AUSTRALIAN **EGG**
CORPORATION LIMITED

An Egg:*Salmonella* Quantitative Risk Assessment Model

for the Australian Egg Industry

A report for the Australian Egg Corporation Limited

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A Salmonella Quantitative Risk Assessment Model for the Australian Egg Industry
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In submitting this report, the researcher has agreed to AECL publishing this material in its edited form.

Foreword

This project aimed to conduct a quantitative risk assessment of *Salmonella* contamination of eggs in Australia. A key outcome was the development of a tool to predict how changes in industry practices may impact on the occurrence of Salmonellosis in humans. The risk assessment process provides a scientifically objective process for identifying risk and appropriate options for control. The approach is increasingly used to develop public health standards and as a basis for international market access.

The report provides a comprehensive review of food-borne outbreaks in which eggs along with other ingredients were implicated (Hazard Identification). These outbreaks were evaluated in terms of the level of certainty of attribution to eggs. The occurrence and serovar of *Salmonella* contaminants from layer flocks, unpasteurised and pasteurised egg products were compared with serovars implicated in outbreaks potentially attributable to eggs. The source of eggs, type of foods, settings of outbreaks and populations exposed were evaluated to define the circumstances in which eggs may be the source of contamination. Such information serves as a guide for the implementation of appropriate controls from farm to consumption.

To gain an estimate of the level of egg contamination (Exposure Assessment), a pilot survey of *Salmonella* prevalence on ungraded (off-farm), graded/washed and contents of graded eggs produced in cages was conducted. In addition, the external contamination of barn laid and free-range eggs was established. This data was used to develop a modular exposure assessment model for each stage of production from point of lay, collection, storage, transportation and processing to the end of retail storage. A survey of egg production and processing practices in Australia was used to obtain necessary data representative of the Australian layer industry practices. The modular approach to design of the Exposure Assessment was similar to previously published Exposure Assessments (FAO-WHO, 2002 and USDA-FSIS, 1998).

Risk of Salmonellosis to consumers was estimated for foods prepared with three different cooking effects and is expressed in terms of probability of illness per million servings under Australian conditions to model options for reduction of consumer risk.

An exposure assessment and risk characterisation for egg products was also undertaken.

This project was funded from industry revenue which is matched by funds provided by the Federal Government.

This report is an addition to AECL's range of research publications and forms part of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

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Printed copies can be purchased by faxing or emailing the downloadable order form from the web site or by phoning (02) 9409 6999.

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Abbreviations

AECL	Australian Egg Corporation Limited
AQIS	Australian Quarantine Inspection Service
AVA	Australian Veterinary Association
CAC	Codex Alimentarius Commission
CCP	Critical Control Point
CDNA	Communicable Diseases Network - Australia
CFU	Colony Forming Unit
CI	Confidence Interval
DHS (Vic)	Department of Human Services (Victoria)
EA	Exposure Assessment
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organisation (United Nations)
FSANZ	Food Standards Australia New Zealand (formerly ANZFA)
FSIS	Food Safety and Inspection Service (USA)
HACCP	Hazard Analysis and Critical Control Point
IMVS	Institute of Medical and Veterinary Science
MDU	Microbiological Diagnostic Unit
MRA	Microbiological Risk Assessment
NEPSS	National Enteric Pathogen Surveillance Scheme
NNDSS	National Notifiable Diseases Surveillance System
PHLS	Public Health Laboratory Service (UK)
PT	Phage Type
QRA	Quantitative Risk Assessment
R&D	Research and Development
RDNC	'Reacts Does Not Conform'
RIRDC	Rural Industries Research and Development Corporation
SARDI	South Australian Research and Development Institute
SE	*Salmonella enterica* serotype Enteritidis
ST	*Salmonella enterica* serotype Typhimurium
USDA	United States Department of Agriculture
WHO	World Health Organisation
WTO	World Trade Organisation
YMT	Yolk Mean Time

Executive Summary

Introduction

Egg products contaminated with *Salmonella* are a recognised cause of food-borne illness. The Australian egg industry is untroubled by the strains of *Salmonella* Enteritidis that have caused human illness abroad. Nevertheless, Australian layer flocks can be infected with other types of *Salmonella* (also found abroad) and from time to time outbreaks of Salmonellosis in the Australian community are attributed to foods containing Australian egg products. However, the extent and nature of circumstances in which eggs produced in Australia are the cause of food-borne illness have not been comprehensively assessed.

The work reported here therefore aimed to describe the risk to human health from the occurrence of *Salmonella* in the Australian commercial egg industry. A second aim was to develop a credible basis for scientifically quantifying the above risks. The resulting quantitative risk assessment model may have a future role in international trade by allowing assessment of 'equivalence' of risk between Australian eggs and those produced by other countries where layer flocks are infected with SE. The approach taken to achieve these aims was based on the risk assessment process promulgated by the Codex Alimentarius Commission, FAO and WHO.

Hazard Identification

The hazard identification section aimed to identify the types of egg-foods and *Salmonella* that have been linked to human Salmonellosis in Australia. This involved assessing the source of eggs used to produce foods incriminated in outbreaks, the microbiological classification of *Salmonella* found in such foods (species, phage type etc) and any other evidence on the role of the particular *Salmonella* hazard or food type, so far as it contributes to human Salmonellosis. Methods consisted of reviewing data from public health, food regulation and veterinary activities and by considering the likely impact of practices occurring during egg production, egg marketing, egg processing, food preparation and consumption.

From this process it was evident that few if any outbreaks of food-borne Salmonellosis in Australia can be unequivocally attributed to the ingestion of eggs that are produced under an authoritative quality control system, graded and retailed commercially. Conversely, there is evidence pointing to the use of non-commercially produced (ungraded) eggs and cracked/seconds eggs sold direct off-farm as the cause of some outbreaks of Salmonellosis. The evidence is sufficient to recommend avoiding the use of the latter type of eggs in high-risk circumstances and to advocate that during future outbreaks of food-borne Salmonellosis authorities acquire information on the source of eggs present in any incriminated foods.

Consumption of food containing raw or undercooked egg was identified as an important factor contributing to egg-related outbreaks of Salmonellosis. Such foods often included egg-milk drinks, mayonnaise and egg-based desserts. These foods are often subjected to temperature abuse and potentially can also acquire *Salmonella* from cross-contamination during preparation, storage and handling. Unfortunately, these foods are repeatedly associated with outbreaks in vulnerable populations (health care, aged care and infant care settings). Greater use of pasteurised egg pulp in meals prepared by commercial caterers and institutions caring for vulnerable individuals could be used to reduce this risk.

Data from veterinary surveillance shows that *Salmonella* Typhimurium is uncommon in the environment of Australian layer flocks although it is the most common serotype isolated from egg products implicated in outbreaks of human Salmonellosis. In general, the *Salmonella* serovars obtained from poultry and their environment are not the same as those obtained in investigations of food-borne illness in humans, including those where egg products are incriminated as the vehicle of

infection. However, this serovar is isolated from non-commercial farms and backyard operations where there is likely to be less emphasis on maintaining layer-feed free of *Salmonella*.

Hazard Characterisation

A quantitative relationship between dose of ingested *Salmonella* and probability of illness (dose response relationship) was derived from outbreak data published by FAO-WHO (2002). These data were re-analysed to derive a new dose-response model that enhanced earlier attempts to model dose response (eg USDA-FSIS, 1988; FAO-WHO, 2002). The new model accounts for uncertainties in dose and attack rates for the source data.

The enhanced dose-response (D2TAP) model, developed in this section, has an important role in predicting the proportion of exposed individuals who develop disease. Furthermore, it is integrally associated with the exposure assessment model.

Exposure Assessment

The goal of exposure assessment is to describe the number of *Salmonella* that consumers are exposed to from contaminated food and the frequency of exposure to such food. Exposure was quantified using a mathematical model constructed to reflect the process of on-farm production, egg processing, egg distribution, egg retailing and food preparation. Simulation techniques were used to predict the level and frequency of exposure. The outputs of the simulation model are dependent on a range of 'input variables' describing egg production and the behavior of *Salmonella* in that system. These inputs include estimates of prevalence of *Salmonella* in eggs in Australia, the conditions under which eggs are handled (eg temperature) and the impact of any control measures present in the production process.

Information about the prevalence of *Salmonella* in eggs was estimated on the basis of a pilot, purpose designed Australian study and from other published experimental data. Descriptions of Australian egg production and handling practices were obtained by survey of egg producers across Eastern and Southern Australia. A total of 73 producers covering 4.2 million birds were included in the survey including producers using alternative production systems (eg barn and free range). An important finding was that the majority of producers hold eggs on-farm for less than four days and at temperatures below 20°C. Only a small proportion of producers reported using refrigeration[1] for eggs stored on-farm. An additional survey of egg grading and processing facilities was conducted to gather information on shell egg and egg product processing. A total of nine processors across all states were included in this survey. A key finding was that most processors meet or exceed the FSANZ temperature and time guidelines for egg pasteurisation.

Risk Characterisation

Information in the previous sections was combined in the risk assessment model to study the probability of illness in consumers of egg products. Risk estimates for *Salmonella* were predicted for each of these foods. The consumer risk is expressed as illnesses per million servings.

The results indicated that the risk of illness is low, provided commercially produced and graded eggs are used. However, our model indicated that inappropriate storage during wholesale and retail holding could substantially increase risk. On-farm mitigations (eg on-farm storage time, refrigeration and egg collection frequency) will extend the time before growth of *Salmonella* in eggs but the small benefit is negated by not maintaining refrigeration during wholesale and retail storage. Therefore, the 37 day shelf-life[2] may result in a potential risk to consumers if eggs are stored at 20ºC.

The completed exposure assessment model was also used to assess the FSANZ requirements for processing of egg products. Results revealed that the current pasteurisation requirements for whole egg

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[1] Refrigeration is defined as maintenance of eggs at or below 7°C.

[2] Estimate based on survey of producers (Appendix 3), communication with egg producers (pers. comm. to A. Pointon) and 'Best before' use by dates on Julian coded stamped eggs.

pulp are satisfactory. By contrast, the processing requirements for yolk and albumen may not be sufficient to ensure elimination of all *Salmonella* from these products. Our analysis indicated that if eggs are to be held for extended times before processing, they should only be processed for whole egg pulp. The standards for the pasteurisation of yolk and albumen should be re-evaluated.

Summary of Recommendations

Introduction of new mitigation strategies into commercial flocks producing graded eggs is unlikely to reduce the risk of human Salmonellosis.

Food standards should be reviewed to minimise the use of cracked and ungraded eggs in products likely to be provided to individuals susceptible to food-borne Salmonellosis.

Refrigeration of eggs after processing and during wholesale and retail storage could substantially reduce the risk of human Salmonellosis. This option exerts the greatest impact on risk reduction compared to other strategies.

General improvement to hygiene and food storage practices in catering operations is required. Special attention to the prevention of cross-contamination and temperature abuse of egg products is needed.

FSANZ standards for pasteurisation of egg products should be re-evaluated

1. Introduction

1.1 Terms of Reference and Risk Assessment

The development of a Quantitative Risk Assessment (QRA) model for *Salmonella* in eggs would provide industry with:

- A predictive tool to assess the impact of changes in industry practices on the occurrence of Salmonellosis in humans, and deliver a method for optimisation of the benefits from HACCP.
- A tool to define (Critical) Control Points and Critical Limits through-chain as production systems change and processing technologies evolve.
- A tool to assist nation-wide harmonisation of Quality Assurance systems.
- A risk-based tool for prioritising food safety R&D for *Salmonella* in the egg industry.
- A model to evaluate the effects of alternative control measures and production systems on food safety risk.
- A risk-based tool for review of standards for microbiological monitoring of eggs.

The ability to draw on outputs from an Australian QRA model for *Salmonella* contamination of eggs would provide industry with an international food safety benchmark process needed to meet public health and international trade obligations. This project addresses food safety in the AECL R&D priorities.

During the decade previous to 2003, Risk Analysis (a process consisting of risk assessment, risk management and risk communication) emerged as a structured model for improvement of food control systems. The objectives of this process are production of safer food, reduction in the numbers of food-borne illnesses and facilitation of domestic and international trade in food. Risk analysis has led to a more holistic approach to food safety, where the entire food chain is considered in efforts to produce safer food.

Microbiological risk assessment is a tool that can be used in the management of risks posed by food-borne pathogens and the elaboration of standards for food in international trade. However, undertaking a microbiological risk assessment (MRA), particularly quantitative MRA, is recognised as a resource-intensive task that requires a multidisciplinary approach. The work described in this report has been greatly facilitated by the contribution of people with expertise in industry, microbiology, mathematical modelling, epidemiology, veterinary public health and food technology.

