In Scenario 3, the effect on mean risk of illness from a shift in cooking temperature by Australian consumers to match those used by US consumers was considered. The mean risk was 28.96 times higher than that estimated for the baseline Australian scenario. Cooking temperatures also had the largest correlation in the sensitivity analysis (see Figure 6.1). It is clear that the cooking preferences of Australian consumers contribute greatly towards the relatively lower estimated risk of illness. From Scenarios 3 and 4, we can conclude that interventions based on influencing consumers to cook moisture-infused pork products similar to how they currently cook pork burgers would have a limited impact on the risk of illness from consumption of moisture-infused pork steaks. It would appear that the high cooking temperatures of pork currently common in Australia adequately protect Australian consumers from the risk of salmonellosis posed by moisture-infused pork. Moisture-infused pork, however, poses a greater risk of illness compared to intact pork and industry risk managers will need to balance the organoleptic benefits of moisture-infused pork against the increased risk, especially if consumer preference for pork shifts towards less thoroughly cooked pork.

Comparing the growth observations presented in Section 6.4.3 to those presented in Section 5.6.1 for Salmonella growth in pork burgers, estimated growth in the moisture-infused pork steaks risk assessment is less than the estimated growth in the pork burger risk assessment. This is likely to be due to the lower concentration of Salmonella in the moisture-infused pork steaks compared to the pork burgers. The sensitivity analysis presented in Section 6.4.4 is also similar to that presented in Section 5.6.2. In both risk assessments in the variability dimension, the
input estimated to have the greatest impact on risk was the cooking temperature, followed by the *Salmonella* concentration, though the mean correlations for both variables were lower for the moisture-infused pork risk assessment and the credible intervals were wider. These wider confidence intervals are likely due to the two types of meat simulated in this risk assessment. For the uncertainty dimension, the prevalence of *Salmonella* in the meat and the dose-response model parameters have a large impact on salmonellosis risk, though the impact of the *Salmonella* concentration is lower in the moisture infusion model due to the reduced *Salmonella* concentration estimated in the infusion module.

Due to the lack of empirical data describing the change in *Salmonella* prevalence and concentration caused by the moisture infusion process, these changes were modelled stochastically. This modelling has allowed the prevalence and concentration of *Salmonella* on these products to be estimated, though large uncertainties arise in this method. Uncertainty could be better understood and reduced by conducting a national survey of the prevalence and concentration of *Salmonella* on moisture-infused pork and intact pork steaks to allow our stochastic estimates to be validated against survey results. We have estimated that 100g infused pork steaks are 2.5 times more likely to be contaminated with *Salmonella* compared to 100g intact steaks. This difference in risk is lower than that assumed by Smith et al. (2013) in their risk assessment of non-intact beef, which assumed that a single beef cut would contaminate four uncontaminated cuts via cross contamination during tenderisation, but their work did not include any details on contamination via the brines. Increased prevalence of *Salmonella* in moisture-infused pork was found in USA (Miller et al., 2008), with 18 detections of *Salmonella* from 1,350 moisture-infused pork chop samples tested (1.33%) but no detections from 685 intact chops. The prevalence values presented here are high because they represent the probability of 100g pork steaks being contaminated with *Salmonella*, which is a larger weight than typically tested under laboratory conditions. Our modelling of the consequences of infusion also suggests that, while the prevalence of *Salmonella* may increase in moisture-infused pork compared to intact pork, the concentration of *Salmonella* in contaminated steaks is decreased compared to intact pork. In this study, we observed that the
mean concentration of *Salmonella* on infused steaks was 0.96 log_{10} CFU/g lower than that of intact steaks. This decrease in concentration highlights how the infusion process redistributes *Salmonella* between infused pork loins and previously uncontaminated loins, causing previously uncontaminated loins to become contaminated with *Salmonella*. The decreased *Salmonella* concentration is consistent with previous studies. Smith et al. (2013), based on findings by Huang and Sheen (2011), assumed that the concentration of pathogens (in their case, *E. coli* O157:H7) on four newly contaminated primals would roughly decrease for each primal processed after the contaminated primal. To investigate the probable effect of changes to the transfer ratios on the estimated prevalence and concentration of *Salmonella* on infused pork steaks, spider plots were constructed. From Figures 6.3 and 6.4, it is evident that increases in transfer from the exterior to the interior decrease the prevalence and concentration of *Salmonella* on infused pork steaks and that increases in the transfer from exterior to brine increase the prevalence and concentration, with changes to these two transfer coefficients appearing to have the greatest impacts. The maximum volume of brine in the balance tank also appears to have a small effect on the prevalence and concentration of *Salmonella* in infused pork steaks (see Table 6.2). Based on Table 6.2 and Figures 6.3 and 6.4, the capacity of the balance tank was estimated to have a relatively small effect on the *Salmonella* prevalence and concentration compared to the effect of changes to the transfer coefficients.

One challenge in predicting the inactivation of *Salmonella* in infused pork steaks is determining the distribution of organisms within each steak and the effect that this has on *Salmonella* survival post cooking. The inactivation model employed in the stochastic risk model (Smith et al., 2013) assumes a specific distribution of *Salmonella* in steaks and likely leads to conservative estimates when predicting survival in infused steaks. This is because the model assumes that the dispersion of *Salmonella* inside the steak is consistent with a steak that has been needle tenderised only. The transfer of pathogens by needle/blade tenderisation is largely limited to areas of the meat nearer to the surface, with limited contamination reaching the centre of the primal (Huang and Sheen, 2011; Johns et al., 2011).
6. A Quantitative Microbial Risk Assessment of Salmonellosis From Consumption of Moisture-Infused Pork

This stochastic model would benefit from an inactivation model capable of accurately modelling the variable distributions of Salmonella in steaks under varying injection conditions. Such models, based on partial differential equations and, specifically, the heat equation, have been used previously in complex risk assessment models (VLA/DTU/RIVM, 2011). While these models were explored for use in this risk assessment, a lack of data describing certain aspects of the equations, and the variable shape of steaks were the reasons for using the simpler models.

In this study, the proportion of pork steaks that are moisture-infused was estimated from expert opinion. Quantifying the proportion of meat products that are non-intact has been reported to be difficult (Muth et al., 2012), and expert opinion was used to estimate the proportion of the various types of non-intact products on the market. This aspect of the model presented in this Chapter could be strengthened by performing a survey of Australian pork processors to better determine this proportion. Scenarios 1 and 2 were included to explore the extremes of the effect of moisture infusion on the mean probability of illness and estimate the bounds of the mean probability of illness under these extremes.

6.6. Conclusion

The risk of salmonellosis from the consumption of pork steaks in Australia appears to be lower than in other developed nations. As the uptake of moisture infusion in pork increases in Australia, the per serving risk of illness posed by pork steaks will also increase. The relatively low risk in Australia is largely due to the high cooking temperatures used by Australian consumers when cooking fresh pork products.

6.A. Comparison of Inactivation Rates

In using an inactivation model for E. coli O157:H7 in beef steaks (infused and intact) to describe the inactivation of Salmonella in pork steaks, we assumed similar inactivation rates for both
6.A. Comparison of Inactivation Rates

meat-pathogen combinations. To justify this assumption, inactivation rates and temperatures for inactivation experiments conducted in meat product and collated in ComBase (www.combase.cc) were extracted. A linear mixed effects model was fitted to the log₁₀ of the reported inactivation rates (log₁₀ CFU/g) and the inactivation temperatures (°C) using the R ‘nlme’ package (Pinheiro et al., 2016). In this model, each experiment conducted was treated as a random effect to account for variation between experiments. No significant differences in inactivation rates were observed between *Salmonella* in pork and *E. coli* O157:H7 in beef in either the intercept (\( p = 0.9628 \)) or slope (\( p = 0.9886 \)) or the fitted regression model. Based on this, we have assumed that any differences in inactivation that may exist between *Salmonella* in pork and *E. coli* O157:H7 would be small and that the cooking method modelled would have the greatest impact on estimated *Salmonella* survival.
Table 6.4: Parameters used in the infusion module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_i$</td>
<td>Prevalence of <em>Salmonella</em> in intact pork steaks</td>
<td>U</td>
<td>Beta($\alpha = 5, \beta = 432$)</td>
<td>Probability</td>
</tr>
<tr>
<td>$C_i$</td>
<td>Concentration of <em>Salmonella</em> for intact steak</td>
<td>VU*</td>
<td>Normal($\mu = -0.78, \sigma = 0.65$)</td>
<td>log$_{10}$CFU g$^{-1}$</td>
</tr>
<tr>
<td>$w_{\text{loin}}$</td>
<td>Weight of a pork loin</td>
<td>V</td>
<td>Normal($\mu = 4.5, \sigma = 0.51$)</td>
<td>kg</td>
</tr>
<tr>
<td>$l_{\text{loin}}$</td>
<td>Length of a pork loin</td>
<td>V</td>
<td>Normal($\mu = 52.5, \sigma = 1.28$)</td>
<td>cm</td>
</tr>
<tr>
<td>$\rho$</td>
<td>Density of pork</td>
<td>C</td>
<td>$9.3 \times 10^{-4}$</td>
<td>kg cm$^{-3}$</td>
</tr>
<tr>
<td>$r_{\text{loin}}$</td>
<td>Radius of pork loin</td>
<td>V</td>
<td>$\sqrt{\frac{w_{\text{loin}}}{\rho \pi}}$</td>
<td>cm</td>
</tr>
<tr>
<td>$S_{A_{\text{loin}}}$</td>
<td>Surface area of pork loin</td>
<td>V</td>
<td>$2\pi r (l + r)$</td>
<td>cm$^2$</td>
</tr>
<tr>
<td>$P_{\text{loin}}$</td>
<td>Prevalence of <em>Salmonella</em> on intact pork steaks based on surface area</td>
<td>U</td>
<td>$1 - (1 - P_i) \frac{S_{A_{\text{loin}}}}{S_{A_{\text{loin}}}}$</td>
<td>Probability</td>
</tr>
<tr>
<td>$Z_{\text{loin}}$</td>
<td>Indicator variable for a loin being <em>Salmonella</em> contaminated</td>
<td>VU</td>
<td>Bernoulli($p = P_{\text{loin}}$)</td>
<td>Boolean</td>
</tr>
<tr>
<td>$N_{\text{loin}</td>
<td>\text{cont}}$</td>
<td>Number of <em>Salmonella</em> on the surface of each contaminated loin</td>
<td>VU</td>
<td>ZTP($\lambda = 10^{C_i} \times S_{A_{\text{loin}}}$)</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 6.4: Parameters used in the infusion module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N_{loin}$</td>
<td>Number of <em>Salmonella</em> on the surface of all contaminated and uncontaminated loins</td>
<td>VU</td>
<td>$N_{loin</td>
<td>cont} \times Z_{loin}$</td>
</tr>
<tr>
<td>prop&lt;sub&gt;inf&lt;/sub&gt;</td>
<td>Proportion of weight of uninfused loin weight increased by injection</td>
<td>V</td>
<td>Triangular (min = 0.08, mode = 0.10, max = 0.15)</td>
<td>Probability</td>
</tr>
<tr>
<td>$V_{inj}$</td>
<td>Volume of brine injected into primal</td>
<td>V</td>
<td>$w_{loin} \times \text{prop}_{\text{inf}}$</td>
<td></td>
</tr>
<tr>
<td>$T_{EI}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the exterior to the interior of the loin</td>
<td>U</td>
<td>Triangular (min = 0.37, mode = 0.455, max = 0.55)</td>
<td></td>
</tr>
<tr>
<td>$T_{EN}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the exterior to the needles of the injection machine</td>
<td>U</td>
<td>Beta ($\alpha = 1.8, \beta = 8.89$)</td>
<td></td>
</tr>
<tr>
<td>$T_{EB}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the exterior to the brine</td>
<td>U&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Triangular (min = 0.2, mode = 0.257, max = 0.532)</td>
<td></td>
</tr>
</tbody>
</table>

Continued on next page
Table 6.4: Parameters used in the infusion module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{IE}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the interior to the exterior</td>
<td>U</td>
<td>0</td>
<td>Proportion</td>
</tr>
<tr>
<td>$T_{II}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the interior to the interior of the loin</td>
<td>U</td>
<td>1</td>
<td>Proportion</td>
</tr>
<tr>
<td>$T_{IN}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the interior to the needles of the injection machine</td>
<td>U</td>
<td>0</td>
<td>Proportion</td>
</tr>
<tr>
<td>$T_{IB}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the exterior to the brine</td>
<td>U</td>
<td>0</td>
<td>Proportion</td>
</tr>
<tr>
<td>$T_{NE}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the needles of the injection machine to the exterior of the primal</td>
<td>U</td>
<td>Beta($\alpha=7.43, \beta=11.62$)</td>
<td>Proportion</td>
</tr>
<tr>
<td>$T_{NI}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the needles to the interior of the primal</td>
<td>U</td>
<td>Beta($\alpha=7.43, \beta=11.62$)</td>
<td>Proportion</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 6.4: Parameters used in the infusion module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

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<tr>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{NB}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the needles to the brine</td>
<td>U</td>
<td>Triangular (min=0, mode=0.05, max=0.8) Proportion</td>
<td></td>
</tr>
<tr>
<td>$T_{BE}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the brine to the exterior</td>
<td>U</td>
<td>Triangular (min=0, mode=0.05, max=0.8) Proportion</td>
<td></td>
</tr>
<tr>
<td>$T_{BI}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the brine to the interior</td>
<td>U</td>
<td>Triangular (min=0.2, mode=0.9, max=1) Proportion</td>
<td></td>
</tr>
<tr>
<td>$T_{BN}$</td>
<td>Proportion transfer of <em>Salmonella</em> from the brine to the needles</td>
<td>U</td>
<td>Triangular (min=0, mode=0.05, max=0.8) Proportion</td>
<td></td>
</tr>
<tr>
<td>$N_{ij}$</td>
<td>The number of <em>Salmonella</em> at the exterior, interior, needles and brine after processing</td>
<td>VU</td>
<td>See text</td>
<td>CFU</td>
</tr>
<tr>
<td>$t_{serve}$</td>
<td>Thickness of serve cut from loin</td>
<td>V</td>
<td>$W_{serve}/(r_{loin}^2 \rho \pi)$</td>
<td>cm</td>
</tr>
<tr>
<td>$S_{A_{serve}}$</td>
<td>The surface area of sides of the steak cut from the loin</td>
<td>V</td>
<td>$2\pi r_{loin} t_{loin}$</td>
<td>cm²</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 6.4: Parameters used in the infusion module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>( W_{serve} )</td>
<td>Weight of serving</td>
<td>C</td>
<td>100</td>
<td>g</td>
</tr>
<tr>
<td>( P_i )</td>
<td>Prevalence of ( Salmonella ) on intact pork</td>
<td>U</td>
<td>( 1 - \left( 1 - \frac{SA_{serve}}{SA_{loin}} \right)^{N_{loin}} )</td>
<td>Probability</td>
</tr>
<tr>
<td>( P_{ext} )</td>
<td>Prevalence of ( Salmonella ) on exterior of infused pork</td>
<td>U</td>
<td>( 1 - \left( 1 - \frac{SA_{serve}}{SA_{loin}} \right)^{N_{ext}} )</td>
<td>Probability</td>
</tr>
<tr>
<td>( P_{int} )</td>
<td>Prevalence of ( Salmonella ) in interior of infused pork</td>
<td>U</td>
<td>( 1 - \left( 1 - \frac{W_{serve}}{W_{loin}} \right)^{N_{int}} )</td>
<td>Probability</td>
</tr>
<tr>
<td>( P_{mi} )</td>
<td>Prevalence of ( Salmonella ) on/in infused pork</td>
<td>U</td>
<td>( P_{ext} + P_{int} \times P_{ext}P_{int} )</td>
<td>Probability</td>
</tr>
<tr>
<td>( C_i )</td>
<td>Concentration of ( Salmonella ) on intact pork</td>
<td>VU</td>
<td>See text</td>
<td>( \log_{10} \ CFU \ g^{-1} )</td>
</tr>
<tr>
<td>( C_{mi} )</td>
<td>Concentration of ( Salmonella ) on/in infused pork</td>
<td>VU</td>
<td>See text</td>
<td>( \log_{10} \ CFU \ g^{-1} )</td>
</tr>
</tbody>
</table>

Continued on next page
### Table 6.4: Parameters used in the infusion module.

The type column describes how this variable was modelled. Parameters of type "C" were modelled as a constant, point estimate. Type "V" parameters changed in the variability dimension only. Type "U" parameters changed in the uncertainty dimension only and type "VU" parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>*</td>
<td>a Distribution truncated at zero.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>b Truncated to a maximum of 10 days.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>c Based on the mcprobtree function in mc2d</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Uncertainty in this parameter was included using non-parametric bootstrap sampling.

*a* Distribution truncated at zero.