The public health risk posed by food products contaminated with bacterial pathogens has fuelled need to better estimate the potential impact in terms of both safety and quality-related issues. During 1983 to 2003, predictive microbiology mathematical modelling techniques have been used to describe growth, survival and inactivation of pathogenic bacteria. Buchanan and Whiting proposed that these models could be integrated with dose-response models to develop a unit operations approach for conducting risk assessments (Buchanan & Whiting, 1996; Whiting, 1995). The value of this approach is that quantitative analyses of the hazards associated with a food can be developed. Furthermore, by assessing changes in the numbers of food-borne pathogens during processing operations, manufacturers can assemble risk assessment protocols and robust scientific evidence for the stringency required for HACCP programs. These models provide an adaptable framework for a *Salmonella* risk assessment for the Australian egg industry. The potential advantages of this approach are:

- Increased public confidence;
- Increased export opportunity;
- Increased importation barrier rationale;
- A capacity to prevent importation of products not meeting Australian safety standards;
- Identification of food safety R&D priorities.

Risk Assessment

The Codex Alimentarius Commission (1999) has the Draft Principles and Guidelines for the Conduct of Microbiological Risk Assessment that applies to risk assessment of microbiological hazards in food. Risk assessment, along with risk management and risk communication, forms the process of risk analysis. Risk assessment itself comprises the four steps:

1. Hazard identification,
2. Hazard characterisation,
3. Exposure assessment, and
4. Risk characterisation.

The approach used for this project followed a risk assessment framework.

Hazard identification – The identification of biological, chemical and physical agents capable of causing adverse health effects and which may be present in a particular food or group of foods.

Hazard characterisation – The qualitative and/or quantitative evaluation of the nature of the adverse effects associated with biological, chemical and physical agents which may be present in food. A dose response assessment may be undertaken if appropriate data is available and it is necessary to address the questions asked by risk managers.

Exposure assessment – The qualitative and/or quantitative evaluation of the likely intake of biological, chemical and physical agents via food as well as exposures from other sources if relevant.

Risk characterisation – The qualitative and/or quantitative estimation, including attendant uncertainties, of the probability of occurrence and severity of known or potential adverse health effects in a given population based on hazard identification, hazard characterisation and exposure assessment.

Figure 1.1: Schematic representation of the risk assessment process
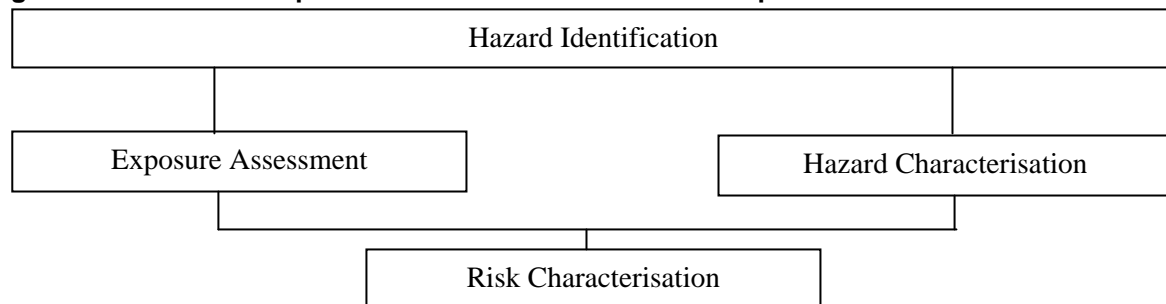
1.2   International Egg:*Salmonella* Risk Assessment Perspective

Contamination of egg products by *Salmonella*, but especially with SE, is an important cause of enteric infections in humans. In addition, contamination has potential for imposition of technical barriers in the international trade of egg products and result in unwanted introduction of specific *Salmonella* into populations of humans and poultry hitherto free of infection. In order to improve the safety of eggs and the trade of egg products, United Nations bodies (FAO and WHO) have produced a generic quantitative risk assessment (QRA) framework for *Salmonella* contamination of chicken meat and eggs (FAO-WHO, 2000; FAO-WHO, 2001). Foundations for this work were data and expertise volunteered from the international community and elements of earlier studies performed by USDA FSIS and Health Canada. (Whiting & Buchanan 1997; Whiting *et al*, 2000, Fazil unpublished).

In Australia, sizeable outbreaks of Salmonellosis have been reported following ingestion of foods containing eggs, along with other ingredients (Ashbolt *et al*, 2002; 2003). State health agencies and industry organisations have also conducted risk prioritisation exercises indicating improved

organisation and management of the information about *Salmonella* associated with Australian eggs is required to improve the potential for export, to protect existing egg markets and to safeguard public health outcomes. Meeting these challenges will depend on conduct of a rigorous assessment of the human health risks arising from the occurrence of *Salmonella* in the egg production and consumption chain in Australia.

The SE risk assessments developed by Whiting and others and the USDA-FSIS have provided the basis for modelling the public health effects associated with consumption of *Salmonella* contaminated eggs and egg products. For example, Whiting and Buchanan (1997) established that the time and temperature of pasteurisation of egg pulp is a critical point. Testing combinations of these parameters emphasised the importance of thermal processing temperature. Even minor variations greatly altered risk from SE. More recently, Whiting and others (Whiting *et al*, 2000) employed a stochastic model to estimate growth of SE during egg collection, processing, storage and transportation. This model accounted for internal egg temperature, yolk membrane integrity and exponential growth of SE. Importantly, the model indicated that under normal conditions, no growth would occur during the progression from point of lay through transportation. Furthermore, the impact of temperature abuse on infected eggs was modelled and scenarios developed to demonstrate the relative importance of handling on potential for growth of *Salmonella*.

Major findings from the FAO-WHO *Salmonella* Enteritidis QRA (FAO-WHO, 2002) included:
- Reduction of flock prevalence of SE will result in a proportional reduction in human health risk in countries where SE occurs in commercial flocks.
- The risk of human illness per food serving appears to be insensitive to the number of SE in contaminated eggs at the time of lay.
- The amount (and reliability) of data on the number of SE cells per infected egg is a major limitation in hazard evaluation.
- There is uncertainty about the effectiveness of cleaning and disinfecting eggs in controlling SE-related human illness.
- The efficacy of vaccination for SE has not been measured.

Developments in the global trade of food have exposed egg producers to a new set of opportunities and risks that are best managed with risk assessment. Estimating 'equivalence' is now the process used to determine whether or not:
- Australian egg products can penetrate foreign markets
- Egg products produced abroad can penetrate Australian markets.

This involves an appraisal of whether the imported product presents the same or lesser magnitude of human-health risk as posed by the domestic product. Under the guidelines produced by the World Trade Organisation (WTO), the assessment of equivalence demands the conduct of a food safety risk assessment by the importing country. A country can deny entry of a product if it fails to meet the equivalence standard. Thus nations wishing to trade in egg products require a pool of scientific expertise to conduct their own risk assessments on eggs and also to appraise the appropriateness of those produced by their trading partners. At the national and domestic level, food safety legislation and food standards are based on the risk assessment approach. It is timely, therefore, for industry to develop this risk assessment capacity to assist the development of responses to these new food safety challenges.

1.3   Scope of Risk Assessment

This project aimed to provide an objective, transparent and scientifically robust basis for the management of *Salmonella* in the egg industry. The internationally accepted approach for the conduct of food safety risk assessment that is promulgated by Codex, FAO and WHO was used throughout the work described in this report. This entailed satisfying hazard identification, exposure assessment, hazard characterisation and risk characterisation elements of study. The project complemented recently completed RIRDC work on defining a national surveillance program for SE in poultry. The

latter project focussed on the farm-end of the production chain and recommended control measures at flock, regional and national levels that can be described in terms of their impact on bird and flock prevalence of SE infection. This project has delivered a means of propagating those estimates through the remainder of the production and consumption chain describing their impact on public health outcomes and importance relative to post-farm factors.

In practical terms the models are jointly held by AECL and the research organisations. Industry policy makers (eg Risk Managers) wishing to use the models, can ask a wide range of "what if" risk assessment questions eg the impact of egg washing, the failure of a CCP, CCPs for *Salmonella* through-chain etc. The models can assist development of answers to questions that cannot be addressed by conventional research because of cost and logistical constraints. They may also greatly assist processors to optimise physical and nutritional attributes of products without compromising safety. For example, manipulation of conditions of pH, time and temperature during pasteurisation of eggs may achieve a safe product but with minimal thermal damage to proteins and loss of vitamins and other thermolabile nutrients.

The aim of risk assessment is to understand how the incidence of human Salmonellosis is influenced by various factors, from the agricultural phase of chicken meat and egg production, through on-farm handling and storage, transport, processing, distribution, retail storage, consumer storage and meal preparation, to final consumption. This approach is appealing because it permits the study of the broadest range of intervention strategies. However, as the work progressed in this project it became evident that the quantity and quality of information available from all sources was not sufficient to allow the construction of a full and expansive model. Thus, the final scope of the *Salmonella* risk assessment and the components of the food production and consumption continuum that were considered became point-of-lay to end-of-retail, and the risk estimates were limited to selected food products.

In this work risk estimates for *Salmonella* were modelled for shell eggs and egg products. The model enabled the proportional impact of different management procedures at the industry sector level and across sectors to be evaluated as a guide to the selection of risk management options. In addition, the model provides flexibility for evaluation and optimisation of controls appropriate for different existing production and marketing systems, and a food safety benchmarking process to allow the industry to meet its public health and international trade obligations.

2. Hazard Identification

2.1 Introduction

Public health data are reviewed and summarised in this section to identify the *Salmonella*:Egg and Egg Product combinations that can be attributed to food-borne illness in Australia. These data include serovars and phage types of all *Salmonella* infections. In relation to outbreaks, details of the population affected, suspect foods in which eggs were suspected, whether the outbreak serovar was recovered from the suspect food, other contributing factors and whether there is a microbiological link to the source of eggs are discussed. Where available, information on the incidence and relative prevalence of *Salmonella* serovars from layer flock environments, stockfeeds, egg and egg products and outbreaks are reviewed to assist interpretation.

In Australia, doctors and laboratories are required to notify health departments when patients are diagnosed with *Salmonella* infection. States and Territories record details about infected patients and forward this information to the National Notifiable Disease Surveillance System (NNDSS), maintained by the Commonwealth Department of Health and Ageing. In addition, the National Enteric Pathogen Surveillance Scheme (NEPSS), situated at the Microbiological Diagnostic Unit Public Health Laboratory (MDU) at the University of Melbourne, receives reports directly from laboratories on all human *Salmonella* isolates and other bacterial pathogens in Australia. NEPSS also receives reports on *Salmonella* recovered from animals, foods and the environment submitted by participating laboratories. The Melbourne Diagnostic Unit (MDU) and the Institute of Medical and Veterinary Science (IMVS) in Adelaide provides a *Salmonella* typing service to laboratories throughout Australia.

2.2 Summary of Data

For the purpose of the Hazard Identification, 26 outbreaks investigated by OzFoodNet (*www.ozfoodnet.org.au*) in which the investigators identified an epidemiological link with eggs were reviewed.

The 26 outbreaks are summarised in Table 2.1 by cross-tabulating the egg-specific criteria with details of other contributing factors. The findings listed below and inferences drawn are derived principally from Table 2.1, however, additional supporting data across the supply continuum is provided in detail in Appendix 2.

The main findings are;

Serovars
- Among the 26 outbreaks, presented by OzFoodNet for review, the most common serovar was *Salmonella* Typhimurium (73% of the outbreaks) and *S.* Heidelberg (11.5%).
- A range of Phage Types were involved (Table 2.1)
These results may indicate a greater capacity for *S.* Typhimurium to cause illness in humans.

Food Sources
- There were 10 outbreaks (5, 6, 8, 13, 19, 20, 23, 28, 31, 36) in which the "outbreak serovar" was isolated from the implicated food but not the egg source (6 layer sources negative and 4 not investigated). There were 2 outbreaks (1, 18) in which the outbreak serovar was isolated from the food source and the implicated egg source. There were 3 outbreaks where the food vehicle (containing eggs) was identified by analytical epidemiology (outbreaks 3, 22, 34 with no layer source investigation), and 1 outbreak (30) where the food source was implicated by both food culture and epidemiology (this layer source was negative).
- An examination of outbreaks associated with cooked egg product (n=9) reveals the serovars isolated are either not, or are uncommonly, isolated on-farm (*S.* Typhimurium PT 135 and 135a, *S.* Heidelberg PT16, *S.* Hadar PT22, *S.* Typhimurium RDNC/AO41). However, *S.*

Typhimurium, in particular, is regularly isolated from egg products (Appendix 2, Table 2.6), poultry and other meats (Appendix 2, Table 2.12).