*b* Truncated to a maximum of 10 days.
Table 6.5.: Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Model</td>
<td>$a$</td>
<td>Coefficient of MPD equation</td>
<td>C</td>
<td>9.01</td>
<td></td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{min1}$</td>
<td>$x$-intercept of MPD equation</td>
<td>C</td>
<td>8.81</td>
<td>Coefficient $(°C)$</td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{submin}$</td>
<td>Value of MPD equation where MPD is 0 CFU/g</td>
<td>C</td>
<td>6.64</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Growth Model</td>
<td>$b$</td>
<td>Coefficient of Ratkowsky equation</td>
<td>U*</td>
<td>0.0306</td>
<td></td>
</tr>
<tr>
<td>Growth Model</td>
<td>$T_{min}$</td>
<td>Minimum temperature at which growth of <em>Salmonella</em> is estimated to occur</td>
<td>U*</td>
<td>6.09</td>
<td>°C</td>
</tr>
<tr>
<td>Growth Model</td>
<td>GR$^{a}$</td>
<td>Maximum growth rate</td>
<td>VU</td>
<td>$[b(T - T_{min})]^2$</td>
<td>$\log_{10} \text{CFU g}^{-1} \text{ h}^{-1}$</td>
</tr>
<tr>
<td>Growth Model</td>
<td>MPD</td>
<td>Maximum population density</td>
<td>V</td>
<td>$a \frac{(T - T_{min2})}{(T - T_{submin})}$</td>
<td>$\log_{10} \text{CFU g}^{-1}$</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 6.5: Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

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<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Model</td>
<td>$MP_{serve}$</td>
<td>Maximum population density per serve</td>
<td>VU</td>
<td>$10^{MPD(T)} \times W_{serve}$</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>$prop_{mi}$</td>
<td>The prevalence of MI in Australian pork</td>
<td>U</td>
<td>Triangular(min = 0.05, mode = 0.075, max = 0.10)</td>
<td>Proportion</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>$is_{mi}$</td>
<td>Is this steak moisture infused?</td>
<td>VU</td>
<td>Bernoulli($prop_{mi}$)</td>
<td>Boolean</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>$P_{all}$</td>
<td>The weighted prevalence of $Salmonella$ for intact and moisture infused pork.</td>
<td>VU</td>
<td>$(P_{mi} \times prop_{mi}) + [P_{i}(1 - prop_{mi})]$</td>
<td>Probability</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>$N_{i}$</td>
<td>Population of $Salmonella$ for intact steaks</td>
<td>VU</td>
<td>$ZTP(\lambda = 10^{C_{i}})$</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Initial Conditions</td>
<td>$N_{mi}$</td>
<td>Population of $Salmonella$ for infused steaks</td>
<td>VU</td>
<td>$ZTP(\lambda = 10^{C_{mi}})$</td>
<td>CFU/serve</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 6.5.: Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

<table>
<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Conditions</td>
<td>( N_{all} )</td>
<td>The population of <em>Salmonella</em> for intact and moisture infused pork</td>
<td>VU</td>
<td>( \begin{cases} N_i &amp; i_s = 0 \ N_{mi} &amp; i_s = 1 \end{cases} )</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Retail</td>
<td>( \theta_r )</td>
<td>Uncertainty in the mean duration of product storage at retail</td>
<td>U</td>
<td>Unif(0.5, 1.5)</td>
<td>h</td>
</tr>
<tr>
<td>Retail</td>
<td>( t_r^b )</td>
<td>Time that product remains on retail shelf</td>
<td>VU</td>
<td>Exp(( \theta = \theta_r )) \times 24</td>
<td>h</td>
</tr>
<tr>
<td>Retail</td>
<td>( T_r )</td>
<td>Temperature of product on retail shelf</td>
<td>VU</td>
<td>( \text{SNormal}(\mu = 3.41, \sigma = 2.11, \xi = 0.77) )</td>
<td>°C</td>
</tr>
<tr>
<td>Retail</td>
<td>( N_r )</td>
<td>Population of <em>Salmonella</em> in mince after retail storage</td>
<td>VU</td>
<td>( \text{min}(C_{\text{mince}} + \text{GR}(T = T_r) \times t_r, \text{MPD}) )</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Consumer Transport</td>
<td>( t_{ct} )</td>
<td>Time taken for transport from retail to home by consumer</td>
<td>VU</td>
<td>( \text{Gamma}(\alpha = 7.37, \beta = 6.30) )</td>
<td>h</td>
</tr>
</tbody>
</table>

Continued on next page
Table 6.5.: Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

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<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumer Transport</td>
<td>$\Delta T_{ct}$</td>
<td>Change in temperature during consumer transport</td>
<td>VU*</td>
<td>Gamma($\alpha = 1.77, \beta = 0.55$)</td>
<td>°C</td>
</tr>
<tr>
<td>Consumer Transport</td>
<td>$T_{ct}$</td>
<td>Temperature of pork mince after consumer transport</td>
<td>VU</td>
<td>$T_r + \Delta T_{ct}$</td>
<td>°C</td>
</tr>
<tr>
<td>Consumer Transport</td>
<td>$N_{ct}$</td>
<td>Population of <em>Salmonella</em> in mince after consumer transport</td>
<td>VU</td>
<td>See text for description</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Domestic Storage</td>
<td>$t_{ds}$</td>
<td>Time that product is in domestic storage</td>
<td>VU</td>
<td>Exp($\theta = 1.53 \times 24$)</td>
<td>h</td>
</tr>
<tr>
<td>Domestic Storage</td>
<td>$T_{ds}$</td>
<td>Temperature of domestic storage</td>
<td>V</td>
<td>Normal($\mu = 3.35, \sigma = 1.94$)</td>
<td>°C</td>
</tr>
<tr>
<td>Domestic Storage</td>
<td>$N_{ds}$</td>
<td>Population of <em>Salmonella</em> in mince after domestic storage</td>
<td>UV</td>
<td>min($C_{ct} + GR(T = T_{ds}) \times t_{ds}, MPD$)</td>
<td>CFU/serve</td>
</tr>
<tr>
<td>Cooking</td>
<td>$T_{int}$</td>
<td>The internal temperature for each degree of doneness</td>
<td>C</td>
<td>{63, 71, 74, 77}</td>
<td>°C</td>
</tr>
</tbody>
</table>

*Continued on next page*
Table 6.5: Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

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<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking</td>
<td>done_weights</td>
<td>The percentage of consumers who prefer each degree of doneness</td>
<td>C</td>
<td>{5.4, 38.3, 43.3, 13.0}</td>
<td>Percent</td>
</tr>
<tr>
<td>Cooking</td>
<td>$T_{cook}$</td>
<td>Final internal temperature for each serving</td>
<td>V</td>
<td>Normal($\mu \sim$ \text{EmpericalD($T_{int,done_weights}$), $\sigma = 1$})</td>
<td>°C</td>
</tr>
</tbody>
</table>
| Cooking            | cook\_red   | The reduction number of $Salmonella$ inactivated during cooking | V    | \[
\begin{cases}
\max(-1.24 + 0.091T_{cook}, 0) & i_{smi} = 0 \\
\max(-1.72 + 0.070T_{cook}, 0) & i_{smi} = 1
\end{cases}
\] | CFU/serve |
| Cooking            | $N_{cook}$  | Population of $Salmonella$ after cooking | V    | $C_{dr} - \text{cook\_red}$ | CFU/serve |
| Dose-response      | $\alpha$   | Parameter 1 of the beta Poisson dose response model | U    | Triangular(min = 0.0763, mode = 0.1324, max = 0.2274) | Coefficient |

Continued on next page
Table 6.5.: Parameters used in the supply chain and consumption module. The type column describes how this variable was modelled. Parameters of type “C” were modelled as a constant, point estimate. Type “V” parameters changed in the variability dimension only. Type “U” parameters changed in the uncertainty dimension only and type “VU” parameters changed in both the variability and uncertainty dimensions.

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<thead>
<tr>
<th>Supply Chain Stage</th>
<th>Parameter</th>
<th>Description</th>
<th>Type</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose-response</td>
<td>$\beta$</td>
<td>Parameter 2 of the beta Poisson dose response model</td>
<td>U</td>
<td>Triangular(min = 38.49, mode = 51.45, max = 57.96)</td>
<td>Coefficient</td>
</tr>
<tr>
<td>Dose-response</td>
<td>$p_{ill</td>
<td>cont}$</td>
<td>The probability of illness given that the serving is contaminated with <em>Salmonella</em>.</td>
<td>UV</td>
<td>$1 - \left(1 + \frac{N_{\text{serve}}}{\beta}\right)^{-\alpha}$</td>
</tr>
<tr>
<td>Dose-response</td>
<td>$p_{ill}$</td>
<td>The overall probability of illness</td>
<td>UV</td>
<td>$p_{ill</td>
<td>cont} \times P_{all}$</td>
</tr>
</tbody>
</table>

* Uncertainty in this parameter was included using non-parametric bootstrap sampling.

a No growth occurs at temperatures less than $T_{\text{min}}$.

b Truncated to a maximum of 10 days.

c Based on the mcprobtree function in mc2d
7. Discussion

In this thesis, a number of novel pieces of research have been conducted, including:

- conducting experiments to quantify the inactivation kinetics of *Salmonella* in pork burger patties cooked on an electric skillet;
- generating and collecting data on, creating and evaluating a predictive model for the growth of *Salmonella* in moisture-infused pork, and
- developing stochastic simulation models and conducting quantitative risk assessments for the risk of salmonellosis from consumption of pork burgers and moisture-infused pork in Australia.

7.1. Interpretation to Pork Products in General

The aim of this thesis was to quantify the risk of salmonellosis from pork products and was achieved by investigating in detail two pork products: pork burgers and pork steaks, infused or intact. The former two products were chosen as both potentially allow *Salmonella* to be internalised, either by grinding or injection. These two products represent the ‘worst case scenario’ for pork products in general and other pork serving methods would be expected to have a lower relative risk. The objectives were in response to the potential for novel risk management strategies for protection of consumers and to support risk managers in those assessments and decisions.
Based on results presented in this thesis, the overall per serving risk of salmonellosis in Australia from the pork products investigated appears to be lower than that of other developed nations (see Table 7.1) and reflects favourably on the Australian pork industry. Direct comparison of the risk estimates presented in this thesis and the EU risk estimates should be performed with caution because of different data and assumptions underlying these two risk assessment models. While the same or similar foodstuffs are modelled, the production processes that occur in these industries are different. Results presented here are dependent on the inputs used: changes to these inputs could invalidate the results and interpretations presented and new predictions from the model, based on new input values, would need to be made to support further risk assessment decisions based on changed circumstances.

Table 7.1.: Comparison of estimates of the risk of salmonellosis from pork products per serving presented here and from VLA/DTU/RIVM (2011) for four European Union member countries.

<table>
<thead>
<tr>
<th>Nation</th>
<th>Pork Burgers</th>
<th>Pork Steaks/Chops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>$1.54 \times 10^{-8}$</td>
<td>$4.12 \times 10^{-8}$</td>
</tr>
<tr>
<td>EU Member State 1</td>
<td>$8.84 \times 10^{-7}$</td>
<td>$7.65 \times 10^{-7}$</td>
</tr>
<tr>
<td>EU Member State 2</td>
<td>$2.24 \times 10^{-5}$</td>
<td>$1.86 \times 10^{-5}$</td>
</tr>
<tr>
<td>EU Member State 3</td>
<td>$2.32 \times 10^{-7}$</td>
<td>$3.88 \times 10^{-7}$</td>
</tr>
<tr>
<td>EU Member State 4</td>
<td>$2.58 \times 10^{-7}$</td>
<td>$2.55 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

While it is not possible to accurately validate the risk estimates presented in this thesis, it is possible to place these results into context against other studies. The low-risk estimates are in agreement with the results of a recent source attribution study conducted for the proportion of salmonellosis cases attributed to pork, beef, sheep, chicken meat or eggs in the Australian state of South Australia (Glass et al., 2016). Pork was estimated to be the smallest source of salmonellosis cases in this state, with only 2.5% of cases attributed to pork, though these proportions may differ between states and nationally.

A number of scenarios were investigated using both risk assessment models presented in this thesis and these scenarios highlighted the effect that changes from current supply chain processes would
7. Discussion

have on the overall risk of salmonellosis. For example, in both risk assessments, scenarios were included to examine the effect that changes to current Australian consumer cooking ‘doneness’ preferences could have on salmonellosis risk, particularly if those preferences moved towards USA consumer preferences for ‘rarer’ servings. These scenarios were included to assist Australian food industry risk managers to predict potential changes in risk due to changing culinary behaviours and to develop appropriate risk management strategies, as needed, proactively. For the same reasons, other scenarios were developed and explored to assess the risk associated with increased availability and consumption of minced pork or moisture-infused pork products in Australia.

7.2. Outcomes for Industry

Proactive management of potential changes to the risk of salmonellosis from the consumption of Australian pork products will reduce the likelihood that the Australian pork industry will be exposed to the negative consequences of a foodborne disease incident, which could involve considerable expense in product recalls, lost market share, lost consumer confidence and compensation to victims. Product recalls costs include the initial cost related to the recall and also subsequent disposal of the product. Further losses occur due to missing product on retail shelves, both in lost income but also disposal costs. Consumer confidence in the product and related products can take a long time to return, with reduced sales and reduced sale prices, and increased costs of marketing to regain share. Outbreaks can also lead to hospitalisations, expensive lawsuits and in extreme cases, death. The ultimate cost of an outbreak can be the closure of the company implicated, as was the case for Garibaldi Smallgoods (CDC 1995; Fewster 2011), which was forced to close as a consequence of an outbreak of E. coli in its fermented meat products. Managing and minimising the risk of salmonellosis from pork products is, therefore, vital to maintaining a profitable pork industry.

This project has provided the Australian pork industry with valuable information on the factors that influence the risk of salmonellosis from consumption of their products that can be used to
make informed decisions to mitigate the risk of illness. It also informs industry as to the effect that potential changes to retail temperatures or cooking temperatures could have on the risk of illness, allowing risk managers to weigh the costs and benefits of any processing or marketing changes against potential increases in risk.

As reinforced in the risk assessments presented here, increased internal endpoint cooking temperatures reduced the risk of salmonellosis from pork products. While increased final cooking temperatures have a beneficial effect in reducing the risk of salmonellosis, they have the opposite effect on eating quality. Channon et al. (2013b) showed that, for some pork cuts, reducing the endpoint cooking temperature from 75 °C to 70 °C increases juiciness, flavour and overall consumer liking. For industry risk managers, the risk of illness needs to be balanced against consumer acceptability to ensure that marketing messages result in a safe, yet appetising product. Increases in prevalence and concentration of *Salmonella* in either pork mince or pork steaks also had a large estimated influence on risk, hence reducing prevalence and concentration could potentially offset any changes caused by a shift towards lower endpoint cooking temperatures. Growth of *Salmonella* on product during retail, consumer transport and consumer storage of product had a small estimated impact on the overall risk of illness and, hence interventions to reduce *Salmonella* growth potential would appear to result in small reductions in mean risk. Increasing temperatures during retail storage, however, was shown to affect the mean risk (see Table 5.4), with the relative impact on risk greater at higher temperatures.

For moisture-infused pork, only a small reduction in risk was observed if consumers were to cook steaks similarly to how they would cook pork burger patties. This is probably related to the small differences between the reported Australian consumer preferences for ‘fresh pork’ versus doneness preference for pork burger patties. Australian food standards state that ‘a statement of ingredients must list each ingredient in the food for sale’ (FSANZ 2014), which would include all moisture-infused pork products. While these products must be labelled as infused, it is unclear how effective this labelling is in informing consumers that these products require more thorough cooking than intact pork steaks. If consumer preference for pork shifts towards ‘rarer’ pork
and consumers mistake infused pork for intact pork, this could potentially have a large effect on consumer risk. This scenario was investigated and it was estimated that a shift towards US consumer preference for pork steaks by Australian consumers would increase the risk of salmonellosis by 2,900%.

While the results of the risk assessments presented here reflect favourably on the Australian pork industry, industry cannot afford complacency. Process control and improvement need to be continued to ensure that industry provides the safest possible product to consumers. As demonstrated by the scenarios considered, changes to consumer handling and preparation practices could potentially cause harm to pork’s current low-risk status.

7.3. Risk Modelling Considerations

The risk assessments presented here used the statistical programming language R (R Core Team, 2016) and the ‘mc2d’ R package (Pouillot and Delignette-Muller, 2010), which contains tools that simplify the process of conducting risk assessments and presenting the results. Two problems with mc2d were uncovered during its use in these risk assessments.

The first is that mc2d provides two estimates of the mean risk for two-dimensional models. The package constructs its summary statistics by calculating the mean of each iteration in the uncertainty dimension, resulting in a vector of mean estimates covering the entire spectrum of uncertainty. The package then calculates summary statistics on this vector, resulting in a grand mean risk estimate, as well as the median and 95% quantiles of the uncertainty around the mean variability of the risk estimate. It is not clear what the best representation of the average risk is, the mean or the median. The authors of the mc2d package use the median as it is always between the 95% uncertainty quantiles (R. Pouillot, 2015, Center for Food Safety and Applied Nutrition, Food and Drug Administration, Pers. Comm.). The problem with this method is that the median is lower than the risk estimate that would be obtained by reducing the problem to one dimension by combining variability and uncertainty. This may lead to a risk estimate that under-represents
the true risk per serving. In the risk assessment presented here, we have chosen to report the
mean of the means and report asymmetric credible intervals.

R is not the most efficient language to construct solutions to high-performance computing
problems. In the R language, operations over a vector or matrix are generally faster if they
are ‘vectorised’, i.e. a function is applied to all values in a vector simultaneously instead of
looping over all the values (Burns, 2012; Ligges and Fox, 2008), though exceptions exist. This
creates problems when running large simulations because the data for all iterations must be
stored in memory and means that when computer memory is at a premium in R, performance
suffers and simulation durations increase greatly. Mc2d has a method that loops over each
uncertainty iteration while performing vector operations on the variability dimension. The way
that this algorithm is implemented is slow, especially if sensitivity analysis is performed during
the simulation.