- Among *S.* Typhimurium outbreaks, 6/19 (31%) were associated with eating cooked foods including hard boiled eggs, fried batter, and baked dishes (Table 2.1; Appendix 2, Table 2.7). These may represent cases of cross-contamination from other ingredients or from kitchen practices as the primary source of contamination with temperature abuse, or insufficient cooking. "Cooking" of egg sauces and custards, usually on light heat, may not be sufficient to reliably eliminate all contamination (Humphrey *et al* 1989; Bates and Spencer 1995), which could again increase rapidly with temperature abuse.

- Foods containing uncooked raw egg/egg products (n=14) seem to be an important contributing factor in egg-related outbreaks. Uncooked food vehicles repeatedly incriminated in outbreaks include: egg/milk drinks, mayonnaise, pies where egg is added post-cooking and desserts (Appendix 2, Table 2.7). However, it is extremely difficult to verify whether eggs are the primary source of contamination or whether there is cross-contamination from other ingredients or the food preparation environment. The latter is indicated in several reports. Outbreak investigations indicate that these foods are also often subjected to temperature abuse. As egg ingredients provide extremely good media for growth and multiplication of bacteria, the temperature abuse may contribute significantly to the finding of *Salmonella* in food items containing eggs.

Egg sources

- The outbreak serovar was recovered in 38% (5) of 13 outbreaks where the egg source was investigated, however, in only 2 of these outbreaks was the serovar isolated from the implicated food.

- Four of these 5 outbreaks were due to *Salmonella* Typhimurium with all 4 being associated with non-commercial or unconfirmed egg sources (Table 2.1).

- There is a low incidence of *S.* Typhimurium detected by routine monitoring of commercial layer flock environments. The sensitivity of this type of sampling, has been deemed as appropriate by Sergent *et al* (2003). In routine flock environment monitoring in NSW only 3.1% of 2252 shed tests (2.4% - 3.9%, C.I. 95%) were positive for *Salmonella*, with only 2.9% of 69 isolates recovered over 3 years of monthly tests on 45 farms being *S.* Typhimurium untypable (Appendix 2; Table 2.4).

- In none of the outbreaks was the age of the egg and its storage time/temperature history reported. While this may be very difficult it may shed valuable insight into whether the internal defences of the eggs had been exceeded enabling growth of internal contamination to infective levels (Section 4).

- There is some linkage between serovars isolated from egg products by NEPSS (Appendix 2, Table 2.6) and outbreak serovars (Table 2.1). While serovars commonly found in stockfeeds do appear in egg products (Cox *et al* 2002), there is an unexplained increase in the relative proportion represented by various *S.* Typhimurium phage types from raw egg pulp samples.

Serovars from commercial layer environments and stockfeeds

- Layer flock environment serovars generally reflect those found in stockfeeds (Appendix 2, Tables 2.4 and 2.6).

- Serovar incursions in commercial layer flocks appear transient rather than representative of longer-term colonisation. This, and delays in layer source investigations may be reflected in the disparity between human outbreak data and on-farm monitoring of *Salmonella.* Data over 3 years from NSW supports this contention as only 3.1% of 2252 (2.4% - 3.9%, 95% CI) monthly shed drag swab tests were positive, with only 8.7% (3.3% - 18.0%, 95% CI) of these testing positive with the same serovars at the following monthly test.

Cross-contamination

- Whenever possible thorough investigation of hygiene practices and other ingredients as the source of contamination is reinforced. Thirteen outbreaks (Table 2.1; Appendix 2 Table 2.7)

have case assessments in which potential for cross-contamination from the kitchen environment was specifically recorded by the investigator. In some, more than one food type was contaminated with the outbreak serovar.

- Examination of the 8 outbreaks where eggs were traced to a commercial production facility (Table 2.1) also reveals that in 6 of these outbreaks the investigators specifically detailed likely cross-contamination of foods. Substantiation, however, is often very difficult.

Vulnerable populations
- Eggs from backyard and unidentified sources were fed in uncooked foods to vulnerable populations in 4 outbreaks (outbreaks 2, 7, 9, 18); a practice that should be discontinued due to the predicted increased risk from cracked eggs (Todd 1996). Furthermore, cross-contamination was highlighted in 3 outbreaks (outbreaks 3, 9, 25) where vulnerable populations were affected.

In relation to certainty of attribution, proving the chain-of-evidence in investigations is often difficult due to the retrospective and non-point source circumstances of some outbreaks, and the low level of contamination in eggs (Section 4). In addition, the review of outbreaks highlighted a lack of consensus with regard to attribution criteria between public health, food technology and veterinary public health experts. From the expert opinions obtained in relation to attribution of these outbreaks, "strong evidence" is claimed for a third or less. Consequently, summarised data and general observations are presented to enable an overview and selection of the target hazard for the project, rather than a detailed analysis of individual cases. The complexities in attribution where eggs are suspected are highlighted in recent reports in relation to outbreak 5 (Unicomb *et al* 2003; Arzey 2004; Unicomb *et al* 2004) and should be considered by an appropriate expert panel.

While these outbreaks provide information for hazard identification it must be borne in mind that outbreak data must be regarded as partial, since outbreaks represent only a fraction of the total number of food poisoning cases notified (Sumner *et al* 2003). In the meantime information on contributing factors in outbreaks can be used to identify steps in the process that should be controlled to prevent or eliminate a food safety hazard or reduce it to an acceptable level (Codex 1997; NACMCF 1998).

Further data from a survey in the 1990s on levels of contamination of pooled unpasteurised egg pulp from multiple farms in Qld, found a high proportion of batches to be contaminated (95%), with 23% of individual farm egg pulp samples positive (Cox *et al* 2002). Serovars and phage types detected in most part did not reflect those implicated in human cases. Outbreaks that might be attributed to commercially processed egg pulp were not evident in the outbreaks (up to mid-2003) submitted for this Hazard Identification. Subsequent data from NEPSS (Appendix 2, Table 2.6) showed a range of *S.* Typhimurium phage types isolated from commercial raw egg pulp samples from unspecified egg sources, including PT9 and PT135, the cause of 3 and 5 of the 26 outbreaks reviewed, respectively. It is noted that PT135 is commonly isolated from a range of other animal sources (Appendix 2, Table 2.12). In addition, PT9 has also recently been isolated from 4 of 27 commercial raw egg pulp samples submitted for pre-pasteurisation monitoring (Murray 2004). While it is acknowledged laboratory data are not based on any statistical sampling process and tested pulp may not always be limited to eggs from commercial layers, the qualitative impact of *Salmonella* was considered sufficiently important to identify this organism as the target pathogen of concern in egg products.

Information on contributing factors detailed in this review support the need to consider mitigations wider than additional on-farm controls in the commercial egg production sector. Many of the outbreaks apparently have much to do with the combination of the type of foods, poor hygiene, cross contamination and temperature abuse in the food preparation settings. This introduces the potential of other food ingredients or the food preparation environment being the primary source of contamination, though it is difficult to quantify the contribution of each factor. Additional information on egg storage history (time and temperature) and the degree of cooking of the implicated dish are important factors in attribution when eggs may be implicated.

For the purposes of this quantitative risk assessment project conclusions regarding serovar and food types of priority for modelling could be drawn. It is concluded *Salmonella*:egg and egg product combinations for modelling are *Salmonella* associated with;

- Egg nog using uncooked eggs
- Egg based sauces that are high in fat and lightly cooked, and
- Dishes that are lower in fat than cooked sauces, but may experience greater cooking eg scrambled eggs (eggs plus milk).

2.3 Australian Consumer Risk from Potential *Salmonella* Enteritidis PT4 Exposure

Salmonella Enteritidis PT4 has not been isolated from intact eggs in Australia and extensive government agency epidemiological evidence does not support locally acquired infection with this phage type (Appendix 2; Tables 2.13, 2.14, 2.15). If introduced and not effectively controlled, the presence of contaminated contents in commercially produced and graded shell eggs would expose the general population. This would represent a substantial increase in risk. The lesson from abroad is, if SE becomes established in Australian flocks then the public health impact and economic loss arising from the occurrence of *Salmonella* in eggs are both likely to increase by (at least) several fold.

While infection with SE PT4 has not been associated with the consumption of shell eggs produced in Australia, there is evidence of environmental contamination with SE PT26 in Queensland flocks (Appendix 2, Table 2.16). Non-human data from north Queensland also shows SE PT26 has been isolated from snakes, possums, crocodiles, cane toads, bovines and water, indicating this is a regional serovar (J Powling, *pers comm*; NEPSS 2003b). This raises the potential for contamination of eggs from this region. Further research into flock incidence may be warranted. Current exposure may, therefore, arise from a number of foods or environmental reservoirs. Outbreaks associated with eggs with this serovar have not been recorded. OzFoodNet is conducting a case-control study to explore potential risk factors for acquiring SE in Australia.

Table 2.1: Cross-tabulation of outbreak criteria

	Non-Commercial										Both		Commercial								Unknown					
Case ID	1[1]	6	7	8	9	13	20	28	37	38	18[2]	22	5	19	23[3]	25	30	31	34	36	2	3	4	16	17	24
Serovar	TM	H	H	TM	H	M	TM	TM	TM	Had	TM	TM	P	TM	TM	V	TM	TM	TM	TM	TM	TM	TM	TM	TM	TM
PT	9	16	1	8	1		135a	170	135a	22	135	9		8	135 145	34	135	64	126	RDNC A041	9	135	135	135a	64	U307
Food isolate	Y	Y		Y	Y	Y	Y	Y			Y		Y	Y	Y		Y	Y		Y						
Source Invest.	Y	Y	Y	Y	Y		Y	Y	Y		Y		Y	Y		Y	Y			Y						Y
Source +/-	+	-		-	-		-		+		+		-	-		+	-			-						+
Analytical epidemiol.												Y					Y		Y			Y				
Cracked/ Dirty		Y	Y	Y					Y				Y		Y					Y					Y	
Cooked/ Uncooked	U	C	U	U	U	C	U	U	C	C	U	U	U	U	U	?	U	C	?	C	U	C	C	C	?	U
Temp. Abuse	Y	Y		Y	Y								Y	Y											Y	
Vulnerable population		Y		Y					Y		Y					Y				Y	Y	Y				
Cross-Contam.		Y	Y	Y	Y							Y	Y	Y		Y	Y	Y	Y			Y				Y
Serovar Layer Environ	N	N	N	N	N	Y	N	N	N	N	Y	N	N	N	Y N	Y	Y	N	N	N	N	Y	Y	N	N	N

Serovars abbreviations: TM Typhimurium, H Heidelberg, M Mbandaka, Had Hadar, P Potsdam, V Virchow

PT Phage Type

Food isolate: Outbreak serovar isolated from the implicated food containing egg as an ingredient

Source investigated: Whether egg layer source environment investigated using drag swab for *Salmonella* detection

Source: +/- : Whether egg layer source *Salmonella* spp. positive or negative

Analytical epidemiol.: Analytical epidemiological methods demonstrated a statistically significant association with a food vehicle containing eggs as an ingredient

Cracked/Dirty: Either cracked or dirty eggs used for implicated food or in associated food preparation premise

Cooked/Uncooked: Egg or egg product in implicated food in either cooked or uncooked state

Temperature abuse: Investigators recorded inappropriate storage temperature for the implicated food

Vulnerable population: Outbreak principally affected aged care, hospital or child care cohorts

Cross-contamination: Investigators recorded the likelihood of cross-contamination from either the food preparation environment, other ingredients or food handlers

Serovar Layer Environ: Isolated previously from commercial layer environments in Australia; Data from NSW (Table 2.4) and Queensland investigations (Cox, 1993; Cox *et al* 2002) and commercial layer source investigations detailed in this Table.

[1] Both non-commercial and commercial source, but positive isolation only from backyard source

[2] Positive egg source not specified

[3] Note this case identifies two isolates.

3. Hazard Characterisation

3.1 Introduction

This section describes the development of a dose response model for *Salmonella* that represents a significant advance on previous approaches. The model is based on summary information of accepted characteristics of *Salmonella*, host (ie human) factors and composition of foods that are known to influence the outcome of exposure to this organism. As the health effects of dietary exposure to *Salmonella* have been extensively reviewed (FAO-WHO, 2002), this aspect will be only briefly summarised in this document.

The new dose response model (D2TAP) is based on a reanalysis of the food related outbreak data published by FAO-WHO (2002). Uncertainties in dose and attack rate[3] were included in the development of the D2TAP dose response model. This is a significant modification of the approach taken by FAO-WHO (2002) models.