7.4. Further Work

One area for further research is to investigate other cooking methods, with pork roasts being
the most obvious priority. Salmonellosis outbreaks attributed to pork in Australia are mostly
attributable to pork roasts (see Table 1.2). An outbreak in South Australia consisting of eight
cases of salmonellosis was attributed to a ‘roast pork meal from a food stall during a festival’
(South Australian Health, 2014, p. 24). One large recent overseas outbreak was attributed to
whole pig roasts (CDC, 2015), in which 152 illness occurred from Salmonella 4,[5],12:i:-.

This apparently higher risk is either because there is a high risk of salmonellosis per serving,
which is currently unknown, or the high number of pork roast servings consumed in Australia,
which is the highest selling fresh pork product (K. Pindsle, Australian Pork Limited, 2015, Pers.
Comm.), or a combination of both. Based on the high consumption of pork roasts in Australia,
risk managers would benefit from the availability of a quantitative risk assessment to determine
the factors that influence the risk and to help identify optimal risk reduction options. A difficulty
7. Discussion

with assessing the risk of salmonellosis from pork roasts is that there are a wide variety of ways that they can be prepared and cooked. While whole intact portions of pork should be the safest type of roast to cook, any type of roast where pathogens are internalised require extra care when cooking, e.g. rolled roasts, when the surface of the meat becomes internalised by the deboning and rolling of the roast. One outbreak in Australia has been attributed to rolled pork roasts by Delpech et al. (1998) who considered that the core of the meat was still frozen prior to cooking, which may have also exacerbated the situation further.

Additionally, extending the risk assessment studies to cover more of the supply chain, from farm-to-fork, similar to the recent European Union risk assessment (VLA/DTU/RIVM, 2011) would benefit the Australian industry’s ability to manage food safety risks and industry sustainability. In the risk assessments presented here, no attempt was made to model the influence on risk of the supply chain before retail because of a lack of data describing the distribution chain before retail and the complexities of the supply chain prior to that point. A particular difficulty will be in characterising and describing the influence of variables in the transport of pork between the boning rooms and retail on the risk of salmonellosis to final consumers. As discussed in Section 1.3.5, the supply chain between the boning room and retail is complex, with large variation in duration, temperature, intermediary steps and food handlers (distribution centres, wholesale butchers etc.). These intermediaries can have a large effect on the transport time from boning room to the retailer (Hamilton et al., 2012), and can also have a large influence on the temperature profile of the product during transport. Details required to accurately model this part of the supply chain would be difficult to obtain because of the number of enterprises that would need to provide data.

7.5. Conclusion

A number of novel findings were made in this thesis, including:

- *Salmonella* survival in cooked pork patties was affected by fat content, with increasing fat content leading to increased *Salmonella* survival, though this effect diminished as the tem-
temperature approached 62 °C. A 5-log₁₀ reduction in *Salmonella* concentration is estimated to occur when the geometric centre of the patty reaches 63 °C. The *Salmonella* serovar had no significant effect on *Salmonella* survival between the three serovars examined (*S*. 4,[5],12:i:-, *S*. Senftenberg and *S*. Typhimurium).

- Growth of *Salmonella* can occur in moisture-infused pork steaks, with the growth potential similar to that in non-infused pork.

- The risk of salmonellosis from pork burgers and moisture-infused pork in Australia appears to be lower than other developed nations (See Table 7.1). Cooking temperature had the largest contribution towards this low risk and changes to these temperatures by Australians towards rarer pork is predicted to have a large effect on that risk. A change in cooking temperatures towards those preferred by US consumers was predicted to result in an 11,300-fold increase in salmonellosis risk from pork burgers and a 29-fold increase for moisture-infused pork.


References


References


References


References


References


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References


References


References


References


References

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References


A. Supplementary Inactivation Data for Chapter 3

In this section, the supplementary data for Chapter 3, provided on the journal publisher’s website are reproduced. The inactivation data and the R code used to fit the generalised linear model are reproduced in Table A.1 and Listing A.1.

Table A.1.: Inactivation data for *Salmonella* in pork burger patties. Column headings are the same as those required by the R code in Listing A.1

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Inactivation data for *Salmonella* in pork burger patties. Column headings are the same as those required by the R code in Listing A.1

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A. Supplementary Inactivation Data for Chapter 3

Inactivation data for *Salmonella* in pork burger patties. Column headings are the same as those required by the R code in Listing A.1

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Listing A.1: R code to used to fit generalised linear model to inactivation data in Table A.1.

```r
# GLM to Salmonella inactivation data
# Phillip Gurman 19/08/2015
library(MASS)
library(glm2)
library(readxl)
cooked.by.plate <- read_excel("cooked.by.plate.xlsx")

# Extract to vectors
temp <- cooked.by.plate$Temperature
y <- cooked.by.plate$Plate.Count
dil <- cooked.by.plate$Dilution.Factor
fat.percent <- cooked.by.plate$Fat.Percentage
sero <- cooked.by.plate$Serovar
batch.id <- cooked.by.plate$Batch.ID

# Function to calculate z
```
z <- function(model) {
  # Find all coefficients that do not contain "batch"
  keep.coefs <- grep("lm.", names(coef(model)), value = T)
  lin.coefs <- coef(model)[keep.coefs] # Remove batches
  lin.coefs <- lin.coefs # Remove (intercept) ie batch A.
  exp(model.matrix(formula.old) %%*% lin.coefs)
}

# This is the formula for the "linear" components of the model (not multiplied by 1/(1/z)). Order must match below.
formula.old <-
  ~ temp + sero + fat.percent + temp:sero + temp:fat.percent

# This is the full model including the nu and its batch offsets. Components inside the logit part of the model have to be prefaced with 'lm.'
formula <-
  "y ~ lm.intercept + lm.temp + batch.id + lm.intercept:sero + lm.intercept:fat.percent + lm.temp:sero + lm.temp:fat.percent"

lm.intercept <- ifelse(temp == 4, 0, 1)
lm.temp <- lm.intercept * temp

#init.coefs <- c(coefs,rep(0,17))

glm.1 <-
  glm.nb(
    as.formula(paste(formula, " + offset(dil*log(10))", sep = "")),
    link = log,
    control = glm.control(maxit = 5000, trace = F),
    method = "glm.fit2"
  )
  
#" + offset("dil.off,"),
A. Supplementary Inactivation Data for Chapter 3

# glm.1 <- glm2(as.formula(paste(formula,sep="")),
    family=negative.binomial(0.50),control=glm.control(maxit=5000,trace=F))

#summary(glm.1)

off <- -log(1 + 1 / z(glm.1)) - log(z(glm.1)) / (1 + z(glm.1))

lm.intercept <- 1 / (1 + z(glm.1))

lm.temp <- lm.intercept * temp

# Set dummy values for the new and old deviance. Values should be large enough to allow
# the loop to proceed.

dev.new <- -10

dev.old <- -1000

# Set the loop counter

i <- 1

# Save the previous coefficient estimates.

coefs <- coef(glm.1)

# Once we have initial parameter estimates, we can use these values to iterate the model
# until the change in the coefficient estimates is 'sufficiently small'.

while (abs(dev.new - dev.old) / (abs(dev.new) + 0.1) > 1E-8) {

# Fit the model using the previous coefficient estimates as the starting values. Also
# add the Taylor series offset into the total offset component.

glm.2 <-

    glm.nb(
        as.formula(paste(
            formula, " + offset(off + dil*log(10))", sep = ""
        )),
        link = log,
        control = glm.control(maxit = 10000, trace = F),
        start = coefs,
    )
}
method = "glm.fit2"
)

# Update the deviance values
dev.old <- dev.new
dev.new <- glm.2$deviance

# Update the values based on the new fit
off <- -log(1 + 1 / z(glm.2)) - log(z(glm.2)) / (1 + z(glm.2))

# Each loop print out the values for the deviance (old and new), the absolute difference, the AIC value, the current estimate of the dispersion parameter and whether the previous iteration converged.

cat(
  "dev.old=",
  dev.old,
  ", dev.new=",
  dev.new,
  "abs.diff=",
  abs(dev.old - dev.new),
  "AIC=",
  AIC(glm.2),
  "theta=",
  glm.2$theta,
  "converged=",
  glm.2$converged
)
A. Supplementary Inactivation Data for Chapter 3

# ANOVA output

```
anova(glm.2, test = "Chisq")
```

# Regression table including coefficient estimates

```
summary(glm.2)
```

# We can obtain confidence intervals by

```
ci <- confint(glm.2, trace = TRUE)
summary(ci)
```

# We can obtain the coefficient estimates log_10 by

```
coef(glm.2) / log(10)
```
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## B. ComBase Data Used in Chapters 4 and 5

**Table B.1.:** Data extracted from the ComBase database used to generate growth rate and maximum population density predictive models

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### B. ComBase Data Used in Chapters 4 and 5

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### B. ComBase Data Used in Chapters 4 and 5

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</tbody>
</table>
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

In this section, R and batch command files are included that are used to evaluate the salmonellosis from pork burger consumption risk assessment model, described in Chapter 5. The contents of these files are presented in Listings C.2 to C.7 and the model evaluation is triggered from the code in Listing C.7.

**Listing C.2:** The R code contained in the file ‘mc2d.boots - Pork.Burgers.R’. Contains the R commands to perform any bootstrapping of data sources used in the model.

```r
# Purpose: fit distributions to the datasets and, if required, generates bootstrap samples from the distributions required.

library(minpack.lm)
library(nlstools)
library(readxl)

ratk <- function(b, c, temp, T_min, T_max){
  (b * (temp - T_min)) * sqrt(1 - exp(c * (temp - T_max)))
}

#Function to convert fahrenheit to celsius
fahr.to.cel <- function(farenheit) { (farenheit - 32) * 5/9}
```
# Beta Poisson Dose Response Model FAO/WHO

b.p.dose.resp <- \textbf{function}(dose, alpha, beta) \textbackslash{}^1 \textbackslash{}{} (-1 + dose/beta)^(-alpha)

\textbf{source}("model.functions.R")

# Read in the process control results and summarise the results for prevalence ect.

# Initial Contamination ----
C.mince.fit <- log.cens.meat.data \%\% filter(Sample.Site \in c("Belly Strip", "Trim") & Salmonella.Presence == "detected") \%\% as.data.frame \%\% fitdistcens("norm", optim.method = "BFGS", control = \textbf{list}(ndeps = c(1E-6, 1E-6)))
C.mince.boots <- bootdistcens(C.mince.fit, niter = ndunc())

# Growth Model ----
\textbf{source}("../Combase Secondary Growth Model/read.growth.inact.data.R")
growth.data.subset <- growth.data.ss \%\% filter(b_f \in c("Beef", "Pork", "Poultry") \%\% select(rate, temp, b_f, yEnd) \%\% mutate(rate.sqrt = \textbf{sqrt}(rate))
GR_fit <- \textbf{nlsLM}(formula = rate.sqrt \sim \textbf{ratk}(b, c, temp = temp, T_min, T_max), data = filter(growth.data.subset, b_f \in c("Beef", "Pork", "Poultry")), start = \textbf{list}(b = 0.03, c = 0.05, T_min = 5, T_max = 52))
MPD_func <- \textbf{function}(a, T, T_min, T_submin, T_max, T_supmax)
a \times (T - T_min) \times (T - T_max) / ((T - T_submin) \times (T - T_supmax))
MPD_data <- growth.data.subset \%\%
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```
47    filter(b_f %in% c("Beef", "Pork", "Poultry") & !is.na(yEnd))
48    MPD_fit <- nlsLM(yEnd ~ MPD_func(a, temp, T_min, T_submin, T_max, T_supmax),
49                   data = MPD_data,
50                   start = c(a = 1, T_min = 5, T_submin=2, T_max = 55, T_supmax = 60),
51                   control = nls.lm.control(maxiter = 1024))
52
53    # Retail Temperatures
54    retail.temp.data <- read.csv("data/retail.temp.data - Poultry Survey.csv")
55    require(fGarch)
56    T.r.fit <- retail.temp.data$temp.shelf %>%
57                    fitdist(distr = "snorm", start = list(mean = 1, sd = 1, xi = .5))
58    T.r.boots <- T.r.fit %>%
59                    bootdist(niter = ndunc(), bootmethod = "nonparam")
60    rm(retail.temp.data)
61
62    # Consumer Transport ----
63    load("data/ecosure.data.RData")
64    meat.data <- meat.data %>%
65        filter(!is.na(f.meat.temp.final)) %>%
66        mutate(time.change = as.numeric(f.meat.time.final - f.meat.time.init) / 60,
67                     temp.change = as.numeric(na.omit(fahr.to.cel(as.numeric(f.meat.temp.final)) -
68                       fahr.to.cel(as.numeric(f.meat.temp.init)))))
69
70    # Consumer transport time bootstrap
71    t.ct.fit <- meat.data %>%
72        getElement("time.change") %>%
73        fitdist(distr = "gamma")
74    t.ct.boots <- t.ct.fit %>%
75        bootdist(niter = ndunc(), bootmethod = "nonparam")
76
77    #Bootstrap of temperature change during consumer transport
78    T.change.ct.fit <- meat.data %>%
79        getElement("temp.change") %>%
```

245
fitdist(distr = dgamma, start = list(shape = 1.7, rate = 0.5454), method = "mme")

T.change.ct.boots <- T.change.ct.fit %>% bootdist(niter = ndunc(), bootmethod = "nonparam")

# What is the correlation between the time of transport and the temp.change in cons.trans

corr.ct <- cor(meat.data$time.change, meat.data$temp.change)

# Domestic Storage ----

#Bootstrap samples for the storage time in domestic refrigeration.
data.mince <- data.frame(left = c(rep(0, 216), rep(2.5, 62), rep(4.5, 14), rep(7, 1)),
                         right = c(rep(2, 216), rep(4, 62), rep(7, 14), rep(14, 1)))

t.ds.fit <- data.mince %>% fitdistcens(distr = "exp")
t.ds.boots <- t.ds.fit %>% bootdistcens(niter = ndunc())

#Bootstraps for domestic storage temperature

nswfa.stats <- read.csv("data/NSWFA - summary.data.csv")

T.ds.fit <- nswfa.stats$mean %>% fitdist(dist = "norm")

T.ds.boots <- T.ds.fit %>% bootdist(niter = ndunc(), bootmethod = "nonparam")

# Inactivation

ecosure.cook.temps <- read.csv("data/ecosure.2007.cooking.data.csv")

T.cook.ecosure.temps <- ecosure.cook.temps %>% filter(Category == "Ground Beef") %>%
                          getElement("Final_Temperature") %>% fahr.to.cel
# Fat Percentage

For the pork burger inactivation model, the fat percentage of the mince is required.

```r
amazingRibs.cens <- data.frame(
  left = c(rep(54, 53), rep(57, 261),
           rep(63, 442), rep(68, 374)),
  right = c(rep(57, 53), rep(63, 261),
            rep(68, 442), rep(73, 374)))
```

```r
T.cook.amazingRibs.boots <- amazingRibs.cens %>%
  fitdistcens(distr = "norm") %>%
  bootdistcens(niter = ndunc())
```

# Inactivation Model

```r
inact.model <-
  function(int.temp, fat, sero, init = exp(inact.coefs["(Intercept)"])) {
    # Function to return the reduction in salmonella concentration
    if (sero %in% c("monophasic Salmonella 1,4,[5],12:i:-",
                    "S. Senftenberg",
                    "S. Typhimurium")) {
      inact.coefs <-
        coef(readRDS(
          "./Inactivation Salmonella Pork Burgers/inact.model.v2.RDS")
        ))
      z <- exp(
        -(inact.coefs["lm.intercept"] +
          int.temp * inact.coefs["lm.temp"] +
          ifelse(sero == "S. Senftenberg", inact.coefs["lm.intercept:seroS.
                                         Senftenberg"], 0) +
          ifelse(sero == "S. Typhimurium", inact.coefs["lm.intercept:seroS.
                                                         Typhimurium"], 0) +
          fat * inact.coefs["lm.intercept:fat.percent"] +
          ifelse(sero == "S. Senftenberg", int.temp * inact.coefs["lm.temp:seroS.
                                                        Senftenberg"], 0) +
```
ifelse(sero == "S. Typhimurium", int.temp * inact.coefs["lm.temp:seroS.Typhimurium"], 0) + int.temp * fat * inact.coefs["lm.temp:fat.percent"]

remaining <- exp(log(init) - log(1 + z))
return(remaining)

Listing C.3: The R code contained in the file ‘mc2d.boots - Pork.Burgers.R’. Contains the R commands that define the parameters of the model in the format required by the ‘mc2d’ package. This file also defines parameters used by the various ‘alternative’ scenarios. This file is included below.

W.serve <- 100
W.lab <- 25

GR_b.params <- function()
list(data = coef(GR_fit)["b"], type = "b")

GR_c.params <- function()
list(data = coef(GR_fit)["c"], type = "c")
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
  type = "0"

GR_T_min.params <- function()
  list(data = coef(GR_fit)["T_min"],
       type = "0")

GR_T_max.params <- function()
  list(data = coef(GR_fit)["T_max"],
       type = "0")

MPD_a.params <- function()
  list(data = coef(MPD_fit)["a"],
       type = "0")

MPD_T_min.params <- function()
  list(data = coef(MPD_fit)["T_min"],
       type = "0")

MPD_T_submin.params <- function()
  list(data = coef(MPD_fit)["T_submin"],
       type = "0")

MPD_T_max.params <- function()
  list(data = coef(MPD_fit)["T_max"],
       type = "0")

MPD_T_supmax.params <- function()
  list(data = coef(MPD_fit)["T_supmax"],
       type = "0")

P.mince.params <- function()
  list(
       func = "rbeta",
       type = "0")
```
type = "U",
lhs = use.lhs,
shape1 = 2 + 1,
shape2 = 148 - 2 + 1
)

P.serve.function <-
  function(P.mince, W.serve, W.lab)
  1 - (1 - P.mince) ^ (W.serve / W.lab)

P.serve.params <-
  function(P.mince, W.serve, W.lab)
  list(P.mince, W.serve, W.lab)

Z.serve.func <- "mcstoc"
Z.serve.params <- function(P.serve)
  list(
    func = "rbern",
    type = "VU",
    lhs = use.lhs,
    prob = P.serve
  )

C.mince.mean.params <- function()
  list(type = "U",
    data = C.mince.boots$estim$mean)

C.mince.sd.params <- function()
  list(type = "U",
    data = C.mince.boots$estim$sd)

C.mince.params <- function(C.mince.mean, C.mince.sd)
  list(
    func = "rnorm",
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

type = "VU",
lhs = use.lhs,
mean = C.mince.mean,
sd = C.mince.sd
)

N.serve.func <- "mcstoc"
N.serve.params <- function(C.mince, W.serve)
  list(func = "rpois",
       type = "VU",
       lhs = use.lhs,
       rtrunc = TRUE,
       linf = 0,
       lambda = 10 ^ C.mince * W.serve
  )

# Retail ----
t.r.rate.params <- function()
  list(func = "runif",
       type = "U",
       lhs = use.lhs,
       min = 0.5,
       max = 1.5
  )

t.r.params <- function(t.r.rate)
  list(func = "rexp",
       type = "VU",
       lhs = use.lhs,
       rate = 1 / (t.r.rate),

rtrunc = TRUE,
lup = 10
)

T.r.mean.params <- function()
  list(type = "U",
       data = T.r.boots$estim$mean)

T.r.sd.params <- function()
  list(type = "U",
       data = T.r.boots$estim$sd)

T.r.xi.params <- function()
  list(type = "U",
       data = T.r.boots$estim$xi)

T.r.params <- function(T.r.mean, T.r.sd, T.r.xi)
  list(
    func = "rsnorm",
    type = "VU",
    lhs = use.lhs,
    mean = T.r.mean,
    sd = T.r.sd,
    xi = T.r.xi
  )

N.r.params <- function(N.serve,
           t.r,
           T.r,
           W.serve,
           GR_b,
           GR_c,
           GR_T_min,
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```
GR_T_max,
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax)

list(
  N.in = N.serve,
duration = t.r,
temp = T.r,
W.serve = W.serve,
GR_b = GR_b,
GR_c = GR_c,
GR_T_min = GR_T_min,
GR_T_max = GR_T_max,
MPD_a = MPD_a,
MPD_T_min = MPD_T_min,
MPD_T_submin = MPD_T_submin,
MPD_T_max = MPD_T_max,
MPD_T_supmax = MPD_T_supmax
)

N.r.growth.model <- "growth.const.alt"

# Consumer transport ----
t.ct.shape.params <- function()
  list(type = "U",
       data = t.ct.boots$estim$shape)

t.ct.rate.params <- function()
  list(type = "U",
       data = t.ct.boots$estim$rate)
```
t.ct.params <- function(t.ct.shape, t.ct.rate)
list(
  func = "rgamma",
  type = "VU",
  lhs = use.lhs,
  shape = t.ct.shape,
  rate = t.ct.rate
)

T.change.ct.shape.params <- function()
list(type = "U",
data = T.change.ct.boots$estim$shape)

T.change.ct.rate.params <- function()
list(type = "U",
data = T.change.ct.boots$estim$rate)

T.change.ct.params <-
function(T.change.ct.shape, T.change.ct.rate)
list(
  func = "rgamma",
  type = "VU",
  lhs = use.lhs,
  shape = T.change.ct.shape,
  rate = T.change.ct.rate
)

N.ct.params <- function(N.r,
t.ct, T.r, T.change.ct, W.serve,
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

\[208\]
\[\text{GR}_b,\]
\[\text{GR}_c,\]
\[\text{GR}_{\text{T}\_\text{min}},\]
\[\text{GR}_{\text{T}\_\text{max}},\]
\[\text{MPD}_a,\]
\[\text{MPD}_{\text{T}\_\text{min}},\]
\[\text{MPD}_{\text{T}\_\text{submin}},\]
\[\text{MPD}_{\text{T}\_\text{max}},\]
\[\text{MPD}_{\text{T}\_\text{supmax}}\]
\[\text{list(}\]
\[\text{N.in = N.r,}\]
\[\text{duration = t.ct,}\]
\[\text{temp_init = T.r,}\]
\[\text{temp_change = T.change.ct,}\]
\[\text{W.serve = W.serve,}\]
\[\text{GR}_b = \text{GR}_b,\]
\[\text{GR}_c = \text{GR}_c,\]
\[\text{GR}_{\text{T}\_\text{min}} = \text{GR}_{\text{T}\_\text{min}},\]
\[\text{GR}_{\text{T}\_\text{max}} = \text{GR}_{\text{T}\_\text{max}},\]
\[\text{MPD}_a = \text{MPD}_a,\]
\[\text{MPD}_{\text{T}\_\text{min}} = \text{MPD}_{\text{T}\_\text{min}},\]
\[\text{MPD}_{\text{T}\_\text{submin}} = \text{MPD}_{\text{T}\_\text{submin}},\]
\[\text{MPD}_{\text{T}\_\text{max}} = \text{MPD}_{\text{T}\_\text{max}},\]
\[\text{MPD}_{\text{T}\_\text{supmax}} = \text{MPD}_{\text{T}\_\text{supmax}}\]
\[\text{)}\]
\[\text{N.ct.growth.model <- } \text{"growth.step.alt"}\]
\[\text{# Domestic Storage ----}\]
\[\text{t.ds.rate.params <- function()}\]
\[\text{list(type = } \text{"U"},\]
\[\text{data = t.ds.boots$estim$rate)}\]
t.ds.params <- function(t.ds.rate)
  list(
    func = "rexp",
    type = "VU",
    lhs = use.lhs,
    rate = t.ds.rate
  )

T.ds.mean.params <- function()
  list(type = "U",
       data = T.ds.boots$estim$mean)

T.ds.sd.params <- function()
  list(type = "U",
       data = T.ds.boots$estim$sd)

T.ds.params <- function(T.ds.mean, T.ds.sd)
  list(
    func = "rnorm",
    type = "VU",
    lhs = use.lhs,
    mean = T.ds.mean,
    sd = T.ds.sd
  )

N.ds.params <- function(N.ct, 
  t.ds, 
  T.ds, 
  W.serve, 
  GR_b, 
  GR_c, 
  GR_T_min, 
  GR_T_max, 

C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```
N.in = N.ct,
duration = t.ds,
temp = T.ds,
W.serve = W.serve,
GR_b = GR_b,
GR_c = GR_c,
GR_T_min = GR_T_min,
GR_T_max = GR_T_max,
MPD_a = MPD_a,
MPD_T_min = MPD_T_min,
MPD_T_submin = MPD_T_submin,
MPD_T_max = MPD_T_max,
MPD_T_supmax = MPD_T_supmax

list(
N.in = N.ct,
duration = t.ds,
temp = T.ds,
W.serve = W.serve,
GR_b = GR_b,
GR_c = GR_c,
GR_T_min = GR_T_min,
GR_T_max = GR_T_max,
MPD_a = MPD_a,
MPD_T_min = MPD_T_min,
MPD_T_submin = MPD_T_submin,
MPD_T_max = MPD_T_max,
MPD_T_supmax = MPD_T_supmax
)
```

```
N.ds.growth.model <- "growth.const.alt"

# Inactivation ----

# The 4 internal temps in de santos (2007)
int.temps <- c(63, 71, 74, 77)

# Consumer Preference Weighting from Channon (2014)
done.weights <- c(4.7, 23.1, 39.1, 33.1)

T.cook.mean.params <- function()

list(
```
func = "rempiricalD",
type = "V",
lhs = use.lhs,
values = int.temps,
prob = done.weights
)

T.cook.func <- "mcstoc"

T.cook.params <- function(T.cook.mean)
  list(
    func = "rnorm",
type = "V",
 lhs = use.lhs,
  mean = T.cook.mean,
  sd = 1
 )

F.mince.params <- function()
  list(
    func = "rtriang",
type = "V",
 lhs = use.lhs,
  min = 2.5,
  mode = 9.4,
  max = 14.6
)

inact.model.params <- function(T.cook, F.mince, init)
  list(
    int.temp = T.cook,
    fat = F.mince,
    sero = "monophasic Salmonella 1,4,[5],12:i:-"),
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
init = init

P.ill.alpha.params <- function()
list(
  func = "rtriang",
  type = "U",
  lhs = use.lhs,
  min = 0.0763,
  mode = 0.1324,
  max = 0.2274
)

P.ill.beta.params <- function()
list(
  func = "rtriang",
  type = "U",
  lhs = use.lhs,
  min = 38.49,
  mode = 51.45,
  max = 57.96
)

dose.resp.model <- "b.p.dose.resp"

growth_amount <-
  function(duration,
           temp,
           GR_b,
           GR_c,
           GR_T_min,
           GR_T_max) {
    rate_sqrt <-
```
\[
GR_b \times \left\{ \begin{array}{l}
\text{temp} - GR_T_{\text{min}} \\
\text{temp} - GR_T_{\text{max}}
\end{array} \right\} \times \sqrt{1 - \exp(GR_c \times \left\{ \begin{array}{l}
\text{temp} - GR_T_{\text{max}} \\
\text{temp} - GR_T_{\text{min}}
\end{array} \right\})}
\]

rate <-
(temp > GR_T_{\text{min}}) \times (temp < GR_T_{\text{max}}) \times (rate_{\text{sqrt}} > 0) \times rate_{\text{sqrt}} ^ 2
rate[is.na(rate)] <- 0
return(rate \times duration)

MP_value <-
\text{function(temp,}
MPD_a,
MPD_T_{\text{min}},
MPD_T_{\text{submin}},
MPD_T_{\text{max}},
MPD_T_{\text{supmax}}) \{
mpd <-
(MPD_a \times (\text{temp} - MPD_T_{\text{min}}) \times (\text{temp} - MPD_T_{\text{max}})) / ((\text{temp} - MPD_T_{\text{submin}}) \times (\text{temp} - MPD_T_{\text{supmax}}))
mp_{\text{serve}} <- 10 ^ \mpd
mpd_{\text{serve\_capped}} <-
(\text{temp} > MPD_T_{\text{submin}}) \times (\text{temp} < MPD_T_{\text{supmax}}) \times mp_{\text{serve}}
return(round(mpd_{\text{serve\_capped}} \times W_{\text{serve}}))
\}

# Alternate growth model for cut versin
growth.const.alt <- \text{function(N.in,}
duration,
temp,
W_{\text{serve}},
GR_b,
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
GR_c,
GR_T_min,
GR_T_max,
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax)
{
  change <-
    round(10 ^ (log10(N.in) + growth_amount(duration, temp, GR_b, GR_c, GR_T_min, GR_T_max))
)
MP_serve <-
  MP_value(temp, MPD_a, MPD_T_min, MPD_T_submin, MPD_T_max, MPD_T_supmax)
  change[which(N.in > MP_serve)] <- N.in[which(N.in > MP_serve)]
  change[which(N.in < MP_serve &&
    change > MP_serve)] <-
    MP_serve[which(N.in < MP_serve && change > MP_serve)]
  return(change)
}

growth.step.alt <-
  function(N.in,
    duration,
    temp_init,
    temp_change,
    Wserve,
    GR_b,
    GR_c,
    GR_T_min,
    )
```
GR_T_max,
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax) {

steps <- 100

delta_temp <- temp_change / steps
delta_time <- duration / steps

new_growth <- N.in

growth_change <- mcdata(0, type = "VU")

for (i in 1:steps)

  growth_change[, ,] <-
    growth_change[, ,] + growth_amount(
      duration = delta_time[, ,],
      temp = temp_init[, ,] + i * delta_temp[, ,],
      GR_b[, ,],
      GR_c[, ,],
      GR_T_min[, ,],
      GR_T_max[, ,]
    )

new_growth <- 10 ^ (log10(new_growth) + growth_change)

MP_serve <-
  MP_value(temp_init + temp_change,
    MPD_a,
    MPD_T_min,
    MPD_T_submin,
    MPD_T_max,
    MPD_T_supmax)

new_growth <- round(new_growth)

new_growth[which(N.in > MP_serve)] <-
  N.in[which(N.in > MP_serve)]
new_growth[which(N.in < MP_serve &&
    new_growth > MP_serve)] <-
    MP_serve[which(N.in < MP_serve && new_growth > MP_serve)]
return(new_growth)
}

# Scenarios
switch(
    scenario,
    "C.mince.inc" = {
        # Covers the estimate of the salmonella concentration based on prev.
        C.mince.params <- function(C.mince.mean, C.mince.sd)
            list(
                func = "rnorm",
                type = "VU",
                lhs = use.lhs,
                mean = C.mince.mean + 1,
                sd = C.mince.sd
            )
    },
    "C.mince.dec" = {
        # Concentration based on the ecoli concentration data
        C.mince.params <- function(C.mince.mean, C.mince.sd)
            list(
                func = "rnorm",
                type = "VU",
                lhs = use.lhs,
                mean = C.mince.mean - 1,
                sd = C.mince.sd
            )
    },
    "T.ct.cons" = {
        N.ct.growth.model <- "growth.const.alt"
    }
)
N.ct.params <- function(N.r,
  t.ct,
  T.r,
  T.change.ct,
  W.serve,
  GR_b,
  GR_c,
  GR_T_min,
  GR_T_max,
  MPD_a,
  MPD_T_min,
  MPD_T_submin,
  MPD_T_max,
  MPD_T_supmax)
list(
  N.in = N.r,
  duration = t.ct,
  temp = T.change.ct + T.r,
  W.serve = W.serve,
  GR_b = GR_b,
  GR_c = GR_c,
  GR_T_min = GR_T_min,
  GR_T_max = GR_T_max,
  MPD_a = MPD_a,
  MPD_T_min = MPD_T_min,
  MPD_T_submin = MPD_T_submin,
  MPD_T_max = MPD_T_max,
  MPD_T_supmax = MPD_T_supmax)

"T.r.3" = {
  T.r.params <- function(T.r.mean, T.r.sd, T.r.xi)
  list(}
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
func = "rsnorm",
type = "VU",
lhs = use.lhs,
mean = T.r.mean - (mean(T.r.mean)) + 3,
sd = T.r.sd,
xi = T.r.xi
}

"T.r.5" = {
  T.r.params <- function(T.r.mean, T.r.sd, T.r.xi)
  list(
    func = "rsnorm",
    type = "VU",
    lhs = use.lhs,
    mean = T.r.mean - (mean(T.r.mean)) + 5,
    sd = T.r.sd,
    xi = T.r.xi
  )
}

"T.r.6" = {
  T.r.params <- function(T.r.mean, T.r.sd, T.r.xi)
  list(
    func = "rsnorm",
    type = "VU",
    lhs = use.lhs,
    mean = T.r.mean - (mean(T.r.mean)) + 6,
    sd = T.r.sd,
    xi = T.r.xi
  )
}

"T.r.7" = {
  T.r.params <- function(T.r.mean, T.r.sd, T.r.xi)
  list(
```

265
```r
func = "rsnorm",
type = "VU",
lhs = use.lhs,
mean = T.r.mean - (mean(T.r.mean)) + 7,
sd = T.r.sd,
xi = T.r.xi
)

"T.change.ct.from.data" = {
  T.change.ct.params <- function(dummy1, dummy2)
    list(
      func = "rempiricalD",
      type = "VU",
      lhs = use.lhs,
      value = meat.data$temp.change
    )
  },

"T.cook.ecosure" = {
  T.cook.params <-
    function(T.cook.wrongmeans)
      list(
        #Means for the baseline scenario still need to be passed in.
        func = "rempiricalD",
        type = "V",
        lhs = use.lhs,
        values = T.cook.ecosure.temps
      )
  },