The application of the dose response model developed for exposure assessment and risk characterisation of *Salmonella* in eggs is described in Sections 4, 5 and 6 of this report.

3.2 Epidemiological Data used to Develop Dose Response Models

Dose response models are the link between exposure in food by pathogenic microorganisms and adverse human health outcome. The development of human dose response models for pathogenic microorganisms has been limited by the lack of suitable data. Human feeding trials conducted under experimentally controlled conditions are rare. The exceptions are a number of studies where *S. Typhimurium* strain Ty21a has been used for live vaccine efficacy studies. As a consequence, dose response models have been developed using either surrogate microorganisms (eg *Shigella dysenteriae*), or epidemiological investigations based on human food-related outbreaks (see reviews published by the FAO-WHO, 2002; USDA-FSIS, 1998 and Blaser & Newman, 1982).

In the absence of suitable SE human feeding trial data, the USDA-FSIS developed a dose response model based on human trials of *Shigella dysenteriae* as the test pathogen (Levine, 1973; Teunis *et al*, 1996). *S. dysenteriae* was chosen as a surrogate organism as it was known to have lower infective doses when compared with *Salmonella* strains. Estimates for the dose response for *Salmonella* in a normal sub-population, including healthy children (>5 years) and adults with no pre-existing health conditions, were developed from the *S. dysenteriae* data. Dose responses, were also adapted to include susceptible sub-populations (the very young, aged persons and immuno-compromised individuals) and uncertainties in the dose response model. However, there are several important deficiencies in this model. These include:

- The model is based on the surrogate organism, *Shigella dysenteriae*, not *Salmonella*.
- The bounds of the model prediction are not realistic. For example, the upper boundary of the attack rate is predicted to be 100%, irrespective of dose.
- The model does not incorporate a consideration of the influence of dietary factors such as fat content.

Unlike the USDA-FSIS model, the FAO-WHO dose response model was based on available *Salmonella* outbreak data for foods and water. Information from 23 disease outbreaks (selected from >30) were selected for appropriate inclusion in the development of a model. Essential epidemiological criteria considered for inclusion were: the number of individuals exposed, the number of individuals

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[3] Attack rate is defined as the percentage of the number of people ill divided by the total number of people who consumed the implicated food. Attack rates may vary between 0% (no illness) to 100% (all people exposed become ill). Probability of illness is defined as the proportion of the exposed population that become ill.

becoming ill, numbers of *Salmonella* in the implicated food and the quantity of food consumed. All data included was collected from either published literature or Japanese surveillance monitoring data (Table 3.1).

Figure 3.1: Summary of FAO-WHO outbreak data (FAO-WHO, 2002). The legend numbers refer to the case number in Table 3.1.
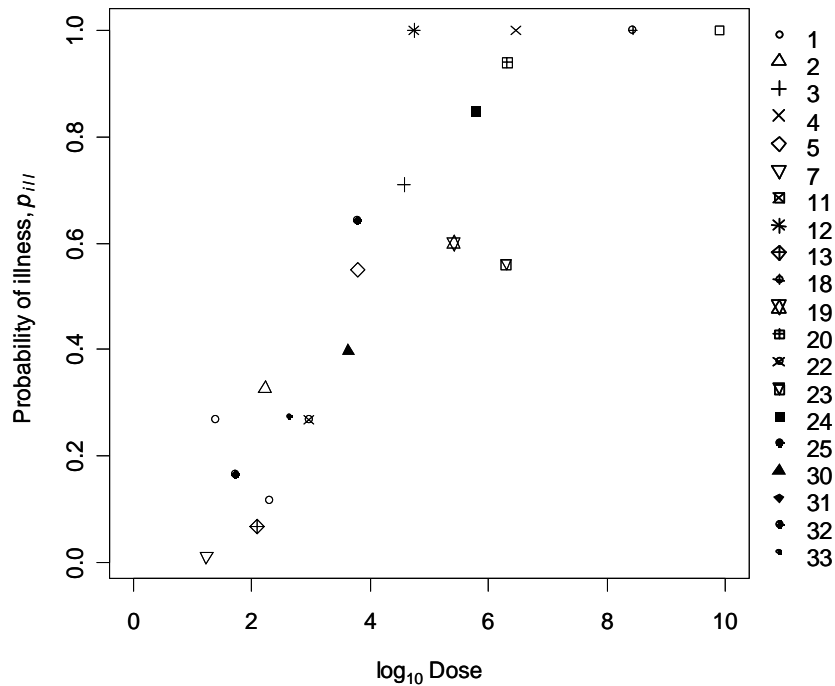
The FAO-WHO outbreak data (FAO-WHO, 2002) (see Table 3.1) were qualitatively analysed for epidemiological evidence indicative of differences in attack rates for SE compared with other *Salmonella* serotypes and for Susceptible vs Normal sub-populations.

Graphical summaries of outbreak data that highlight differences between populations (Normal vs Susceptible) (Figure 3.2) and *Salmonella* serovars (Figure 3.3) are presented. Probability of illness for Normal and Susceptible populations show no obvious differences (Figure 3.2). However in the case of three outbreaks (Cases 1, 13 and 25), individuals from both sub-populations ingested the same foods implicated in disease. All individuals involved in outbreak 13 become ill. For outbreaks 1 and 25 the attack rate for the Susceptible population was greater than that for the Normal population in both cases (Figure 3.2). When assessed against the balance of the outbreak data, the FAO concluded that the available outbreak data did not support the proposition that there are differences in susceptibility (attack rates) for Susceptible and Normal populations. Nevertheless, although the FAO noted that attack rates may not be different between populations, it is possible that the severity and range of complications resulting from Salmonellosis may be different.

For comparative purposes, the probability of illness (rather than attack rate) and corresponding dose for different *Salmonella* serovars is presented in Figure 3.3. The *Salmonella* serovars were grouped as SE and other *Salmonella* spp. Differences between non-SE serovars was not investigated due to the limited range of serovars in the outbreaks investigated. The FAO concluded that no differences between different *Salmonella* serovars were evident from the available outbreak data and that the dose response model developed was applicable for SE and other *Salmonella* serovars.

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Figure 3.2: Comparison of the probability of illness from outbreaks where 'Normal' (filled squares) and 'Susceptible' (open squares) sub-populations were identified (FAO-WHO, 2002). Boxed points represent different populations from the same outbreak.
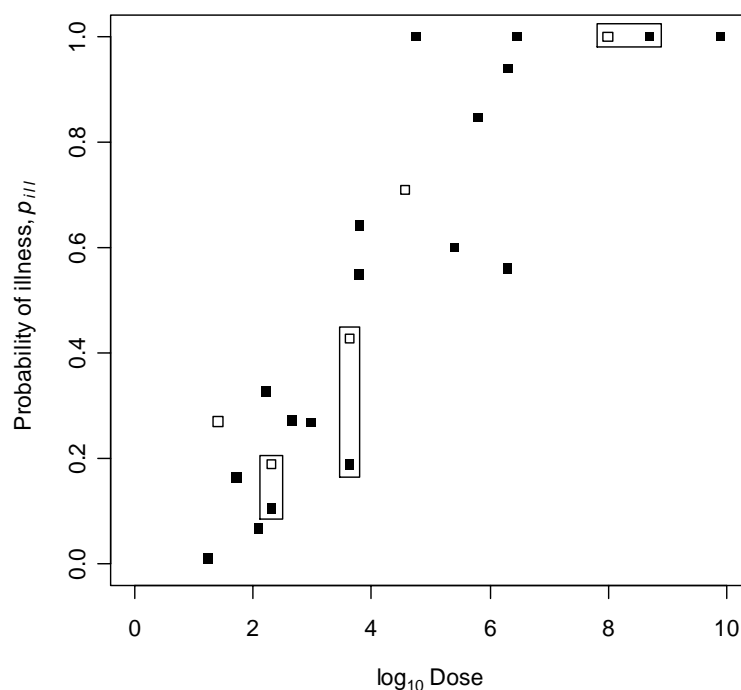
Figure 3.3: Probability of illness and corresponding dose for outbreaks with *Salmonella* serovar identified (FAO-WHO, 2002).
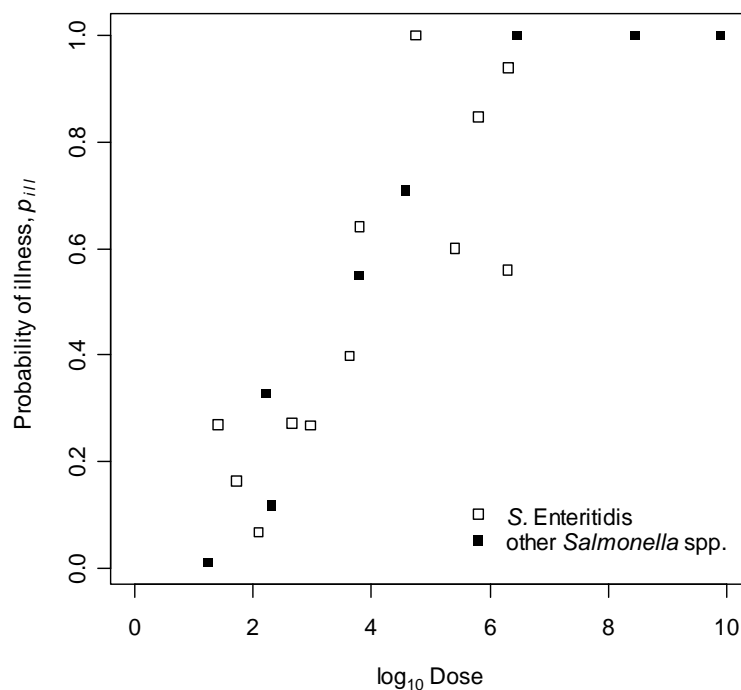
3.3   Dose Response Model Formulation

Beta-Poisson models have been used to evaluate human dose responses for *Salmonella* (Haas *et al*, 1999; Holcomb *et al*, 1999; USDA-FSIS, 1998; FAO-WHO, 2002). These models have the form:

$$p_{ill} = 1 - \left(1 + \frac{dose}{\beta}\right)^{-\alpha} \qquad \textbf{(Equation 3.1)}$$

Where p_{ill} is the probability of becoming ill from the consumption of a dose of a pathogenic microorganism, α is a shape factor and β is a location factor.

The FAO-WHO Dose Response model was based on qualitative assessment of the outbreak data (differences in the virulence of different *Salmonella* serovars [Figure 3.2]). However the effects of other factors (eg fat content of foods) were not considered. Uncertainties associated with each of the total number exposed, the number ill, the quantity of food consumed and the concentration of *Salmonella* was randomly selected for each outbreak by Monte Carlo simulation. The Beta-Poisson dose response model was fitted using a maximum likelihood technique.

Each time that the dose response model (Equation 3.1) is fitted to the re-sampled outbreak data the result is a pair of values of α and β. The FAO-WHO report notes that the ideal approach for using dose response relationships in a risk assessment would be to use each pair of α and β to recreate the dose response curves. However, the final dose response model proposed by the FAO-WHO uses (poorly defined) probability distributions for α and β rather than the actual values.

Table 3.1: Summary of FAO-WHO outbreak data including food vehicle (FAO-WHO, 2002)

Case no.	Serovar	Food	Dose log$_{10}$ CFU	Probability of Illness (%)
1	*S.* Typhimurium	Water	2.31	11.78
2	*S.* Heidelberg	Cheddar cheese	2.22	32.76
3	*S.* Cubana	Carmine dye	4.57	70.93
4	*S.* Infantis	Ham	6.46	100
5	*S.* Typhimurium	Imitation ice cream	3.79	55.00
7	*S.* Newport	Hamburger	1.23	1.07
11	*S.* Enteritidis	Hollandaise sauce	4.74	100
12	*S.* Enteritidis	Ice cream	2.09	6.80
13	*S.* Typhimurium	Ice cream	8.44	100
18	*S.* Enteritidis	Roasted beef	5.41	60.00
19	*S.* Enteritidis	Grated yam with soup	6.31	93.93
20	*S.* Enteritidis	Beef & bean sprouts	2.97	26.86
22	*S.* Enteritidis	Scallop with egg yolk	6.3	56.01
23	*S.* Enteritidis	Cake	5.8	84.62
24	*S.* Enteritidis	Peanut sauce	1.72	16.41
25	*S.* Enteritidis	Chicken and egg	3.63	39.85
30	*S.* Enteritidis	Cooked egg	3.8	64.18
31	*S.* Enteritidis	Cake	2.65	27.33
32	*S.* Enteritidis	Egg salad	1.4	26.92
33	*S.* Oranienburg	Grated yam with soup	9.9	100

3.4 The New D2TAP Dose Response Model

In order to better characterise the probability distributions for α and β in the Beta-Poisson Dose Response model for *Salmonella*, the FAO-WHO outbreak data has been re-evaluated. The starting point for the alternative model development was the uncertainty distributions for the dose, the exposed and positive (= ill) populations as reported by the FAO-WHO. The details of the outbreak data were reviewed to avoid anomalies arising from data uncertainties (eg the probability of illness for outbreak 19 was constrained to not exceed one. This situation arose as the uncertainty in the number of people

reported as ill may exceed the total number of people exposed for some simulations - see the maximum uncertainty value in Table 3.15 of the FAO-WHO report).