"Inact.S. Senftenberg" = {
  inact.model.params <- function(T.cook, F.mince, init)
    list(
      int.temp = T.cook,
      fat = F.mince,
    )
  },
```

266
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

603  sero = "S. Senftenberg",
604  init = init
605  
606  ),
607  "Inact.S. Typhimurium" = {
608    inact.model.params <- function(T.cook, F.mince, init)
609      list(
610        int.temp = T.cook,
611        fat = F.mince,
612        sero = "S. Typhimurium",
613        init = init
614      )
615  },
616  "AmazingRibs" = {
617    T.cook.m.r <-
618      do.call(mcstoc, list(
619        func = "runif",
620        min = 54,
621        max = 57,
622        lhs = use.lhs
623      ))
624    T.cook.m <-
625      do.call(mcstoc, list(
626        func = "runif",
627        min = 57,
628        max = 63,
629        lhs = use.lhs
630      ))
631    T.cook.m.w <-
632      do.call(mcstoc, list(
633        func = "runif",
634        min = 63,
635        max = 68,
lhs = use.lhs

T.cook.w <-
do.call(mcstoc, list(
  func = "runif",
  min = 68,
  max = 73,
  lhs = use.lhs
))

T.cook.func <- "mcprobtree"
whichdist <- mcstoc(
  rempiricalD,
  type = "V",
  values = 1:4,
  prob = done.weights / 100
)

T.cook.params <- function(T.cook.mean) {
  list(
    mcswitch = whichdist,
    mcvalues = list(
      "1" = T.cook.m.r,
      "2" = T.cook.m,
      "3" = T.cook.m.w,
      "4" = T.cook.w
    )
  )
}

"AmazingRibs.distr" = {
  T.cook.func <- "mcstoc"
T.cook.params <- function(T.cook.mean) {
  list(
    func = "rnorm",
    type = "VU",
    mean = T.cook.amazingRibs.boots$estim$mean,
    sd = T.cook.amazingRibs.boots$estim$sd
  )
}

"Fat.2.99" = {
  F.mince.params <- function()
  list(
    func = "rnorm",
    type = "V",
    lhs = uselhs,
    mean = 2.99,
    sd = 1
  )
},

"Fat.12.35" = {
  F.mince.params <- function()
  list(
    func = "rnorm",
    type = "V",
    lhs = uselhs,
    mean = 12.35,
    sd = 1
  )
},

"AR+Fat.2.99" = {
  T.cook.func <- "mcstoc"
  T.cook.params <- function(T.cook.mean) {

list(
func = "rnorm",
type = "VU",
mean = T.cook.amazingRibs.boots$estim$mean,
sd = T.cook.amazingRibs.boots$estim$sd
)

F.mince.params <- function()
  list(
    func = "rnorm",
type = "V",
    lhs = use.lhs,
    mean = 2.99,
sd = 1
  )
)

"AR+Fat.12.35" = {
  T.cook.func <- "mcstoc"
  T.cook.params <- function(T.cook.mean) {
    list(
      func = "rnorm",
type = "VU",
mean = T.cook.amazingRibs.boots$estim$mean,
sd = T.cook.amazingRibs.boots$estim$sd
    )
  }
  F.mince.params <- function()
    list(
      func = "rnorm",
type = "V",
    lhs = use.lhs,
    mean = 12.35,
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

\[ sd = 1 \]

\)

"Teunis_2010" = {
    dose.resp.model <- "teunis_2010.dr.model"
}

P.ill.alpha.params <- function()
  list(
    # Means for the baseline scenario still need to be passed in.
    func = "rempiricalD",
    type = "U",
    lhs = use.lhs,
    values = teunis_2010.dr.data$alpha
  )

P.ill.beta.params <- function()
  list(
    # Means for the baseline scenario still need to be passed in.
    func = "rempiricalD",
    type = "U",
    lhs = use.lhs,
    values = teunis_2010.dr.data$beta
  )

P.ill.eta.params <- function()
  list(
    # Means for the baseline scenario still need to be passed in.
    func = "rempiricalD",
    type = "U",
    lhs = use.lhs,
    values = teunis_2010.dr.data$eta
  )

P.ill.rho.params <- function()
  list(
    # Means for the baseline scenario still need to be passed in.
    func = "rempiricalD",
    type = "U",
    lhs = use.lhs,
    values = teunis_2010.dr.data$r
  )
)
)

Listing C.4: The R code contained in the file ‘mc2d.test - Pork.Burgers.R’. Contains the R commands used to verify some of the functions used in the model to ensure that the function results are as expected.
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
label = "Growth amount at 6°C and 10 hours"

expect_equal(object = as.numeric(growth_amount(duration = 1, temp = 50, GR_b = GR_b, GR_c = GR_c, GR_T_min = GR_T_min, GR_T_max = GR_T_max)),
              expected = 0)

equal(object = as.numeric(MP_value(temp = 0, MPD_a, MPD_T_min, MPD_T_submin, MPD_T_max, MPD_T_supmax)),
       expected = 0,
       label = "MPD @ 0°C")

equal(object = as.numeric(MP_value(temp = 60, MPD_a, MPD_T_min, MPD_T_submin, MPD_T_max, MPD_T_supmax)),
       expected = 0,
       label = "MPD @ 60°C")

equal(object = as.numeric(MP_value(temp = 30, MPD_a, MPD_T_min, MPD_T_submin, MPD_T_max, MPD_T_supmax)),
       expected = 22631674120,
       label = "MPD @ 30°C")

equal(object = as.numeric(growth.const.alt(N.in = 10, duration = 1, temp = 0, W.serve, GR_b, GR_c, GR_T_min, GR_T_max, MPD_a, MPD_T_min, MPD_T_submin, MPD_T_max, MPD_T_supmax)),
       expected = 10,
       label = "const temp growth, 10 org, 1 hour @ 30°C")

# While growth shouldn't occur, it shouldn't cap a 'large' N.in with MP
expect_equal(object = as.numeric(growth.const.alt(N.in = 1E20, duration = 1, temp = 0, W.serve, GR_b, GR_c, GR_T_min, GR_T_max, MPD_a, MPD_T_min, MPD_T_submin, MPD_T_max, MPD_T_supmax)),
              expected = 0)
```

273

```r
# Model without using the cut algorithm in mc2d.

# Load required functions

modEC3 <- mcmodelcut({
    GR_b,GR_c,GR_T_min,GR_T_max,
    MPD_a, MPD_T_min, MPD_T_submin,
    MPD_T_max, MPD_T_supmax)),
    expected = 1E20,
    label = "const temp growth, 1E20 org, 1 hour @ 30°C")

expect_equal(object = as.numeric(growth.const.alt(N.in = 10,duration = 240,temp = 30,
    W.serve,
    GR_b,GR_c,GR_T_min,GR_T_max,
    MPD_a, MPD_T_min, MPD_T_submin,
    MPD_T_max, MPD_T_supmax)),
    expected = 22631674120,
    label = "const temp growth, 1E20 org, 240 hours @ 30°C")

expect_equal(object = as.numeric(growth.const.alt(N.in = 1,duration = 100,temp = 12,
    W.serve,
    GR_b,GR_c,GR_T_min,GR_T_max,
    MPD_a, MPD_T_min, MPD_T_submin,
    MPD_T_max, MPD_T_supmax)),
    expected = 3852,
    label = "const temp growth, 1 org, 1 hour @ 6°C")
```


## First block:
## Evaluates all the Θ, V and U nodes.
{
  # Growth Parameters----
  GR_b <- do.call(mcdata, GR_b.params())
  GR_c <- do.call(mcdata, GR_c.params())
  GR_T_min <- do.call(mcdata, GR_T_min.params())
  GR_T_max <- do.call(mcdata, GR_T_max.params())

  MPD_a <- do.call(mcdata, MPD_a.params())
  MPD_T_min <- do.call(mcdata, MPD_T_min.params())
  MPD_T_submin <- do.call(mcdata, MPD_T_submin.params())
  MPD_T_max <- do.call(mcdata, MPD_T_max.params())
  MPD_T_supmax <- do.call(mcdata, MPD_T_supmax.params())

  #Run growth diagnostics
  growth_diag(
    GR_b,
    GR_c,
    GR_T_min,
    GR_T_max,
    MPD_a,
    MPD_T_min,
    MPD_T_submin,
    MPD_T_max,
    MPD_T_supmax
  )

  # Initial Contamination----
  P.mince <- do.call(mcstoc, P.mince.params())
  P.serve <- do.call(P.serve.function,
    P.serve.params(P.mince, W.serve, W.lab))
C.mince.mean <- do.call(mcdata, C.mince.mean.params())
C.mince.sd <- do.call(mcdata, C.mince.sd.params())

# Retail----
t.r.rate <- do.call(mcstoc, t.r.rate.params())
T.r.mean <- do.call(mcdata, T.r.mean.params())
T.r.sd <- do.call(mcdata, T.r.sd.params())
T.r.xi <- do.call(mcdata, T.r.xi.params())

# Consumer Transport
t.ct.shape <- do.call(mcdata, t.ct.shape.params())
t.ct.rate <- do.call(mcdata, t.ct.rate.params())
T.change.ct.shape <- do.call(mcdata, T.change.ct.shape.params())
T.change.ct.rate <- do.call(mcdata, T.change.ct.rate.params())

# Domestic Refrigeration
t.ds.rate <- do.call(mcdata, t.ds.rate.params())
T.ds.mean <- do.call(mcdata, T.ds.mean.params())
T.ds.sd <- do.call(mcdata, T.ds.sd.params())

# Inactivation
F.mince <- do.call(mcstoc, F.mince.params())
T.cook.mean <- do.call(mcstoc, T.cook.mean.params())
T.cook <- do.call(T.cook.func, T.cook.params(T.cook.mean))

# Pill
P.ill.alpha <- do.call(mcstoc, P.ill.alpha.params())
P.ill.beta <- do.call(mcstoc, P.ill.beta.params())
if (scenario == "Teunis_2010") {
}
P.ill.eta <- \textbf{do.call}(\textit{mcstoc}, \textit{P.ill.eta.params})

P.ill.rho <- \textbf{do.call}(\textit{mcstoc}, \textit{P.ill.rho.params})

\#
\#
# Second block:
# Evaluates all the VU nodes
# Leads to the mc object.
#
# Growth Parameters----

# Initial Contamination----
Z.serve <- \textbf{do.call}(\textit{Z.serve.func}, \textit{Z.serve.params(P.serve)})

C.mince <- \textbf{do.call}(\textit{mcstoc}, \textit{C.mince.params(C.mince.mean, C.mince.sd)})

N.serve <- \textbf{do.call}(\textit{N.serve.func}, \textit{N.serve.params(C.mince, W.serve)})

# Retail----
t.r <- \textbf{do.call}(\textit{mcstoc}, \textit{t.r.params(t.r.rate)} \times 24)

T.r <- \textbf{do.call}(\textit{mcstoc}, \textit{T.r.params(T.r.mean, T.r.sd, T.r.xi)})

N.r <- \textbf{do.call}

N.r.growth.model,

N.r.params(

  \textit{N.serve,}
  \textit{t.r,}
  \textit{T.r,}
  \textit{W.serve,}
  \textit{GR.b,}
  \textit{GR.c,}

)
GR_T_min,
GR_T_max,
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax
)

# Consumer Transport
t.ct <- do.call(mcstoc, t.ct.params(t.ct.shape, t.ct.rate))
T.change.ct <- do.call(mcstoc,
  T.change.ct.params(T.change.ct.shape,
  T.change.ct.rate))

# Include correlation between nodes.
ct.cor.nodes <- cornode(t.ct, T.change.ct, target = corr.ct)
t.ct <- ct.cor.nodes$t.ct
T.change.ct <- ct.cor.nodes$T.change.ct
N.ct <- do.call(
  N.ct.growth.model,
  N.ct.params(
    N.r,
    t.ct,
    T.r,
    T.change.ct,
    W.serve,
    GR_b,
    GR_c,
    GR_T_min,
    GR_T_max,
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax
)
)

# Domestic Refrigeration
t.ds <- do.call(mcstoc, t.ds.params(t.ds.rate)) * 24
T.ds <- do.call(mcstoc, T.ds.params(T.ds.mean, T.ds.sd))
N.ds <- do.call(
N.ds.growth.model,
N.ds.params(
N.ct,
t.ds,
T.ds,
W.serve,
GR_b,
GR_c,
GR_T_min,
GR_T_max,
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax
)
)

# Inactivation
N.cook <- do.call(inact.model,
inact.model.params(T.cook, F.mince, init = N.ds))
```
# Pill

if (scenario != "Teunis_2010") {
  P.ill.given.cont <- do.call(dose.resp.model, 
      list(dose = N.cook, 
        alpha = P.ill.alpha, 
        beta = P.ill.beta))
} else {
  P.ill.given.cont <- do.call(dose.resp.model, 
    list(alpha = P.ill.alpha, 
      beta = P.ill.beta, 
      eta = P.ill.eta, 
      rho = P.ill.rho, 
      dose = N.cook))
}

# Differences

N.r.diff <- N.r - N.serve
N.ct.diff <- N.ct - N.r
N.ds.diff <- N.ds - N.ct
N.cook.diff <- N.ds - N.cook

log.N.r.diff <- log10(N.r) - log10(N.serve)
log.N.ct.diff <- log10(N.ct) - log10(N.r)
log.N.ds.diff <- log10(N.ds) - log10(N.ct)
log.N.cook.diff <- log10(N.ds) - log10(N.cook)

P.ill <- P.ill.given.cont * P.serve
res <- mc(
  GR_b, 
  GR_c, 
  GR_T_min, 
  ...
GR_T_max,
MPD_a,
MPD_T_min,
MPD_T_submin,
MPD_T_max,
MPD_T_supmax,
P.mince,
C.mince,
t.r,
T.r,
N.r,
N.r.diff,
log.N.r.diff,
t.ct,
T.change.ct,
N.ct,
N.ct.diff,
log.N.ct.diff,
t.ds,
T.ds,
N.ds,
N.ds.diff,
log.N.ds.diff,
F.mince,
T.cook,
N.cook,
N.cook.diff,
log.N.cook.diff,
P.ill.alpha,
P.ill.beta,
P.ill
)
}
## Third block:

## Leads to a list of statistics: summary, plot, tornado

## or any function leading to a vector (et), a list (minmax),

## a matrix or a data.frame (summary)

```r
if (scenario == "baseline") {
  list(
    sum = summary(res,
    lim = c(0.025, 0.975),
    prob = c(0.025, 0.5, 0.95, 0.975,
      0.99, 0.999, 0.9999, 0.99999)
    ),
    tornado = tornado(res, use = "pairwise.complete.obs")
  )
} else {
  list(sum = summary(res,
    lim = c(0.025, 0.975),
    prob = c(0.025, 0.5, 0.95, 0.975,
      0.99, 0.999, 0.9999, 0.99999))
)}
```

Listing C.6: The R code contained in the file ‘mc2d.runorder_cut - Pork.Burgers.R’. Contains the R commands that are used to evaluate the stochastic model defined in ‘mc2d.model_cut - RA Pork Burgers.R’ for all the alternative scenarios.
```r
source("model.functions.R")
library(filehash)
library(compiler)
enableJIT(3)
compilePKGS(TRUE)
setCompilerOptions(suppressUndefined = TRUE)

ndvar(5E4)
ndunc(5E4)
#ndvar(5E2);ndunc(5E2) # Small run

use.lhs <- TRUE
# Should latin hypercube sampling be used in the simulation?
results <- dbInit("2D results/results", type = "RDS")

if (process == 1) {
  # We only need to write the simulation dimensions once
  results$nsv <- ndvar()
  results$nsu <- ndunc()

  scenario <- "baseline"

  cat("-----------------------------\n")
cat("Scenario =", scenario, "\n")
cat("-----------------------------\n")
seed <- 314
set.seed(seed)
source("mc2d.boots - Pork.Burgers.R")
source("mc2d.params - Pork.Burgers.R")
source("mc2d.test - Pork.Burgers.R")
source("mc2d.model_cut - Pork.Burgers.R")
```
dbInsert(results, scenario, NULL)
evalmccut(modEC3, nsv = ndvar(), nsu = ndunc()) %>% dbInsert(results, scenario, .) gc()

} else {
  scenarios <- c("C.mince.inc",
               "C.mince.dec",
               "T.ct.cons",
               "T.r.3",
               "T.r.5",
               "T.r.6",
               "T.r.7",
               "T.change.ct.from.data",
               "T.cook.ecosure",
               "Inact.S. Senftenberg",
               "Inact.S. Typhimurium",
               "Fat.2.99",
               "Fat.12.35",
               "Teunis_2010"
  )

  for (i in 1:length(scenarios)) {
    if (((i - 1) %% (cores - 1)) + 1 == (process - 1)) {
      scenario <- scenarios[i]
      cat("----------------------------------------\n")
      cat("Scenario =", scenario, "\n")
      cat("----------------------------------------\n")
      seed <- 314
      set.seed(seed)
      source("mc2d.boots - Pork.Burgers.R")
      source("mc2d.params - Pork.Burgers.R")
      source("mc2d.test - Pork.Burgers.R")
C. R Code for Pork Burger Risk Assessment Model (Chapter 5)

```r
source("mc2d.model_cut - Pork.Burgers.R")
dbInsert(results, scenario, NULL)
evalmccut(modEC3, nsv = ndvar(), nsu = ndunc()) %>% dbInsert(results, scenario, .)
gc()
}
}
```

**Listing C.7:** The batch code contained in the file ‘batch_run.bat’. Contains the batch commands that start the various R processes that evaluate the model. This script allows the various scenarios to be evaluated to be distributed over the available computer cores. The number of cores can be adjusted for each computer.

```bash
for /l %%x in (1, 1, 6) do ( 
    START "" Rscript "mc2d.runorder_cut - RA Pork Burgers.R" %%x
)
```

285
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D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

In this section, R and batch commands are included that are used to evaluate the salmonellosis from moisture-infused pork steak consumption risk assessment model, described in Chapter 6. The contents of these files are presented in Listings D.8 to D.13 and the model evaluation is triggered from the code in Listing D.13.

Listing D.8: The R code contained in the file ‘mc2d.boots - MI.R’. Contains the R commands to perform any bootstrapping of data sources used in the model.