The parameter estimates for α and β in Equation 3.1 were determined using a maximum likelihood method (Venables & Ripley, 1994) using the statistical software R (R Development Core Team, 2004). A total of 5000 simulations were performed to obtain 5000 pairs of α and β following the FAO-WHO methodology. Examination of the histograms of the fitted values of α and β revealed that both parameters were skewed with a highly non-linear relationship between the parameters (results not shown).

To improve the statistical properties of the distributions for α and β an alternative form of the Beta-Poisson Dose Response model, D2TAP was used (Equation 3.2):

$$p_{ill} = 1 - \left(1 + \frac{Dose}{10^{(\log_{10}\beta)}}\right)^{-10^{\log_{10}\alpha}}$$
(**Equation 3.2**)

Equation 3.2 uses a commonly used statistical approach of taking logarithms of the parameters to modify their properties; the new model parameters in Equation 3.2 are $\log_{10}\alpha$ and $\log_{10}\beta$. Despite the re-parameterisation the fit of Equation 3.1 and 3.2 are equivalent as the exponential of the new parameters is taken (ie $10^{\log_{10}\beta}$ is equivalent to β). Equation 3.2 was fitted to the FAO-WHO outbreak data using the same statistical approaches as used for Equation 3.1.

Examination of the histograms for $\log_{10}\alpha$ and $\log_{10}\beta$ indicated that both distributions were symmetrical, close to normally distributed (quantile-quantile normality plots not shown) and strongly correlated. The mean, standard deviations and correlation coefficient of $\log_{10}\alpha$ and $\log_{10}\beta$ are presented in Table 3.2. The mean value of α and β are 0.1346 and 53.33, respectively and are similar to the expected values of the FAO-WHO dose response model of 0.1324 and 51.45.

Because of the findings of the favourable behaviour of $\log_{10}\alpha$ and $\log_{10}\beta$, the proposed dose response model used in this Risk Assessment is of the form of Equation 3.2. The parameter estimates of $\log_{10}\alpha$ and $\log_{10}\beta$ are assumed to be jointly Normally distributed with the means, standard deviations and correlation coefficient as listed in Table 3.2.

A comparison of the predictions of the alternative and FAO-WHO dose response models is presented in Figure 3.4. The grey lines represent predictions of the alternative dose response model at the 99.5[th] percentile of the joint Normal distribution, while the dashed black lines are the bound of the FAO-WHO model. The model predictions for both models are similar at low doses (dose $< 10^2$ cells) and at high doses (dose $> 10^6$ cells). At intermediate doses, it is apparent that the alternative dose response model predictions are less variable than the FAO-WHO model.

3.5   Comparison of Dose Response Models

A summary of the predicted attack rates for different doses of *Salmonella* for the D2TAP, FAO-WHO and USDA-FSIS dose response models is presented in Table 3.3. As expected, the predicted attack rates increased with increasing dose. However, differences in the predicted attack rates for the dose response models are apparent from a comparison of models at the same doses. For both the Normal and Susceptible populations the USDA-FSIS dose response model predicts attack rates greater than either the D2TAP or the FAO-WHO models. The greatest difference in predicted attack rates is between the USDA-FSIS and the proposed D2TAP model for a Susceptible population infected with a dose of a single *Salmonella* cell. The attack rate for the USDA-FSIS model is 36.4 times greater than either the FAO-WHO and D2TAP models. The difference in attack rate between all models decreases as the mean dose increases. For example, at a dose of 1000 cells, the ratio between predicted attack rates for the previous example drops to 2.5 times greater for the USDA-FSIS model compared with the

FAO-WHO model. As expected, differences in the predicted attack rates for each of the dose response models at high doses ($>10^{10}$) are small (data not shown).

Figure 3.4: Probability of illness predicted by the D2TAP and FAO-WHO dose response models. Solid line represents expected values for the D2TAP model; dashed lines are the upper and lower bounds of the FAO-WHO dose response model; and the grey lines represent predictions by D2TAP at the 99.5 percentile.
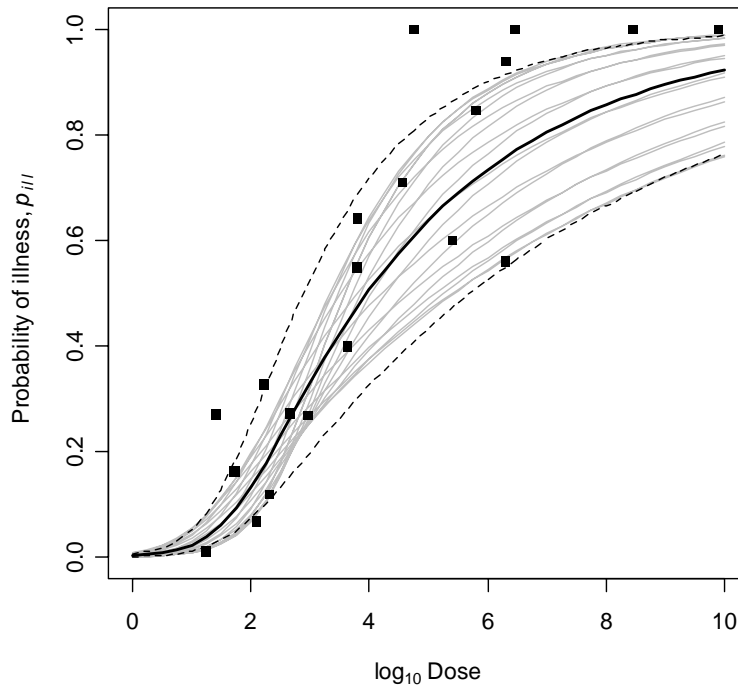
Table 3.2: Predicted $\log_{10}\alpha$ and $\log_{10}\beta$ values for the D2TAP dose response model (after 5000 iterations)

Parameter	Distribution	Expected Value	Standard deviation
$\log_{10}\alpha$	Normal	-0.871	0.089
$\log_{10}\beta$	Normal	1.727	0.227
ρ	Constant	0.892	

ρ = correlation coefficient

Expected values: $\alpha=0.1346$; $\beta=53.33$

Table 3.3: Predicted probability of illness (%) estimated by the FAO-WHO, USDA-FSIS and D2TAP models.

Dose response model	Mean Log Dose (Mean Dose)			
	0 (1 cell)	**1** (10 cells)	**2** (100 cells)	**3** (1000 cells)
FAO-WHO	0.25%	2.30%	13.3%	32.9%
USDA-FSIS (normal population)	1.1%	9.1%	36.4%	64.5%
USDA-FSIS (susceptible population)	9.1%	36.3%	64.4%	81.1%
D2TAP	0.25%	2.3%	13.2%	33.1%

3.6   Discussion

A dose response model for *Salmonella* infections in humans has been developed from "real world" out-break data. The proposed D2TAP dose response models described in this section, were developed using a re-parameterisation of the $\alpha-\beta$ form of the Beta-Poisson model. The impact of factors such as host susceptibility or the fat content of foods on dose response have not been included as preliminary analysis indicated insufficient data in the WHO data set to warrant inclusion. Similarly, the effect of serovar on dose response has not been included because there is insufficient data available that describes infectious doses for a range of different *Salmonella* serovars.

Predicted attack rates for the D2TAP model are generally the same as the FAO-WHO model. The difference between the two models was the assumptions used in dealing with the uncertainty in the outbreak data used in model development. As a result we have used the proposed dose response model for risk assessment purposes (see Section 5).

3.7   Summary and Conclusions

- The proposed model developed for this study is based on the outbreak data from the FAO-WHO risk assessment (FAO-WHO, 2002).
- A *Salmonella* dose response has been developed based on a Beta-Poisson dose response model (D2TAP).
- The D2TAP model includes uncertainties in the number of exposed, the number of people ill, the amount of food consumed and the concentration of *Salmonella* cells.

4. Exposure Assessment – Shell Eggs

4.1 Introduction

This section describes the development of a mathematical model that enables calculation of the probability of illness resulting from the ingestion of foods containing eggs or egg products contaminated with *Salmonella* spp. Construction of this Exposure Assessment (EA) model followed a design similar to that used by the FAO-WHO (2002) and USDA-FSIS (1998). Importantly, this model allows assessment of the effects of production, processing and storage factors from point of lay to preparation and consumption. In view of the fact that SE is not established in Australian layer flocks, this EA model does not specifically consider the impact of this pathogen on the Australian Egg Industry. However, where appropriate, data describing the behaviour of SE in eggs has been used to fill data gaps for non SE *Salmonella*.

The development and application of the EA model to an analysis of the impact of production, processing and storage of eggs on growth of *Salmonella* in egg contents is underpinned by survey data describing egg production and processing practices in Australia covering production of over 4 million birds across 4 states. Furthermore, this EA model can be used as a basis to gauge the ability of pasteurisation practices, that are currently recommended for use in the Australian food industry, to eliminate *Salmonella* from egg pulp, yolk and albumen (Section 6).

4.2 Review of Previous Exposure Assessment Models

Detailed reviews of previous qualitative and quantitative Exposure Assessments have been summarised in recent FAO Risk Assessments (FAO-WHO, 2002). Several quantitative EAs for shell eggs (USDA-FSIS, 1998; Health Canada, unpublished) and processed egg products (USDA-FSIS, 1998; Whiting *et al*, 2000) modelled the impact of processing variables on the number of SE cells in servings of shell eggs or egg products. For example, the USDA-FSIS EA estimated the number of SE cells in 16 different food preparation pathways. The pathways included preparation of foods including eggs, and the location of the preparation (domestic and institution).

4.3 An Exposure Assessment Model for the Australian Egg Industry

4.3.1 Model Structure

The major difference between the USDA-FSIS EA (FAO-WHO, 2002; Whiting *et al*, 2000; USDA-FSIS, 1998) and the EA presented in this report is the use of Australian egg production and processing industry data in the development of the process model (Appendix 3). The EA model is the mathematical description of the steps between the point of lay and consumption. Generalised flowcharts for off-line production, processing and retail storage of shell eggs (Figure 4.1), egg grading (Figure 4.2) and egg product processing (Figure 4.3).

Each step in the continuum from the point of lay to the consumer will contribute to the time, temperature and contamination exposure histories before preparation and consumption. The magnitude of these variables experienced by eggs from "point of lay to plate" will influence the potential for invasion of egg contents and growth of *Salmonella* within the egg.

We have developed an EA to describe Australian shell egg and processed egg product process models from information obtained through pilot surveys of Australian producers (Appendix 3) and processors for caged production only. The egg producer survey[4], that principally involved flocks from NSW, Qld

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[4] The producer survey (covering 4 million layers and 73 producers from NSW, Qld, SA and a small number from Victoria), was not intended to be a comprehensive survey of production practices and flock management issues for all flocks in Australia. Rather, the survey was to gather information on various production and handling

and SA and a small number of flocks from Victoria, gathered information regarding flock size, egg collection frequency, storage and distribution practices. A summary of the survey results and the questionnaire are presented in Appendix 3. A recent survey of the national industry revealed that over 400 producers had a combined layer population of over 13 million (Geoff Runge (Queensland Department of Primary Industries, *pers. comm.*). The processor survey[2] gathered information on the storage times and temperatures pre- and post-processing for shell eggs.

In the building of the EA model the following were considered:
- The influence of egg properties on growth of *Salmonella;*
- The prevalence and concentration of the *Salmonella* in eggs and egg pulp;
- The changes in numbers[5] of *Salmonella* at each stage from production to consumption; and
- Modelling the final steps from preparation and consumption.

As with the USDA-FSIS EA there are three main components in the Australian shell egg production model: production, distribution and storage and preparation and consumption. An additional component for the processing of liquid egg products (whole egg, yolk and albumen) is also presented.

4.3.2   The Defence Mechanism of the Egg
In normal healthy layers, the content of freshly laid eggs is sterile. In order to prevent microbial invasion of eggs and subsequent growth of microorganisms in egg contents, eggs have three layers of anti-microbial defence. These are the shell and shell membranes, the albumen and the vitelline membranes.