```r
# Purpose: fit distributions to the datasets and, if required, generates bootstrap
# samples from the distributions required.
# Required packages:
libary(filehash)
libary(minpack.lm)

ratk <- function(b, c, temp, temp.min, temp.max) {
  (b * (eval.parent(temp) - temp.min))^2 * (1 - exp(c * (eval.parent(temp) - temp.max)))
}

#Function to convert Fahrenheit to celsius
fahr.to.cel <- function(fahrenheit) { (fahrenheit - 32) * 5/9}
```
# Beta Poisson Dose Response Model FAO/WHO

```r
b.p.dose.resp <- function(dose, alpha, beta) 1 - (1 + dose/beta)^(-alpha)

source('model.functions.R')

rm("log.cens.meat.data")
```

# Growth Model ----

```r
graphs <- FALSE

source("../Growth MI Steaks/final_model.R", chdir = TRUE)

Growth_fit <- fixed_fit
```

# Retail ----

# Retail Temperatures

```r
retail.temp.data <- read.csv("data/retail.temp.data - Poultry Survey.csv")

require(fGarch)

T.r.boots <- retail.temp.data$temp.shelf %>%
  fitdist(distr = "snorm", start = list(mean = 1, sd = 1, xi = .5)) %>%
  bootdist(niter = ndunc(), bootmethod = "nonparam")

rm(retail.temp.data)
```

# Consumer Transport ----

```r
load("data/ecosure.data.RData")

meat.data <- meat.data %>%
  filter(!is.na(f.meat.temp.final)) %>%
  mutate(
    time.change = as.numeric(f.meat.time.final - f.meat.time.init) / 60,
    temp.change = as.numeric(na.omit(
      fahr.to.cel(as.numeric(f.meat.temp.final)) -
      fahr.to.cel(as.numeric(f.meat.temp.init))
    ))
```

288
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
# Consumer transport time bootstrap

# Bootstrap of temperature change during consumer transport

# What is the correlation between the time of transport and the temp.change in cons.trans

data.steak <- data.frame(left = c(rep(0, 222), rep(2.5, 68), rep(4.5, 15), rep(7, 1)),
                         right = c(rep(2, 222), rep(4, 68), rep(7, 15), rep(14, 1)))

t.ds.fit <- data.steak %>%
            fitdistcens(distr = "exp")

t.ds.boots <- t.ds.fit %>%
             bootdistcens(niter = ndunc())
```

289
# Bootstraps for domestic storage temperature

```r
nswfa.stats <- read.csv("data/NSWFA - summary.data.csv")
T.ds.boots <- nswfa.stats$mean %>%
  fitdist(dist = "norm") %>%
  bootdist(niter = ndunc(), bootmethod = "nonparam")
```

# Inactivation

```r
source("model.functions.R")
ecosure.cook.temps <- read.csv("data/ecosure.2007.cooking.data.csv")
ecosure.cook.pork <-
  ecosure.cook.temps %>% dplyr::filter(Category == "Pork") %>%
  getElement("Final_Temperature") %>% fahr.to.cel
```

Listing D.9: The R code contained in the file `mc2d.tests - MI.R`. Contains the R commands used to verify some of the functions used in the model to ensure that the function results are as expected.

```r
library(testthat)

test_transfers <- function(EI, EN, IE, IN, IB, NE, NI, NB, BE, BI, BN, TR){
  test_that("exterior to interior is valid", {
    expect_gte(min(EI), 0)
    expect_lte(max(EI), 1)
  })

test_that("exterior to needles is valid", {
  expect_gte(min(EN), 0)
  expect_lte(max(EN), 1)
})

test_that("exterior to brine is valid", {
  expect_gte(min(EB), 0)
  expect_lte(max(EB), 1)
})
```
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
test_that("interior to exterior is valid", {
  expect_gte(min(IE), 0)
  expect_lte(max(IE), 1)
})

test_that("interior to needles is valid", {
  expect_gte(min(IN), 0)
  expect_lte(max(IN), 1)
})

test_that("interior to brine is valid", {
  expect_gte(min(IB), 0)
  expect_lte(max(IB), 1)
})

test_that("needles to exterior is valid", {
  expect_gte(min(NE), 0)
  expect_lte(max(NE), 1)
})

test_that("needles to interior is valid", {
  expect_gte(min(NI), 0)
  expect_lte(max(NI), 1)
})

test_that("needles to brine is valid", {
  expect_gte(min(NB), 0)
  expect_lte(max(NB), 1)
})

test_that("brine to exterior is valid", {
```
```r
expect_gte(min(BE), 0)
expect_lte(max(BE), 1)
}

test_that("brine to interior is valid", {
  expect_gte(min(BI), 0)
  expect_lte(max(BI), 1)
})

test_that("brine to needles is valid", {
  expect_gte(min(BN), 0)
  expect_lte(max(BN), 1)
})

test_that("transfer matrix is valid", {
  expect_gte(min(TR), 0)
  expect_lte(max(TR), 1)
})
}
```

Listing D.10: The R code contained in the file ‘mc2d.params - MI.R’. Contains the R commands that define the parameters of the model in the format required by the ‘mc2d’ package. This file also defines parameters used by the various ‘alternative’ scenarios.
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

12 GR_T_min.params <- function()
13 list(data = coef(Growth_fit)["temp_min"],
14 type = "0")
15
16 MPD_a.params <- function()
17 list(data = coef(Growth_fit)["a"],
18 type = "0")
19
20 MPD_T_min.params <- function()
21 list(data = coef(Growth_fit)["T_min"],
22 type = "0")
23
24 MPD_T_submin.params <- function()
25 list(data = coef(Growth_fit)["T_submin"],
26 type = "0")
27
28 # Initial Contamination
29
30 # The prev concentration of salmonella on the exterior of the primal before MI.
31 P.shoulder.params <- function()
32 list(
33 func = "rbeta",
34 type = "U",
35 shape1 = P.shoulder.shape1,
36 shape2 = P.shoulder.shape2
37 )
38
39 P.serve.function <-
40 function(P.in, W.init, W.final)
41 1 - (1 - P.in) ^ (W.final / W.init)
42 P.serve.params <-
43 function(P.in, W.init, W.final)
list(P.in, W.init, W.final)

C.loin.mean.params <- function()
  list(type = "U",
       data = C.belly.and.trim.boots$estim$mean)

C.loin.sd.params <- function()
  list(type = "U",
       data = C.belly.and.trim.boots$estim$sd)

# Salmonella concentration on loin? *Does not take into account g to cm conversion
C.loin.params <- function(C.loin.mean, C.loin.sd)
  list(func = "rnorm",
       type = "VU",
       mean = C.loin.mean,
       sd = C.loin.sd)

# The weight of the primal.
W.loin.func <- "mcstoc"
W.loin.params <- function()
  list(func = "rnorm",
       type = "V",
       mean = 4.5,
       sd = 0.5102)

D.loin.func <- "mcdata"
D.loin.params <- function()
  list(type = "0",
       data = 9.3E-4) #kg/cm^3)
# Surface area of primal. Based on SA of a cylinder

```r
L.loin.func <- "mcstoc"
L.loin.params <- function()
  list(func = "rnorm",
       mean = 52.5,
       sd = 1.28)

R.loin.func <- function(W.loin, L.loin, D.loin)
  sqrt(W.loin / ((L.loin) * D.loin * pi))

SA.loin.func <- function(R.loin, L.loin)
  2 * pi * R.loin * (L.loin + R.loin)

Z.function <- "mcstoc"
Z.params <- function(P.in)
  list(func = "rbern",
       type = "VU",
       prob = P.in)

N.loin.cont.func <- "mcstoc"
N.loin.cont.params <- function(C.in, SA.primal)
  list(func = "rpois",
       type = "VU",
       rtrunc = TRUE,
       linf = 0,
       lambda = 10 ^ C.in * SA.primal)

N.loin.func <- function(N.loin.cont.in, Z.in)
  N.loin.cont.in * Z.in
```
N.loin.params <-
  function(N.loin.cont.in, Z.in)
  list(N.loin.cont.in, Z.in)

prop.inf.func <- "mcstoc"
prop.inf.params <- function()
  list(
    func = "rtriang",
    min = 0.08,
    mode = 0.10,
    max = 0.15,
    type = "V"
  )
V.brine.balane.max.function <- function()
50

if (scenario_infusion == "baseline") {
  EI.function <- "mcstoc"
  EI.params <- function()
    list(
      func = "rtriang",
      type = "U",
      min = 0.37,
      mode = 0.455,
      max = 0.55
    )
  EI.offset <- function()
    0
  EN.function <- "mcstoc"
  EN.params <- function()
    list(
      func = rbeta,
shape1 = 1.8,
shape2 = 8.89,
type = "U"
)
EN.offset <- function()
  0

EB.function <- "mclust"
EB.params <- function()
  list(
    func = "rtriang",
    min = EB.min,
    mode = EB.mean,
    max = EB.max,
    type = "U"
  )
EB.offset <- function()
  0

IE.function <- "mclust"
IE.params <- function()
  list(data = 0,
       type = "U")

IN.function <- "mclust"
IN.params <- function()
  list(data = 0,
       type = "U")

IB.function <- "mclust"
IB.params <- function()
  list(data = 0,
       type = "U")
NE.function <- "mcstoc"
NE.params <- function()
  list(
    func = "rbeta",
    shape1 = 7.43,
    shape2 = 11.62,
    type = "U"
  )
NE.fraction <- function()
  2
NE.offset <- function()
  0.

NI.function <- "mcstoc"
NI.params <- function()
  list(
    func = "rbeta",
    shape1 = 7.43,
    shape2 = 11.62,
    type = "U"
  )
NI.fraction <- function()
  2
NI.offset <- function()
  0

NB.function <- "mcstoc"
NB.params <- function()
  list(
    func = "rtriang",
    min = 0,
    mode = 0.05,
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
max = 0.8,
type = "U"
)
NB.offset <- function()
0

BE.function <- "mcstoc"
BE.params <- function()
list(
  func = "rtriang",
  min = 0,
  mode = 0.05,
  max = 0.8,
  type = "U"
)
BE.offset <- function()
0

BI.function <- "mcstoc"
BI.params <- function()
list(
  func = "rtriang",
  min = 0.2,
  mode = 0.90,
  max = 1,
  type = "U"
)
BI.offset <- function()
0

BN.function <- "mcstoc"
BN.params <- function()
list(
```

299
```r
func = "rtriang",
min = 0,
mode = 0.05,
max = 0.8,
type = "U"
)
BN.offset <- function()
0

} else {
  EI.function <- "mcdata"
  EI.params <- function()
  list(type = "U", data = 0.455)
  EI.offset <- function()
  0

  EN.function <- "mcdata"
  EN.params <- function()
  list(data = 0.17,
       type = "U")
  EN.offset <- function()
  0

  EB.function <- "mcdata"
  EB.params <- function()
  list(data = EB.mean,
       type = "U")
  EB.offset <- function()
  0

  IE.function <- "mcdata"
  IE.params <- function()
  list(data = 0,
       type = "U")

} else {
  EN.function <- "mcdata"
  EN.params <- function()
  list(data = 0.17,
       type = "U")
  EN.offset <- function()
  0

  EB.function <- "mcdata"
  EB.params <- function()
  list(data = EB.mean,
       type = "U")
  EB.offset <- function()
  0

  IE.function <- "mcdata"
  IE.params <- function()
  list(data = 0,
       type = "U")

} else {
  EN.function <- "mcdata"
  EN.params <- function()
  list(data = 0.17,
       type = "U")
  EN.offset <- function()
  0

  EB.function <- "mcdata"
  EB.params <- function()
  list(data = EB.mean,
       type = "U")
  EB.offset <- function()
  0

  IE.function <- "mcdata"
  IE.params <- function()
  list(data = 0,
       type = "U")
```
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

    type = "U")

IN.function <- "mcdata"
IN.params <- function()
    list(data = 0,
         type = "U")

IB.function <- "mcdata"
IB.params <- function()
    list(data = 0,
         type = "U")

NE.function <- "mcdata"
NE.params <- function()
    list(data = 0.39,
         type = "U")
NE.offset <- function()
    0
NE.fraction <- function()
    2

NI.function <- "mcdata"
NI.params <- function()
    list(data = 0.39,
         type = "U")
NI.offset <- function()
    0
NI.fraction <- function()
    2

NB.function <- "mcdata"
NB.params <- function()
    list(data = 0.05,
         type = "U")
NB.offset <- function()
  0

BE.function <- "mcdata"
BE.params <- function()
  list(data = 0.05,
       type = "U")
BE.offset <- function()
  0

BI.function <- "mcdata"
BI.params <- function()
  list(data = 0.90,
       type = "U")
BI.offset <- function()
  0

BN.function <- "mcdata"
BN.params <- function()
  list(data = 0.05,
       type = "U")
BN.offset <- function()
  0

# Combined MI effect

P.i.params <- function(P.i.shape1, P.i.shape2)
  list(
    func = "rbeta",
    type = "U",
    shapel = P.i.shape1,
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
shape2 = P.i.shape2

P.mi.params <- function(P.mi.shape1, P.mi.shape2)
  list(
    func = "rbeta",
    type = "U",
    shape1 = P.mi.shape1,
    shape2 = P.mi.shape2
  )

C.i.mean.func <- "mcdata"
C.i.mean.params <- function(C.i.mean.boots)
  list(type = "U",
       data = as.numeric(C.i.mean.boots))

C.i.sd.func <- "mcdata"
C.i.sd.params <- function(C.i.sd.boots)
  list(type = "U",
       data = as.numeric(C.i.sd.boots))

C.i.params <- function(C.i.mean, C.i.sd)
  list(
    func = "rnorm",
    type = "VU",
    mean = C.i.mean,
    sd = C.i.sd
  )

C.mi.mean.func <- "mcdata"
C.mi.mean.params <- function(C.mi.mean.boots)
  list(type = "U",
       data = as.numeric(C.mi.mean.boots))
```
C.mi.sd.func <- "mcdata"
C.mi.sd.params <- function(C.mi.sd.boots)
  list(type = "U",
       data = as.numeric(C.mi.sd.boots))
C.mi.params <- function(C.mi.mean, C.mi.sd)
  list(func = "rnorm",
        type = "VU",
        mean = C.mi.mean,
        sd = C.mi.sd)
percentMI.function <- "mcstoc"
percentMI.params <- function()
  list(func = "rtriang",
        type = "U",
        lhs = use.lhs,
        min = 0.05,
        mode = 0.075,
        max = 0.10)
is.mi.func <- "mcstoc"
is.mi.params <- function(percentMI)
  list(func = "rbern",
        type = "VU",
        lhs = use.lhs,
        prob = percentMI)
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
P.all.func <- function(P.mi, P.i, percentMI)
  (P.mi * percentMI) + (P.i * (1 - percentMI))

N.serve.func <- mcstoc
N.serve.params <- function(conc)
  list(
    func = "rpois",
    type = "VU",
    rtrunc = TRUE,
    linf = 0,
    lambda = 10 ^ conc
  )

# Retail ----
t.r.rate.params <- function()
  list(
    func = "runif",
    type = "U",
    lhs = use.lhs,
    min = 0.5,
    max = 1.5
  )

t.r.params <- function(t.r.rate)
  list(
    func = "rexp",
    type = "VU",
    lhs = use.lhs,
    rate = 1 / (t.r.rate * 24),
    rtrunc = TRUE,
    lsup = 10 * 24
  )
```

305
T.r.mean.params <- function()
  list(type = "U",
       data = T.r.boots$estim$mean)

T.r.sd.params <- function()
  list(type = "U",
       data = T.r.boots$estim$sd)

T.r.xi.params <- function()
  list(type = "U",
       data = T.r.boots$estim$xi)

T.r.params <- function(T.r.mean, T.r.sd, T.r.xi)
  list(func = "rsnorm",
       type = "VU",
       lhs = use.lhs,
       mean = T.r.mean,
       sd = T.r.sd,
       xi = T.r.xi)

N.r.params <- function(N.all, t.r, T.r, W.serve, GR_b, GR_T_min, MPD_a, MPD_T_min, MPD_T_submin)
  list(N.in = N.all)
duration = t.r,

temp = T.r,

W.serve = W.serve,

GR_b = GR_b,

GR_T_min = GR_T_min,

MPD_a = MPD_a,

MPD_T_min = MPD_T_min,

MPD_T_submin = MPD_T_submin
}

N.r.growth.model <- "growth.const.alt"

# Consumer transport ----

t.ct.shape.params <- function()

list(type = "U",

    data = t.ct.boots$estim$shape)

t.ct.rate.params <- function()

list(type = "U",

    data = t.ct.boots$estim$rate)

t.ct.params <- function(t.ct.shape, t.ct.rate)

list(

    func = "rgamma",

    type = "VU",

    lhs = use.lhs,

    shape = t.ct.shape,

    #7.395201

    rate = t.ct.rate

)

T.change.ct.shape.params <- function()
```
list(type = "U",
     data = T.change.ct.boots$estim$shape)

T.change.ct.rate.params <- function()
list(type = "U",
     data = T.change.ct.boots$estim$rate)

T.change.ct.params <-
function(T.change.ct.shape, T.change.ct.rate)
list(
    func = "rgamma",
    type = "VU",
    lhs = use.lhs,
    shape = T.change.ct.shape,
    rate = T.change.ct.rate
)

N.ct.growth.model = "growth.step.alt"
N.ct.params <-
function(N.r,
    t.ct,
    T.r,
    T.change.ct,
    W.serve,
    GR_b,
    GR_T_min,
    MPD_a,
    MPD_T_min,
    MPD_T_submin)
list(
    N.in = N.r,
    duration = t.ct,
    T.init = T.r,
```