The shell and shell membranes
The shell represents a physical barrier against invasion by microorganisms. Although this structure contains numerous pores of a size that potentially allow penetration by bacteria and viruses, the cuticle on the external surface, which forms within 3 minutes of lay, creates an protective barrier which encompasses about 97% of the egg surface. Together, the shell and the cuticle form an important first line of defence. Nevertheless, the shell does not eliminate the potential for contamination of the internal content of eggs during egg formation and maturation within the ovaries, uterus and vagina of the hen. There is opportunity for contamination of egg contents from fresh surface associated faeces. Furthermore, washing during processing can substantially disrupt, or completely remove the cuticle thereby compromising the integrity of the shell as a barrier and consequently permitting invasion of egg contents by contact with environmental bacteria.

Two membranes separate the internal surface of the shell and the albumen; both represent a physical barrier to microorganisms.

The albumen
The albumen contains a number of antimicrobial components capable of inhibiting growth of bacteria eg glycoproteins, lysozyme, N-acetylglucosaminidase, ovotransferrin and various proteases. Ovotransferrin is principally responsible for the bacteriostatic activity of albumen. When eggs are freshly laid, the pH of the albumen is in the range 7 to 7.5, but within 3 days of lay, the pH increases to 9 to 9.5. At this pH, ovotransferrin has significant iron binding capacity and is strongly bacteriostatic for bacteria with a high iron requirement. Unless bacteria can express iron scavenging proteins with higher affinity for Fe^{2+}, they will be unable to satisfy nutritional requirements and cease to grow.

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procedures and management practices in the Australian egg industry. Information was gathered for caged, barn and free range production systems.
[5] It is assumed that the prevalence does not change during production and processing. In this context prevalence is defined as the proportion of eggs with contents contaminated by *Salmonella*.

Figure 4.1: Generalised flowchart for the off-line production of shell eggs (Note: transportation steps after processing removed for clarity)
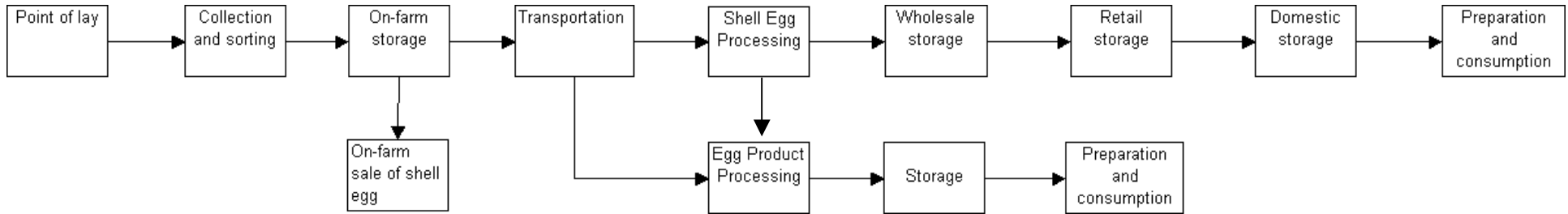
Figure 4.2: Generalised flowchart for shell egg processing
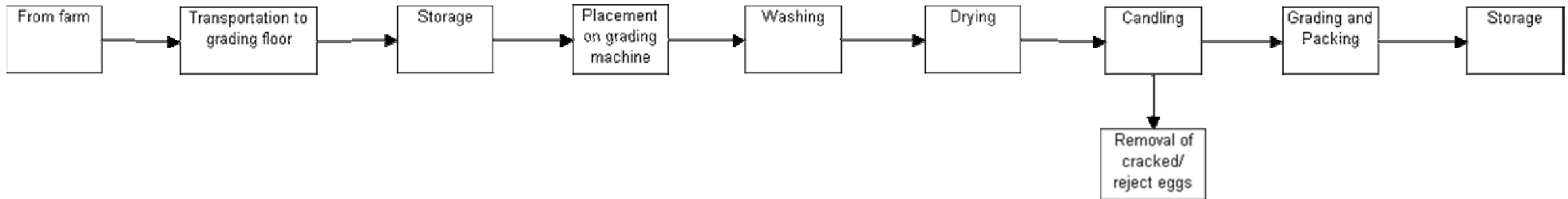
Figure 4.3: Generalised flowchart for egg product processing
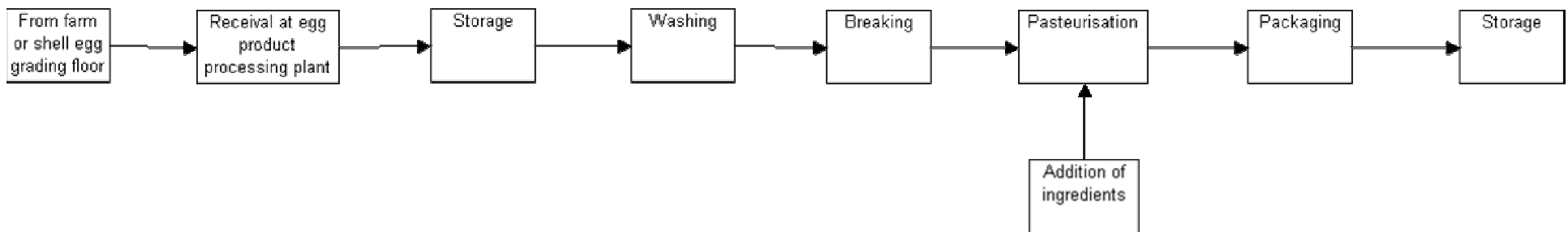
Vitelline membranes

The vitelline membranes (the yolk membrane) consist of two fibrous layers that form a physical barrier. The outer layer also contains insoluble lysozyme which is active against the bacterial cell wall. As membrane stability decreases with egg age, the ability of bacteria to penetrate these membranes increases, particularly at temperatures above 20°C (Whiting *et al,* 2000). This effect is discussed in more detail later in this section.

4.3.3   *Salmonella* in Eggs

In order to mathematically describe the growth and change in numbers of *Salmonella* in the contents of shell eggs, the following events have been assumed:

1. *Salmonella* that contaminate the contents[6] of eggs migrate in small numbers from the shell surface across the shell membrane into the albumen.
2. Growth in the albumen is limited by the innate bacteriostatic activity of that component of the egg.
3. As the yolk membrane degrades with egg age, release of yolk contents results in active growth of *Salmonella.*
4. Cooking steps result in inactivation of *Salmonella.* The resultant reduction in numbers of *Salmonella* is dependent on the severity of the cooking process.

This sequence of events is illustrated in Figure 4.4.

Figure 4.4: Diagrammatic representation of invasion and growth of *Salmonella* in shell eggs from point of lay through to preparation/cooking. N = number of *Salmonella* cells.

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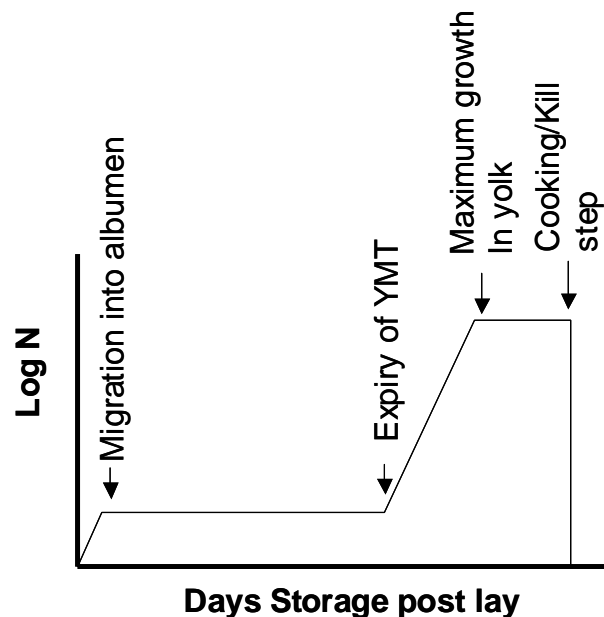
[6] External egg shell *Salmonella* load and the mode of contamination has not been considered (ie vertical *vs* horizontal transmission). Instead the prevalence of *Salmonella* contaminated eggs has been used. Data for prevalence has been sourced from a pilot Australian study and overseas studies (see Table 4.1).

Prevalence of Salmonella in Eggs

Important outcomes of the EA are the prediction of the probability that an egg chosen at random is contaminated with *Salmonella* and the number of *Salmonella* cells within contaminated eggs. The prediction of these values is dependent on an understanding of the prevalence of *Salmonella* contaminated eggs. A summary of the prevalence of *Salmonella* in eggs based on Australian and overseas studies, is presented in Table 4.1. This table includes a summary of published results for the *Salmonella* (SE and non-SE *Salmonella*) prevalence data from multiple flocks for eggs after lay and from retail sources. Analysis of the larger studies (>10000 eggs tested) indicated that SE may be isolated up to 6 to 8 times more frequently than all other *Salmonella*, in countries where SE is endemic.

As has been described previously, the prevalence of *Salmonella* serovars in flocks and flock environments is intermittent, and frequently reflects those identified in animal feeds (Section 2). This evidence supports the contention that *Salmonella* serovars, which are not host adapted, are present in layer flock environments primarily as a result of the use of contaminated stock feed. These serovars do not normally cause persistent infection in layers. The prevalence of *Salmonella* serovars based on isolates from layer sheds in NSW (2000 – 2003) is 2.8% of 1735 sheds tested (representing 47 farms). No seasonality is observed in the surveillance data (2000-2002). Aside from the NSW data, there is no national surveillance program that provides estimates of the prevalence of *Salmonella* in layer shed environments in Australia.

Time Before Growth in Egg Contents

Understanding the effect of storage temperature and time before the initiation of growth of *Salmonella* in egg contents is a key factor in assessing the risk from the consumption of eggs. As noted above, egg albumen can inhibit the growth of *Salmonella*. However, if *Salmonella* cells reach the yolk, rapid growth is possible at storage temperatures above 10°C. The majority of experimental studies undertaken to assess the time before growth of *Salmonella* in eggs relate to studies using SE. For example, Humphrey (1994), Braun and Fehlhaber (1995) and Cogan *et al*, (2001) examined the effect of temperature on the time before growth of SE inoculated directly into the egg albumen. In particular, Humphrey (1994) found that a small percentage of eggs stored at 20°C supported immediate growth of SE in albumen, with subsequent invasion of the yolk (open squares in Figure 4.5). The delay in the growth of *Salmonella* in eggs has been found to be temperature dependent (Humphrey, 1994). For eggs held at room temperature, growth may be inhibited for 2 to 3 weeks. Conversely, for eggs stored at 37°C, growth may occur in a few days. This effect is the result of a temperature and time dependent breakdown in the integrity of the vitelline membrane.

Previous risk assessments for SE in eggs (USDA-FSIS, 1998 and FAO-WHO, 2002) used the Yolk Mean Time (YMT) approach discussed in Whiting *et al*, (2000). The YMT was (arbitrarily) defined as the storage time when 20% of eggs supported growth in the albumen. The equation developed was based on experimental results for artificially contaminated eggs (Humphrey, unpublished; Humphrey, 1994). However, experimental data describing the time before growth of *S*. Typhimurium inoculated into eggs (Cogan *et al,* 2004) (Figure 4.5, full squares) suggest that the YMT may not reflect the behaviour of all *Salmonella* serovars in eggs. The significance of the latter observations is that the use of YMT as an indicator for time before growth of *Salmonella* in eggs may result in conservative risk estimates.

Table 4.1: Summary of SE and non-SE isolation from egg contents from multiple flocks

Location	Number of SE isolates	Number of non-SE isolates	Total number of eggs tested	Country of Origin	Source
Retail					
Contents	1	0	12540	Northern Ireland	Wilson *et al*, (1998)
Shells	2	6	12540		
Contents	16	2	83820	UK	Wall & Ward (1999)
Shells	103	17	83820		
Contents (graded)	na	0	20000	Australia	SARDI (2003, unpublished)
Shells (graded)	na	0	6476		
Off-farm					
Contents	63	10	140000	USA	Saeed (1998)
Shells	nt	nt			
Contents	178	20	647000	USA	Schlosser *et al*, (1999)
Shells	nt	nt			
Contents	6	16	~284715	Japan	Shirota *et al*, (2001)
Shells	nt	nt			

na – not applicable, nt – not tested

Whiting *et al,* (2000) noted that the logarithm of the YMT was linear with respect to temperature. A model describing the effect of temperature on YMT takes the form:

$$\log_{10} YMT = 2.0805 - 0.04217T$$

where YMT is in days and the temperature (*T*) is °C. The residual standard error for this model is 0.1524.

This model assumes no eggs would support the growth of *Salmonella* before expiry of the YMT. Conversely, after the YMT all eggs are assumed to have experienced yolk invasion and the potential for growth of *Salmonella*. The storage temperature of the egg controls the growth rate of the *Salmonella* in the yolk. Inspection of Figure 4.5 shows that the YMT approach underestimates risk prior to the expiry of the YMT, and overestimates the proportion of eggs supporting growth after the Yolk Mean Time. The dashed vertical line in Figure 4.5 is the predicted value (17.8 days at 20°C) from the USDA predictive YMT model. The effect of temperature on the YMT is presented graphically in Figure 4.6. The dashed lines in the figure represent the upper and lower 95% confidence intervals. Thus the YMT at 20°C may lie between ca. 10 and 35 days. Consequently 2.5% of eggs may show growth of *Salmonella* in as little as 10 days or as long as 35 days.

Figure 4.5. Relationship between time and susceptibility to support growth of *Salmonella* at 20°C arising from artificial internal contamination (■, results for *S. Typhimurium*- Cogan *et al,* 2004; □, results for SE - Humphrey, 1994; and solid and dashed line - USDA-FSIS, 1998).
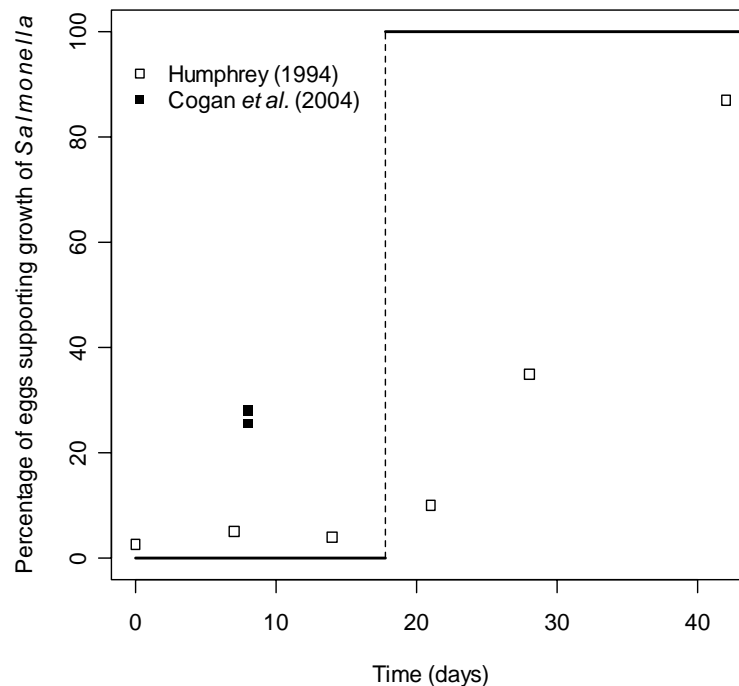
The FAO-WHO SE in Eggs QRA suggested that the USDA approach be modified by including the possibility that egg yolk could be invaded by SE during egg development (FAO-WHO, 2002). The result of the assumption of early yolk invasion was that about 3% of all eggs would support growth immediately after lay, while the remainder of internally contaminated eggs would follow the YMT equation. This modified approach reflects the observed low proportion of artificially contaminated eggs supporting growth soon after inoculation. However, the issue of over estimating risk for storage times beyond the YMT was not dealt with. Alternative model forms are therefore needed to more accurately describe the observed behaviour of *Salmonella* growth in eggs.

For the purpose of this report, estimates of the time before growth of *Salmonella* spp. in Australian shell eggs is assumed to be similar to that of SE. Yolk contamination at point of lay is assumed to not occur in Australian eggs. Consequently, estimates of the Yolk Mean Time remaining have been used as an indicator of time before growth of *Salmonella* in the content of shell eggs.

Growth Rates and Growth Rate Prediction
Published reports of the effect of temperature on the growth rate of *Salmonella* have been compiled in order to develop a mathematical model that allowed prediction of growth after the YMT was exceeded. Most reports available from the published literature described the growth of *Salmonella* in homogenised whole egg (Bradshaw *et al,* 1990; Ruzickova, 1994; Schoeni *et al,* 1995; Gast and Holt, 2000). To ensure that a statistically robust model was developed, additional growth rate data for *Salmonella* Typhimurium on chicken meat (Oscar, 2002) was used.

Figure 4.6: Plot of YMT vs storage temperature. The solid line shows the fitted model with confidence intervals (dashed lines). Data from Whiting *et al*, (2000).
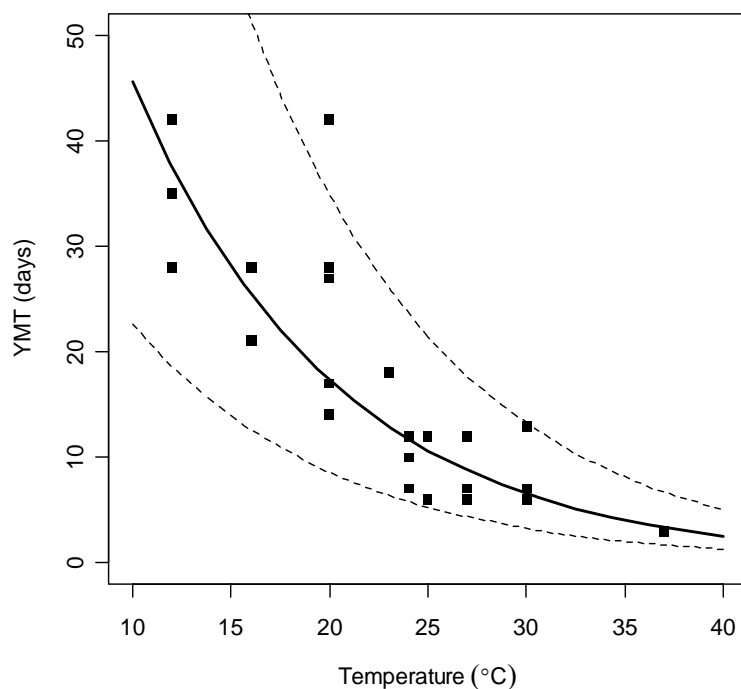
The predictive growth rate model selected was the cardinal temperature equation proposed by Rosso (Rosso *et al,* 1993; Delignette-Muller and Rosso, 2000).

$$k = k_{opt} \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]}$$

where k is the growth rate ($\log_{10}$ h^{-1}) at temperature T (°C), k_{opt} is the optimum growth rate, and T_{min}, T_{opt} and T_{max} are the predicted minimum, optimum and maximum temperatures of growth, respectively. The advantage of this model is that the equation parameters have biological significance. The results of the regression are presented in Table 4.2.

Table 4.2: Predicted values for the growth rate of *Salmonella* Typhimurium on chicken meat using the Rosso equation.

Parameter	Value	Units
k_{opt}	0.7039	$\log_{10}$ h^{-1}
T_{min}	5.567	°C
T_{opt}	39.76	°C
T_{max}	49.59	°C

The residual standard for the regression is 0.02997.

A comparison of the Rosso model predictions to the growth data for *Salmonella* in whole egg and yolk is presented in Figure 4.7. The 95% confidence intervals for this figure are shown. Most of the experimental data used fall within these confidence limits (including those describing growth on chicken meat). Thus the model predictions based on the Rosso equation are unlikely to lead to an underestimation of the observed growth rates of *Salmonella* in egg. Consequently, the Rosso equation was used to estimate growth rates of *Salmonella* in egg contents after the completion of the YMT.

Figure 4.7: Comparison of growth rates for *Salmonella* in whole egg and yolk with the prediction of the Rosso equation for the growth of *Salmonella* Typhimurium on chicken meat (Oscar, 2002) (solid line). Dashed lines represent the 95% confidence interval. Data from: Bradshaw *et al,* (1990), Gast & Holt (2000, 2001), Ruzickova (1994) and Schoeni *et al,* (1995).
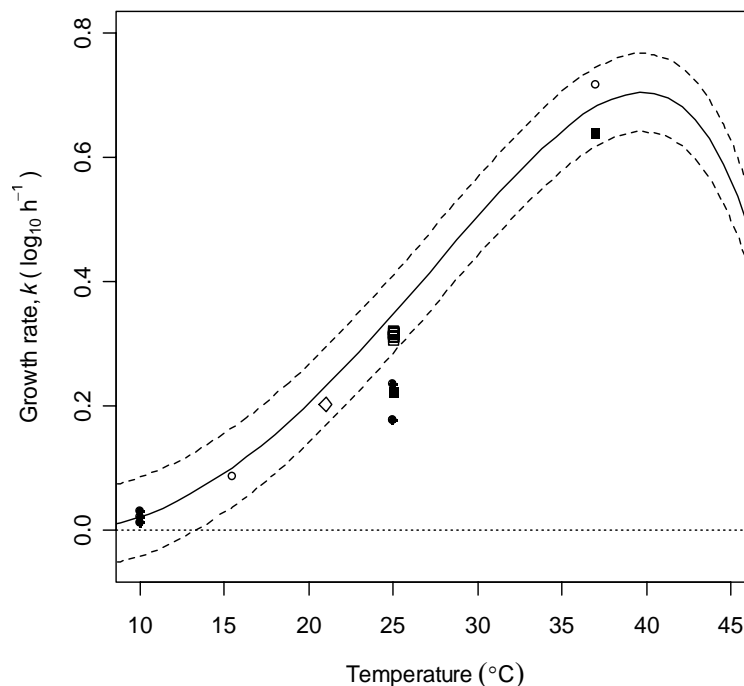
4.3.4   Exposure Assessment Model

The simulation model for the off-line production of shell eggs (eggs sent to a central grading facility) through to retail is based on the flowchart presented in Figure 4.8. This process was selected because it represents the longest chain between point of lay and the end of retail storage.

For each step in the chain, temperature and time distributions must be established. In the USDA-FSIS and Health Canada Exposure Assessments the majority of temperatures and times were based on expert opinion from industry representatives or personal opinion (USDA-FSIS, 1998; Health Canada, unpublished). Unfortunately, these opinions may have reflected beliefs rather than actual practice. This represents an important industry data gap for production and processing steps (FAO-WHO, 2002).

Information gathered from the surveys (Appendix 3) was analysed to determine suitable model inputs and the variability associated with those inputs. Briefly, the relevant survey results important for development of the Exposure Assessment are:

- Egg collection frequency (1 and ≥2 per day)
- Time eggs remain in the layer shed until collection
- Time to reach on-farm cool room after collection
- On-farm storage temperature
- On-farm storage time
- Transportation temperatures
- Transportation times

From the processor survey, information on the following model inputs was acquired:

- Storage temperature (pre- and post-processing)
- Storage time (pre- and post-processing)
- Grading floor temperature
- Processing times
- Wash water temperatures

A summary of the model inputs used for the development of the baseline model is shown in Table 4.3.

The underlying model structure used was based on Whiting *et al,* (2000). The major difference between the model presented here and that of Whiting *et al,* (2000) is the use of Australian production and processing data. In particular, where we have identified differences in practices, distributions (see Table 4.3) have been used to investigate the influence of these factors on risk. This enabled evaluation of the effects of on-farm and processing practices on the time before growth of *Salmonella* in eggs. The first five factors in Table 4.3, represent on-farm factors (time and temperature) and the sixth describes time before processing of eggs on the grading floor (Surveys indicated the temperature of all processing environments was approximately 16ºC). To investigate the effect of both on-farm and processing factors, the performance of the best 10%, median 10% and the worst 10% of farms or processors were analysed to develop statistical distributions as model inputs for the EA model. Collectively, these factors were grouped as the best 10%, median 10% and worst 10% on-farm and processing, giving a total of nine possible scenarios to examine. This enabled comparison of the effects of each category of factor (ie. on-farm *vs* processing) on time before growth of *Salmonella* and risk to consumer.

To examine the impact of egg production and processing practices on growth of *Salmonella* in whole shell eggs, we calculated the effects of different practices (eg egg collection frequency, storage time) on the YMT. Estimates of the predicted YMT for different production scenarios were used to calculate the time before growth of *Salmonella* can occur in eggs produced under best, median and worst on-farm and processing conditions.

The impact of storage times (up to 36 days) and temperatures (4, 16, 22 and 30ºC) on growth of *Salmonella* in contaminated eggs during retail display was then evaluated. Retail storage times and temperatures were selected to represent a range of conditions found in the Australian retail chain. The risk associated with three groups of foods containing eggs, for different degrees of cooking (uncooked, lightly cooked and well cooked) were then simulated. Risk estimates were calculated by combining the probability of consuming contaminated eggs with the probability of illness occurring following the consumption of a contaminated egg using the dose response model developed in Chapter 3.