308
539 \text{T}.change = \text{T}.change.ct,
540 \text{W}.serve = \text{W}.serve,
541 \text{GR}_b = \text{GR}_b,
542 \text{GR}_T_{\text{min}} = \text{GR}_T_{\text{min}},
543 \text{MPD}_a = \text{MPD}_a,
544 \text{MPD}_T_{\text{min}} = \text{MPD}_T_{\text{min}},
545 \text{MPD}_T_{\text{submin}} = \text{MPD}_T_{\text{submin}}
546 )
547
548
549 # Domestic Storage ----
550
551 \text{t}.ds\cdot\text{rate.params} \leftarrow \text{function}()
552 \quad \text{list}(\text{type} = "U",
553 \quad \quad \text{data} = \text{t}.ds\cdot\text{boots}\$\text{estim}\$\text{rate})
554
555 \text{t}.ds\cdot\text{params} \leftarrow \text{function}(\text{t}.ds\cdot\text{rate})
556 \quad \text{list}(\n557 \quad \quad \text{func} = "\text{rexp}",
558 \quad \quad \text{type} = "\text{VU}",
559 \quad \quad \text{lhs} = \text{use.lhs},
560 \quad \quad \text{rate} = \text{t}.ds\cdot\text{rate}
561 \quad )
562
563 \text{T}.ds\cdot\text{mean.params} \leftarrow \text{function}()
564 \quad \text{list}(\text{type} = "U",
565 \quad \quad \text{data} = \text{T}.ds\cdot\text{boots}\$\text{estim}\$\text{mean})
566
567 \text{T}.ds\cdot\text{sd.params} \leftarrow \text{function}()
568 \quad \text{list}(\text{type} = "U",
569 \quad \quad \text{data} = \text{T}.ds\cdot\text{boots}\$\text{estim}\$\text{sd})
570
571 \text{T}.ds\cdot\text{params} \leftarrow \text{function}(\text{T}.ds\cdot\text{mean}, \text{T}.ds\cdot\text{sd})
572 \quad \text{list}(}
func = "rnorm",
    type = "VU",
    lhs = use.lhs,
    mean = T.ds.mean,
    sd = T.ds.sd
  )

N.ds.params <-
  function(N.ct, t.ds, T.ds, W.serve, GR_b, GR_T_min, MPD_a, MPD_T_min, MPD_T_submin)
  list(
    N.in = N.ct,
    duration = t.ds,
    temp = T.ds,
    W.serve = W.serve,
    GR_b = GR_b,
    GR_T_min = GR_T_min,
    MPD_a = MPD_a,
    MPD_T_min = MPD_T_min,
    MPD_T_submin = MPD_T_submin
  )

N.ds.growth.model <- "growth.const.alt"

# Inactivation ----


# The 4 internal temps in de santos (2007)

```rint.temps <- c(63, 71, 74, 77)
```

# Consumer Preference Weighting from Channon (2014)

```r
done.weights.fresh.pork <- c(5.6, 34.6, 38.6, 21.2)
done.weights.pork.burgers <- c(4.7, 23.1, 39.1, 33.1)
```

```r
T.cook.mean.params <- function()
  list(
    func = "rempiricalD",
    type = "V",
    values = int.temps,
    prob = done.weights.fresh.pork
  )
```

```r
T.cook.params <- function(T.cook.mean)
  list(
    func = "rnorm",
    type = "V",
    lhs = use.lhs,
    mean = T.cook.mean,
    sd = 1
  )
```

```r
# inact.redn <- function(T.cook) pmax(-1.24+0.091 * int.cook.temp,0)
inact.redn <-
  function(T.cook, is.mi)
  ifelse(unmc(is.mi == 1),
    pmax(-1.72 + 0.070 * T.cook, 0),
    pmax(-1.24 + 0.091 * T.cook, 0))
```

```r
P.ill.alpha.params <- function()
  list(
```
```
P. ill. beta.params <- function()
  list(
    func = "rtriang",
    type = "U",
    lhs = use.lhs,
    min = 0.0763,
    mode = 0.1324,
    max = 0.2274
  )

## Need to cap growth at 30°C

growth_amount <- function(duration, temp, GR_b, GR_T_min) {
  rate_sqrt <- (GR_b * (temp - GR_T_min))
  rate <- ((rate_sqrt > 0) * rate_sqrt ^ 2)
  rate <- (temp > GR_T_min) * rate
  return(rate * duration)
}

MP_value <- function(temp, MPD_a, MPD_T_min, MPD_T_submin) {
  mpd <- (MPD_a * (temp - MPD_T_min)) / ((temp - MPD_T_submin))
  mpd_capped <- (temp > MPD_T_submin) * mpd
  return(round(10 ^ mpd_capped * W.serve))
}

# Alternate growth model for cut versin
growth.const.alt <- function(N.in, 
  duration, 
  temp, 
  W.serve, 
  GR_b, 
  GR_T_min, 
  MPD_a, 
  MPD_T_min, 
  MPD_T_submin) 
{
  change <- 
    round(10 ^ (log10(N.in) + growth_amount(duration, temp, GR_b, GR_T_min)))
  MP_serve <- MP_value(temp, MPD_a, MPD_T_min, MPD_T_submin)

  change[which(N.in > MP_serve)] <- 
    N.in[which(N.in > MP_serve)]
  change[which(N.in < MP_serve && 
    change > MP_serve)] <- 
    MP_serve[which(N.in < MP_serve && change > MP_serve)]

  return(change)
}

growth.step.alt <- function(N.in, 
  duration, 
  T.init, 
  T.change, 
  W.serve, 
  GR_b, 
  GR_T_min, 
  MPD_a, 
  MPD_T_min, 
  MPD_T_submin) {

steps <- 100
delta_temp <- T.change / steps
delta_time <- duration / steps
new_growth <- N.in
growth_change <- mcdata(0, type = "VU")
for (i in 1:steps)
growth_change[,] <-
growth_change[,] + growth_amount(duration = delta_time[, ,],
  temp = T.init[, ,] + i * delta_temp[, ,],
  GR_b[, ,],
  GR_T_min[, ,])

new_growth <- 10 ^ (log10(new_growth) + growth_change)
MP_serve <-
MP_value(T.init + T.change, MPD_a, MPD_T_min, MPD_T_submin)
new_growth <- round(new_growth)
new_growth[which(N.in > MP_serve)] <-
  N.in[which(N.in > MP_serve)]
new_growth[which(N.in < MP_serve &&
  new_growth > MP_serve)] <-
  MP_serve[which(N.in < MP_serve && new_growth > MP_serve)]
return(new_growth)

switch (scenario_infusion,
  "baseline= 0" = {
  },
  "EI= 0.20" = {
  EI.offset = function()
  0.20
})
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```
737  },
738  "EI = 0.15" = {
739    EI.offset = function()
740    0.15
741  },
742  "EI = 0.10" = {
743    EI.offset = function()
744    0.10
745  },
746  "EI = 0.05" = {
747    EI.offset = function()
748    0.05
749  },
750  "EI = -0.20" = {
751    EI.offset = function()
752    -0.20
753  },
754  "EI = -0.15" = {
755    EI.offset = function()
756    -0.15
757  },
758  "EI = -0.10" = {
759    EI.offset = function()
760    -0.10
761  },
762  "EI = -0.05" = {
763    EI.offset = function()
764    -0.05
765  },
766  "EN = 0.20" = {
767    EN.offset = function()
768    0.20
769  },
```
"EN= 0.15" = {
  EN.offset = function()
  0.15
},

"EN= 0.10" = {
  EN.offset = function()
  0.10
},

"EN= 0.05" = {
  EN.offset = function()
  0.05
},

"EB= 0.20" = {
  EB.offset = function()
  0.20
},

"EB= 0.15" = {
  EB.offset = function()
  0.15
},

"EB= 0.10" = {
  EB.offset = function()
  0.10
},

"EB= 0.05" = {
  EB.offset = function()
  0.05
},

"NE= 0.20" = {
  NE.offset = function()
  0.20
},

"NE= 0.15" = {

NE.offset = function()
  0.15
},
"NE= 0.10" = {
  NE.offset = function()
  0.10
},
"NE= 0.05" = {
  NE.offset = function()
  0.05
},
"NE= -0.15" = {
  NE.offset = function()
  - 0.15
},
"NE= -0.1" = {
  NE.offset = function()
  - 0.1
},
"NE= -0.05" = {
  NE.offset = function()
  - 0.05
},
"NI= 0.20" = {
  NI.offset = function()
  0.20
},
"NI= 0.15" = {
  NI.offset = function()
  0.15
},
"NI= 0.1" = {
  NI.offset = function()
0.1
},

"NI= 0.05" = {
   NI.offset = function()
   0.05
},

"NI= -0.15" = {
   NI.offset = function()
   - 0.15
},

"NI= -0.1" = {
   NI.offset = function()
   - 0.1
},

"NI= -0.05" = {
   NI.offset = function()
   - 0.05
},

"NB= 0.20" = {
   NB.offset = function()
   0.20
},

"NB= 0.15" = {
   NB.offset = function()
   0.15
},

"NB= 0.1" = {
   NB.offset = function()
   0.1
},

"NB= 0.05" = {
   NB.offset = function()
   0.05
}
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
869 },
870 "BE= 0.20" = {
871 BE.offset = function()
872 0.20
873 },
874 "BE= 0.15" = {
875 BE.offset = function()
876 0.15
877 },
878 "BE= 0.1" = {
879 BE.offset = function()
880 0.1
881 },
882 "BE= 0.05" = {
883 BE.offset = function()
884 0.05
885 },
886 "BI= -0.20" = {
887 BI.offset = function()
888 - 0.20
889 },
890 "BI= -0.15" = {
891 BI.offset = function()
892 - 0.15
893 },
894 "BI= -0.1" = {
895 BI.offset = function()
896 - 0.1
897 },
898 "BI= -0.05" = {
899 BI.offset = function()
900 - 0.05
901 },
```
"BN= 0.2" = {
    BN.offset = function()
    0.2
},
"BN= 0.15" = {
    BN.offset = function()
    0.15
},
"BN= 0.1" = {
    BN.offset = function()
    0.1
},
"BN= 0.05" = {
    BN.offset = function()
    0.05
},
"Vmax= 25" = {
    V.brine.balane.max.function <- function()
    25
},
"Vmax= 75" = {
    V.brine.balane.max.function <- function()
    75
},
"Vmax= 100" = {
    V.brine.balane.max.function <- function()
    100
}
)

# Scenarios----
switch(
    scenario,
"all.intact" = {
    # Covers the estimate of the salmonella concentration based on prev.
    percentNI.function <- "mcdata"
    percentNI.params <- function()
        list(0,
            type = "0")
},
"all.mi" = {
    # Concentration based on the ecoli concentration data
    percentNI.function <- "mcdata"
    percentNI.params <- function()
        list(1,
            type = "0")
},
"T.cook.ecosure" = {
    T.cook.params <- function(dummy)
        list(
            func = "rempiricalD",
            type = "V",
            lhs = use.lhs,
            values = ecosure.cook.pork
        )
},
"T.cook.pork.burgers" = {
    T.cook.mean.params <- function()
        list(
            func = "rempiricalD",
            type = "V",
            values = int.temps,
            prob = done.weights.pork.burgers
        )
    }
}
Listing D.11: The R code contained in the file ‘mc2d.cut - MI.R’. Contains the R commands that define the stochastic model as a function.

```r
# Model without using the cut algorithm in mc2d.

# Load required functions

model_infusion <- mcmodelcut({

  # Initial Contamination for intact pork
  P.shoulder <- do.call(mcstoc, P.shoulder.params())
  P.i <- P.shoulder
  C.loin.mean <- do.call(mcdata, C.loin.mean.params())
  C.loin.sd <- do.call(mcdata, C.loin.sd.params())

  W.loin <- do.call(W.loin.func, W.loin.params())
  D.loin <- do.call(D.loin.func, D.loin.params())
  L.loin <- do.call(L.loin.func, L.loin.params())
  R.loin <- do.call(R.loin.func, list(W.loin, L.loin, D.loin))
  SA.loin <- do.call(SA.loin.func, list(R.loin, L.loin))

  # Proportion of weight of loin weight increased by addition of brine
  prop.inf <- do.call(prop.inf.func, prop.inf.params())

  # Weight to volume ratio
  W.V.ratio <- 1

  # Volume of brine injected into each loin
  V.inj <- W.loin * prop.inf * W.V.ratio

})
```
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
# Set the minimum and maximum values for the balance tank volume
V.brine.balance.max <-
V.brine.balance.max.function() # L (based on geoff's guess)
V.brine.balance.min <-
1 # L (guess, there would be a minimum value at which the tank is refilled
otherwise it wouldn't work)
EI <- do.call(EI.function, EI.params()) + EI.offset()
EN <- do.call(EN.function, EN.params()) + EN.offset()
EB <- do.call(EB.function, EB.params()) + EB.offset()

IE <-
do.call(IE.function, IE.params()) # Based on no internal contamination initially
IN <-
do.call(IN.function, IN.params()) # Based on no internal contamination initially
IB <-
do.call(IB.function, IB.params()) # Based on no internal contamination initially

NE <-
do.call(NE.function, NE.params()) / NE.fraction() + NE.offset() # Not zero but
very small
NI <-
do.call(NI.function, NI.params()) / NI.fraction() + NI.offset() # Unknown, linked
to EN
NB <-
do.call(NB.function, NB.params()) + NB.offset() # 'very low'

BE <-
do.call(BE.function, BE.params()) + BE.offset() # 'very low'
BI <- do.call(BI.function, BI.params()) + BI.offset()
BN <-
do.call(BN.function, BN.params()) + BN.offset()# 'very low'

# External, internal, needles, brine. From is rows and to is columns
```
TR <- mcdata(
  data = ,
  c((1 - EI) * (1 - EN) * (1 - EB),
     EI,
     (1 - EI) * EN,
     (1 - EI) * (1 - EN) * EB,
     (1 - IN) * IE,
     (1 - IN) * (1 - IE) * (1 - IB),
     IN,
     (1 - IN) * (1 - IE) * IB,
     NE,
     (1 - NE) * NI,
     (1 - NE) * (1 - NI) * (1 - NB),
     (1 - NE) * (1 - NI) * NB,
     (1 - NI) * (1 - NB) * BE,
     BI,
     (1 - BI) * BN,
     (1 - BI) * (1 - BN) * (1 - BE)
 ),
  type = "U",
  nvariants = 16
)

#Test the transfers are valid
test_transfers(EI, EN, EB, IE, IN, IB, NE, NI, NB, BE, BI, BN, TR)

# Begin process
# How much brine is required in the brine tank? Assuming that the machine requires
# at least 1L of brine at the end
V.brine.total <- sum(V.inj)
#V_brine_primary_max <- 10
V.brine.balance <- V.brine.balance.max
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

V.brine.main <- V.brine.total - V.brine.balance

C.loin <- do.call(mcstoc, C.loin.params(C.loin.mean, C.loin.sd))

P.loin <-
do.call(P.serve.function,
    P.serve.params(P.shoulder, 100, SA.loin))

Z.loin <- do.call(Z.function, Z.params(P.loin))

N.loin.cont <-
do.call(N.loin.cont.func, N.loin.cont.params(C.loin, SA.loin))

N.loin <-
do.call(N.loin.func, N.loin.params(N.loin.cont, Z.loin)) # MP.serve is absurdly large on purpose.

# Population of salmonella in brine tank and on the needles
N.brine <- mcdata(0, type = "VU")
N.needles <- mcdata(0, type = "VU")

# To define the dimensions of the N.inj matrix
N.inj <- N.brine * 0

# N.trans represents the transfers of salmonella from the exterior to the other positions.
N.trans <- array(0, c(ndvar(), 1, 4))
N.trans[, , 1] <- N.loin[, ,]

for (v in 1:ndvar())
{
}
if (V.brine.balance < V.brine.balance.min) {

    # How much brine needs to be removed from the main tank to replenish the balance tank

    V.brine.main <-
        V.brine.main - (V.brine.balance.max - V.brine.balance)

    # Refill the balance tank.

    V.brine.balance <-
        V.brine.balance + (V.brine.balance.max - V.brine.balance)

} # Number of Salmonella in brine to be injected See Nauta 2005 eqn 1

N.inj[v, ,] <-
    vapply(N.brine[v, ,], function(x) {
        rbinom(1, x, V.inj[v, ,] / V.brine.balance)
    }, 1)

    # Add n.inj salmonella to the transfer vector

N.trans[v, , 4] <- N.inj[v, ,]

    # There is an issue here with using round?

N.trans[v, ,] <-
    round(N.trans[v, ,] %*% matrix(
        as.vector(TR[, ,]),
        nrow = 4,
        ncol = 4,
        byrow = TRUE
    ))

    # Remove the used brine from the brine tank

V.brine.balance <- V.brine.balance - V.inj[v, ,]

    # Remove the N.inj salmonella from the brine tank

N.brine[v, ,] <- N.brine[v, ,] - N.inj[v, ,]

    # Add in to the brine tank the returning salmonella

N.brine[v, ,] <- N.brine[v, ,] + N.trans[v, , 4]
if (v != ndvar()) {

  # Carry forward the contamination on the needles
  N.trans[v + 1, , 3] <- N.trans[v, , 3]

  # Carry forward the contamination in the balance tank
  N.brine[v + 1, , ] <- N.brine[v, , ]
}

internal <- mcdata(N.trans[, , 1], type = "VU")
external <- mcdata(N.trans[, , 2], type = "VU")
combined <- internal + external

# What is the surface area of the slice taken
W.serve <- mcdata(0.1, type = "0")
T.serve <- W.serve / (R.loin ^ 2 * D.loin * pi)

SA.serve <- 2 * pi * R.loin * T.serve

# Determine the prevalence on intact steaks
p.cont.i <- 1 - (1 - SA.serve / SA.loin) ^ N.loin
P.i.serve <- mcdata(as.numeric(colMeans(p.cont.i)), type = "U")

p.cont.mi.ext <- (1 - (1 - SA.serve / SA.loin) ^ external)
p.cont.mi.int <-
  (1 - (1 - W.serve / (W.loin * 1000)) ^ internal)
p.cont.mi <-
  p.cont.mi.ext + p.cont.mi.int - p.cont.mi.ext * p.cont.mi.int
P.mi.serve <-
  mcdata(as.numeric(colMeans(p.cont.mi)), type = "U")

per.gram.i <- N.loin / (W.loin * 1000)
per.gram.mi <- combined / (W.loin * 1000)
res <- mc(
  P.shoulder,
  C.loin,
  W.loin,
  L.loin,
  SA.loin,
  prop.inf,
  EI,
  EN,
  EB,
  NE,
  NI,
  NB,
  BE,
  BI,
  BN,
  N.ext = mcdata(N.trans[, , 1], type = "VU"),
  N.int = mcdata(N.trans[, , 2], type = "VU"),
  N.needle.post = mcdata(N.trans[, , 3], type = "VU"),
  N.brine.inj = mcdata(N.trans[, , 4], type = "VU"),
  N.brine.tank = N.brine,
  p.cont.mi.ext,
  p.cont.mi.int,
  P.i.serve,
  P.mi.serve,
  external,
  internal,
  combined,
  per.gram.mi
)
if (scenario == "baseline") {

D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
list(
    sum = summary(res),
    P.i.serve = P.i.serve,
    P.mi.serve = P.mi.serve,
    per.gram.i = per.gram.i,
    per.gram.mi = per.gram.mi,

    tornado = tornado(res, use = "pairwise.complete.obs")
)
)
} else {
    list(sum = summary(res),
         P.i.serve = P.i.serve,
         P.mi.serve = P.mi.serve,
         per.gram.i = per.gram.i,
         per.gram.mi = per.gram.mi)
}
)
)

post_infusion_ops <- function()
{
    results_infusion[[scenario_infusion]]$P.i.unc.fit <-
    fitdist(
        as.numeric(results_infusion[[scenario_infusion]]$P.i.serve),
        "beta",
        start = list(shape1 = 2.5, shape2 = 20),
        optim.method = "BFGS",
        control = list(ndeps = c(1E-9, 1E-9))
    )
    results_infusion[[scenario_infusion]]$P.mi.unc.fit <-
    fitdist(
        as.numeric(results_infusion[[scenario_infusion]]$P.mi.serve),
        "beta",
    )
```

329
start = list(shape1 = 2.5, shape2 = 10),
optim.method = "BFGS",
control = list(ndeps = c(1E-9, 1E-9))
)

results_infusion[[scenario_infusion]]$C.i.mean.boots <-
mcdata(apply(results_infusion[[scenario_infusion]]$per.gram.i[, ,], 2, function(x)
  mean(log10(x[which(x > 0)]))),
type = "U")
results_infusion[[scenario_infusion]]$C.i.sd.boots <-
mcdata(apply(results_infusion[[scenario_infusion]]$per.gram.i[, ,], 2, function(x)
  sd(log10(x[which(x > 0)]))),
type = "U")
results_infusion[[scenario_infusion]]$C.mi.mean.boots <-
mcdata(apply(results_infusion[[scenario_infusion]]$per.gram.mi[, ,], 2, function(x)
  mean(log10(x[which(x > 0)]))),
type = "U")
results_infusion[[scenario_infusion]]$C.mi.sd.boots <-
mcdata(apply(results_infusion[[scenario_infusion]]$per.gram.mi[, ,], 2, function(x)
  sd(log10(x[which(x > 0)]))),
type = "U")
}

modEC3 <- mcmodelcut{
  # Import previous numbers
  P.i.shape1 <-
  results_infusion$baseline$P.i.unc.fit$estimate["shape1"]
P.i.shape2 <-
  results_infusion$baseline$P.i.unc.fit$estimate["shape2"]
P.mi.shape1 <-
  results_infusion$baseline$P.mi.unc.fit$estimate["shape1"]
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```
P.mi.shape2 <- results_infusion$baseline$P.mi.unc.fit$estimate["shape2"]
C.i.mean.boots <- as.numeric(results_infusion$baseline$C.i.mean.boots)
C.i.sd.boots <- as.numeric(results_infusion$baseline$C.i.sd.boots)
C.mi.mean.boots <- as.numeric(results_infusion$baseline$C.mi.mean.boots)
C.mi.sd.boots <- as.numeric(results_infusion$baseline$C.mi.sd.boots)

# Define growth model parameters ----
GR_b <- do.call(mcdata, GR_b.params())
GR_T_min <- do.call(mcdata, GR_T_min.params())
MPD_a <- do.call(mcdata, MPD_a.params())
MPD_T_min <- do.call(mcdata, MPD_T_min.params())
MPD_T_submin <- do.call(mcdata, MPD_T_submin.params())

# Initial conditions for moisture infused pork
P.i <- do.call(mcstoc, P.i.params(P.i.shape1, P.i.shape2))
P.mi <- do.call(mcstoc, P.mi.params(P.mi.shape1, P.mi.shape2))

C.i.index <- sample(seq(from = 1, to = length(C.i.mean.boots)),
                      size = ndunc(),
                      replace = TRUE)
C.i.mean <- do.call(C.i.mean.func, C.i.mean.params(C.i.mean.boots[C.i.index]))
C.i.sd <- do.call(C.i.sd.func, C.i.sd.params(C.i.sd.boots[C.i.index]))
```

331
sample(seq(from = 1, to = length(C.mi.mean.boots)),
        size = ndunc(),
        replace = TRUE)

C.mi.mean <-
do.call(C.mi.mean.func, C.mi.mean.params(C.mi.mean.boots[C.mi.index]))

C.mi.sd <-
do.call(C.mi.sd.func, C.mi.sd.params(C.mi.sd.boots[C.mi.index]))

# Proportion that is MI

percentMI <-
do.call(percentMI.function, percentMI.params())

P.all <- P.all.func(P.mi, P.i, percentMI)

t.r.rate <- do.call(mcstoc, t.r.rate.params())

T.r.mean <- do.call(mcdata, T.r.mean.params())

T.r.sd <- do.call(mcdata, T.r.sd.params())

T.r.xi <- do.call(mcdata, T.r.xi.params())

# Consumer Transport----

t.ct.shape <- do.call(mcdata, t.ct.shape.params())

t.ct.rate <- do.call(mcdata, t.ct.rate.params())

T.change.ct.shape <-
do.call(mcdata, T.change.ct.shape.params())

T.change.ct.rate <-
do.call(mcdata, T.change.ct.rate.params())

# Domestic Refrigeration----

t.ds.rate <- do.call(mcdata, t.ds.rate.params())

T.ds.mean <- do.call(mcdata, T.ds.mean.params())

T.ds.sd <- do.call(mcdata, T.ds.sd.params())

# Inactivation----

T.cook.mean <- do.call(mcstoc, T.cook.mean.params())

T.cook <- do.call(mcstoc, T.cook.params(T.cook.mean))
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
# Illness----
P.ill.alpha <- do.call(mcstoc, P.ill.alpha.params())
P.ill.beta <- do.call(mcstoc, P.ill.beta.params())
}
{
    # Determine the number of salmonellae per serving given contamination
    C.i <- do.call(mcstoc, C.i.params(C.i.mean, C.i.sd))
    C.mi <- do.call(mcstoc, C.mi.params(C.mi.mean, C.mi.sd))
    N.i <- do.call(N.serve.func, N.serve.params(C.i))
    N.mi <- do.call(N.serve.func, N.serve.params(C.mi))
    is.mi <- do.call(is.mi.func, is.mi.params(percentMI))
    N.all <- ifelse(unmc(is.mi), N.mi, N.i) %>% mcdata(type = "VU")

    # Retail
    t.r <- do.call(mcstoc, t.r.params(t.r.rate))
    T.r <-
    do.call(mcstoc, T.r.params(T.r.mean, T.r.sd, T.r.xi))
    N.r <- do.call(
        N.r.growth.model,
        N.r.params(
            N.all = N.all,
            t.r = t.r,
            T.r = T.r,
            W.serve = W.serve,
            GR_b = GR_b,
            GR_T_min = GR_T_min,
            MPD_a = MPD_a,
            MPD_T_min = MPD_T_min,
            MPD_T_submin = MPD_T_submin
        )
    )
```


```r
# Consumer Transport----
t.ct <-
  do.call(mcstoc, t.ct.params(t.ct.shape, t.ct.rate))
T.change.ct <-
  do.call(mcstoc,
        T.change.ct.params(T.change.ct.shape, T.change.ct.rate))
ct.cor.nodes <-
cornode(t.ct, T.change.ct, target = corr.ct) # Include correlation between nodes.
t.ct <- ct.cor.nodes$t.ct
T.change.ct <- ct.cor.nodes$T.change.ct
N.ct <- do.call(
  N.ct.growth.model,
  N.ct.params(
    N.r,
    t.ct,
    T.r,
    T.change.ct,
    W.serve,
    GR_b,
    GR_T_min,
    MPD_a,
    MPD_T_min,
    MPD_T_submin
  )
)

# Domestic Refrigeration----
t.ds <- do.call(mcstoc, t.ds.params(t.ds.rate)) * 24
T.ds <- do.call(mcstoc, T.ds.params(T.ds.mean, T.ds.sd))
N.ds <- do.call(
```
N.ds.growth.model,
N.ds.params(
  N.ct,
  t.ds,
  T.ds,
  W.serve,
  GR_b,
  GR_T_min,
  MPD_a,
  MPD_T_min,
  MPD_T_submin
)

# Inactivation----
cook.red <- mcdata(inact.redn(T.cook, is.mi), type = "VU")
N.cook <- 10 ^ (log10(N.ds) - cook.red)

# Illness----
P.ill.given.cont <-
b.p.dose.resp(N.cook, alpha = P.ill.alpha, beta = P.ill.beta)
P.ill <- P.ill.given.cont * P.all
res <- mc(
  GR_b,
  GR_T_min,
  MPD_a,
  MPD_T_min,
  MPD_T_submin,
  P.i,
  P.mi,
  C.i,
  C.mi)
percentMI, N.i, N.mi, P.all, N.all, t.r, T.r, N.r, 
log.N.r = log10(N.r), 
log.N.r.diff = log10(N.r) - log10(N.all), t.ct, T.change.ct, N.ct, 
log.N.ct = log10(N.ct), 
log.N.ct.diff = log10(N.ct) - log10(N.r), t.ds, T.ds, N.ds, 
log.N.ds = log10(N.ds), 
log.N.ds.diff = log10(N.ds) - log10(N.ct), T.cook, N.cook, P.ill.alpha, P.ill.beta, P.ill.given.cont, P.ill 
) 
} 
{
  if (scenario == "baseline") {
    list(
      sum = summary(
        res,
```r
lim = c(0.025, 0.975),
prob = c(
  0.025,
  0.25,
  0.5,
  0.75,
  0.95,
  0.975,
  0.99,
  0.999,
  0.9999,
  0.99999
)

else {
  list(sum = summary(
    res,
    lim = c(0.025, 0.975),
    prob = c(
      0.025,
      0.25,
      0.5,
      0.75,
      0.95,
      0.975,
      0.99,
      0.999,
      0.9999,
      0.99999
    )
  )))
}
tornado = tornado(res, use = "pairwise.complete.obs")
```
process <- as.numeric(commandArgs(trailingOnly = TRUE)[[1]])
cores <- 6

#sink(paste("output",process,".txt"))

if (process == 1)
  file.create("2D results/wait")

source("model.functions.R")
library(filehash)
library(compiler)

compilePKGS(enable = TRUE)
enableJIT(3)
setCompilerOptions(suppressUndefined = TRUE)

ndvar(5E3)
ndunc(2.5E3)
# ndvar(5E2);ndunc(1E2) # Testing values

use.lhs <- TRUE
# Should latin hypercube sampling be used in the simulation?
results_infusion <- dbInit("2D results/results_infusion", type = "RDS")

Listing D.12: The R code contained in the file ‘mc2d.runorder - MI.R’. Contains the R commands that are used to evaluate the stochastic model defined in ‘mc2d.cut - MI.R’ for all the alternative scenarios.
if (!exists(as.character(substitute(process))) || process == 1) {
  results_infusion$nsv <- ndvar()
  results_infusion$nsu <- ndunc()
}

source("infusion.boots.R")

source("mc2d.tests - MI.R")

source("mc2d.cut - MI.R")

scenario <- "baseline"

scenarios_infusion <- c(
  "baseline= 0",
  "EI=  0.20",
  "EI=  0.15",
  "EI=  0.10",
  "EI=  0.05",
  "EI= -0.20",
  "EI= -0.15",
  "EI= -0.10",
  "EI= -0.05",
  "EN=  0.20",
  "EN=  0.15",
  "EN=  0.10",
  "EN=  0.05",
  "EB=  0.20",
  "EB=  0.15",
  "EB=  0.10",
  "EB=  0.05",
  "NE=  0.20",
  "NE=  0.15",
  "NE=  0.10",
)
if (process == 1) {
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
scenario_infusion <- "baseline"

scenario_infusion <- "baseline"
cat("Scenario Infusion: ", scenario_infusion, "\n")
cat("Scenario Infusion: ", scenario_infusion, "\n")

seed <- 314

set.seed(seed)

source("mc2d.params - MI.R")

seed <- 314

set.seed(seed)

source("mc2d.params - MI.R")

dbInsert(results_infusion, scenario_infusion, NULL)

evalmccut(model_infusion, 
  nsvar = ndvar(),
  nsu = ndunc(),
  seed = NULL) %>% dbInsert(results_infusion, scenario_infusion, .)

evalmccut(model_infusion, 
  nsvar = ndvar(),
  nsu = ndunc(),
  seed = NULL) %>% dbInsert(results_infusion, scenario_infusion, .)

post_infusion_ops()

cat("Scenario Infusion: ", scenario_infusion, "\n")
cat("Scenario Infusion: ", scenario_infusion, "\n")

seed <- 314

set.seed(seed)

source("mc2d.params - MI.R")

dbInsert(results_infusion, scenario_infusion, NULL)

evalmccut(model_infusion, 
  nsvar = ndvar(),
  nsu = ndunc(),
  seed = NULL) %>% dbInsert(results_infusion, scenario_infusion, .)

for (i in 1:length(scenarios_infusion)) {
  if (((i - 1) %% (cores - 1)) + 1 == (process - 1)) {
    cat("i = ", i, "\n")
    scenario_infusion <- scenarios_infusion[i]
    cat("Scenario Infusion: ", scenario_infusion, "\n")
    cat("Scenario Infusion: ", scenario_infusion, "\n")
    seed <- 314
    set.seed(seed)

    source("mc2d.params - MI.R")

    dbInsert(results_infusion, scenario_infusion, NULL)

    evalmccut(model_infusion, 
  nsvar = ndvar(),
  nsu = ndunc(),
  seed = NULL) %>% dbInsert(results_infusion, scenario_infusion, .)

    file.remove("2D results/wait")
  }
}
```
nsv = ndvar(),
nsu = ndunc(),
seed = NULL
)
post_infusion_ops()
}
}

# Supply Chain Module
seed <- 314
set.seed(seed)
ndvar(5E4)
ndunc(5E4)
# ndvar(1E3); ndunc(5E2)

scenario_infusion <- "baseline"
source("mc2d.boots - MI.R")

# while (file.exists("2D results/wait")) {
# print("Waiting for core 1")
# Sys.sleep(60)
# }

results <- dbInit("2D results/results", type = "RDS")

if (process == 1) {

    # We only need to write the simulation dimensions once
    results$nsv <- ndvar()
D. R Code for Moisture Infusion Risk Assessment Model (Chapter 6)

```r
results$nsu <- ndunc()

scenario <- "baseline"

cat("------------------------------\n")
cat("Scenario =", scenario, "\n")
cat("------------------------------\n")

source("mc2d.params - MI.R")

dbInsert(results, scenario, NULL)
evalmccut(modEC3, nsv = ndvar(), nsu = ndunc()) %>% dbInsert(results, scenario, .)
gc()
}
else {

scenarios <-
c("all.intact",
  "all.mi",
  "T.cook.ecosure",
  "T.cook.pork.burgers")

for (i in 1:length(scenarios)) {
  if (((i - 1) %% (cores - 1)) + 1 == (process - 1)) {
    scenario <- scenarios[i]
    cat("------------------------------\n")
cat("Scenario =", scenario, "\n")
cat("------------------------------\n")
    seed <- 314
    set.seed(seed)
    source("mc2d.params - MI.R")

    dbInsert(results, scenario, NULL)
evalmccut(modEC3, nsv = ndvar(), nsu = ndunc()) %>% dbInsert(results, scenario, .)
```

343
Listing D.13: The batch file named ‘batch_run.bat’ starts the various R processes that evaluate the model. This script allows the various scenarios to be evaluated to be distributed over the available computer cores. The number of cores can be adjusted for each computer.

```batch
for /l %%x in (1, 1, 6) do ( 
  START "" Rscript "mc2d.runorder - MI.R" %%x
)
```
E. Published Papers

Chapter 3, as published in the *International Journal of Food Microbiology* is included below.


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