Table 4.3. Summary of on–farm collection, distribution and handling conditions for shell eggs. These factors were used as inputs for development of the EA model. A full description of the model inputs are listed in Appendix 4.

Factor	Rating of industry practice	Distribution[1]
Egg collection frequency	Best 10%	Twice per day
	Median 10%	Once per day
	Worst 10%	Once per day
Time for eggs to reach the storeroom after collection (hours)	Best 10%	Triangle(0.1, 0.2, 1)
	Median 10%	Uniform(1, 3)
	Worst 10%	Triangle(1, 4, 10)
Storage temperature on-farm (°C)	Best 10%	Uniform(4, 10)
	Median 10%	Uniform(13, 16)
	Worst 10% [2]	Normal(26, 2)
Storage time on-farm (hours)	Best 10%	Log Normal(8.2, 5.8)
	Median 10%	Log Normal(46.1, 17.3)
	Worst 10%	Log Normal(65, 30.3)
Temperature during transportation off-farm (°C)	Best 10%	Uniform(10, 12)
	Median 10%	Uniform(14, 18)
	Worst 10% [2]	Normal(26, 2)
Time before processing at a central grading floor (hours)	Best 10%	Triangle(0.5, 6, 24)
	Median 10%	Triangle(18, 24, 48)
	Worst 10%	Triangle(72, 168, 336)

[1]The distributions and values used are as follows: Uniform(minimum, maximum); Triangle(minimum, mode, maximum); Normal(mean, standard deviation); Log Normal(mean, standard deviation)
[2]Assumed Summer Temperature.

4.4   Application of the Exposure Assessment Model

The outputs of the EA for shell eggs are: the number of *Salmonella* in the egg and the probability that an egg has supported growth; and age and percent of YMT expired for eggs at any particular stage of the supply chain relative to point of lay. The former is particularly relevant in estimating the risk associated with consumption of shell eggs (See Section 5).

4.5   Conclusions

The Exposure Assessment models developed in this section allowed an examination of processing and retail storage practices on growth and survival of *Salmonella* in eggs. In particular, the EA was used to determine the impact of processing and storage on growth of *Salmonella* in eggs at the production, processor, wholesale and retail stages.

There is probably little commercial egg producers and processors can do to reduce risk of contamination of eggs at point of lay. Nevertheless, depending on the temperature and length of storage at the wholesale and retail level, there is significant potential for growth of *Salmonella* in contaminated, commercially produced and graded eggs.

Figure 4.8: Process diagram for shell egg production and processing (after Whiting *et al,* 2000).
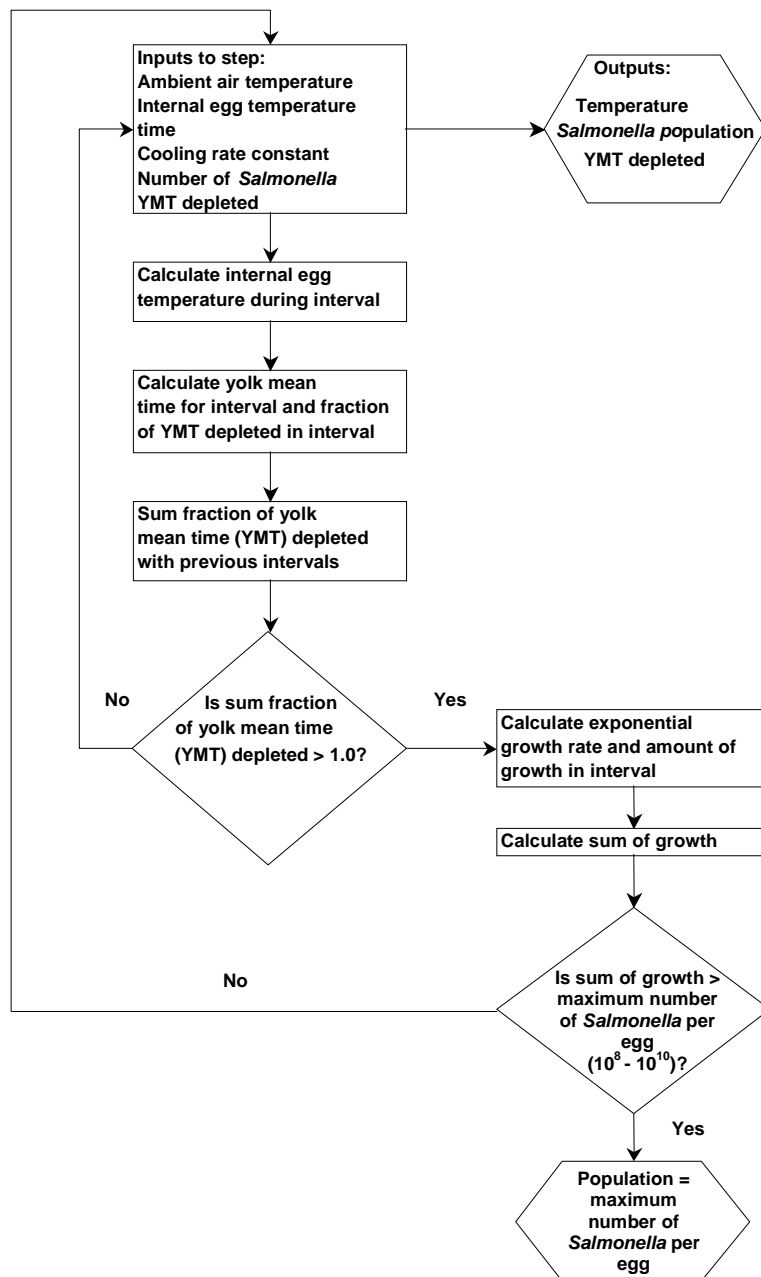
4.6 Summary

The purpose of Exposure Assessment (EA) was to:

1. Estimate the prevalence of *Salmonella* in eggs from Australian commercial producers
2. Predict the proportion of eggs which will support growth of *Salmonella* in egg contents prior to consumption
3. Estimate the number of *Salmonella* in eggs used for preparation of different food types. The information obtained is used in Section 5 to assess the public health outcome (illness).

5. Risk Characterisation of *Salmonella* in Shell Eggs

5.1 Introduction

This section describes the use of predictive modelling methods to characterise the risk associated with the consumption of shells eggs. Importantly, this report assumes that the risk to consumers posed by all *Salmonella* serovars is equivalent. This assumption is required because prediction of serotype involvement is not possible with available data. Consequently, risk estimates presented in this section are indicative of those expected for eggs contaminated by *S*. Typhimurium.

The Quantitative Risk assessment (QRA) model for *Salmonella* in eggs differs from previous risk assessments by the USDA-FSIS and the FAO-WHO in several key aspects:

- All *Salmonella* serovars associated with eggs have been considered to represent risk to the consumer[7]. This assumption is based on review of Australian outbreaks of Salmonellosis associated with foods containing eggs as an ingredient (Section 2).
- The assessment incorporated information from surveys of egg production, transportation and grading/processing practices, including temperature and time estimates gathered from point of lay to the end of wholesale storage. Where differences in egg handling practices on-farm and during processing (eg egg collection frequency, storage time on farm) were identified, the survey results were integrated to identify the worst, the median (middle) and best 10% of each practice.
- The influences of retail storage temperature and time on risk were explicitly considered. Four storage temperatures were included in the model development: 4°, 16°, 22° and 30°C. These temperatures represent refrigeration, cool room, ambient temperatures with air conditioner and warm summer temperatures, respectively. Storage times up to 36 days were considered.
- Risk estimates, expressed as cases of Salmonellosis per million servings, were developed for three common food types that contain eggs as a major ingredient and that are subjected to different degrees of cooking. The three cooking effects were uncooked (eg egg flips and some desserts), lightly cooked (eg egg based sauces) and well cooked (eg scrambled eggs). The selection of these foods was based on the analysis of outbreaks involving foods containing eggs as ingredients.

The approach taken in the QRA and the risk estimates developed, were designed to give the Egg Industry a greater understanding of how egg handling practices influence the risk associated with the consumption of shell eggs in Australia. In addition, this approach allows assessment of changes in risk estimates that are likely to occur during retail storage.

5.2 Risk Estimation for *Salmonella* in Eggs

5.2.1 Model Overview

Production Process models
To examine the impact of egg production practices on growth of *Salmonella* in whole shell eggs, we calculated the effects of different practices (eg egg collection frequency, storage time) on the YMT[8]

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[7] It is recognised that many serovars eg *S*. sofia, have not been implicated in Australian outbreaks. This may be because some or all of these serovars are not pathogenic to humans and do not represent a food safety risk. This issue is recognised as an uncertainty in model development. However, our assumption is supported by the lack of concordance between the very low incidence of contaminated, commercial flock environments with *S*. Typhimurium versus the relatively high incidence of this serovar associated with eggs that are an ingredient of foods implicated in outbreaks (Section 2).

(Section 4). Estimates of the predicted YMT for different production scenarios were used to calculate the time before growth of *Salmonella* can occur in eggs. To investigate the influence of production and processing practices on YMT, the production and processor survey results were sorted into three broad categories for each processing variables: best, median and worst.

Characterisation of Risk for Foods containing Eggs
In order to predict the risk of illness associated with consumption of foods containing contaminated eggs, the following inputs were needed for the risk characterisation model:
- The number of *Salmonella* cells per serving (Section 4);
- The prevalence of *Salmonella* contaminated eggs (Section 4); and
- The probability of illness from consumption of foods containing contaminated eggs/egg product (Dose response models from Section 3).

For eggs contaminated with *Salmonella*, the impact of production, processing and retail storage was evaluated to enable prediction of the number of *Salmonella* cells in eggs, up to and including preparation. The final numbers of cells were calculated for each of the three food products of interest and these estimates used as the infective dose for the Dose Response models. The Dose Response model was then used to calculate the probability of illness per serving for each food type.

Risk in this perspective is not expressed as an estimate of absolute illnesses per annum, but as estimated illnesses per million servings. However, these estimates are characterised by uncertainty arising from:
- Lack of information on retail storage
- Egg usage (shell egg *vs* other)
- The proportion of ungraded eggs used as shell eggs and other egg products
- The impact of catering *vs* home preparation on risk
- The adequacy of storage of foods containing eggs
- Pooling of eggs
- Uncertainties associated with the model (eg all contaminating *Salmonella* are pathogenic, uniform prevalence of *Salmonella*, all *Salmonella* behave the same as *S.* Enteritidis in the albumen of contaminated eggs).

As a consequence, the probability of contamination of servings and the number of cells present in contaminated eggs is used to calculate consumer risk.

5.2.2 Exposure Assessment – Evaluation of On-Farm and Processing Practices
From a producer and processor perspective, methods that allow analysis of the impact of industry practices on risk to consumers are particularly useful. For example, industry can assess the impact of changes or improvements in production practices on risk. Thus the predictive tools described in this report can, for example, be used to leverage industry sector specific changes or as an educational aid for management through to individual employees.

In this part of the report, on-farm and processing practices are evaluated and estimates of risk derived to illustrate the impacts of these industry practices. The Microsoft Excel add-in software @Risk version 4.5.2 (Palisade Corporation) was used for all EA simulations[9]. Results of scenarios for the on-farm and shell egg processing practices are presented in Figures 5.1 to 5.4. Four result sets are presented: (1) the median egg age, (2) median YMT expired, (3) percentage of eggs with YMT>1, and (4) the median time before growth occurs in eggs stored at 16°C and 22°C.

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[8] The Yolk Mean Time (YMT) is a parameter that is used to predict the time at which growth of *Salmonella* in eggs will occur. In this QRA model, the YMT of a freshly laid egg is zero. When the value of the YMT exceeds one, *Salmonella* will grow at a rate controlled by the internal temperature of the egg.
[9] Latin Hypercube sampling of distributions with a default setting of 100, 000 iterations was used for all simulations. Convergence was monitored every 500 iterations and stopped when statistics changed by <1.5%.

Each of the Figures 5.1 to 5.4 illustrate results of the nine scenarios (3 on-farm x 3 processing factors) and describe the effects of best, median and worst case practices for on-farm and grading (processing) of shell eggs. The predicted median age of eggs (in days) at the beginning of retail storage associated with each of the nine scenarios is presented in Figure 5.1. Median egg age ranges from 3 days for best practice on-farm handling and processing times to about 14 days for worst case handling and processing practices. Significantly, the longer eggs are maintained in production and wholesale processing environments, the shorter the residual YMT and hence a shorter time before potential growth of *Salmonella* in contaminated eggs, and concomitantly, the shorter the safe shelf life.

Figure 5.1: Predicted median egg age at the end of wholesale storage (or beginning of retail storage) that result from the best, median and worst on-farm and processing scenarios.
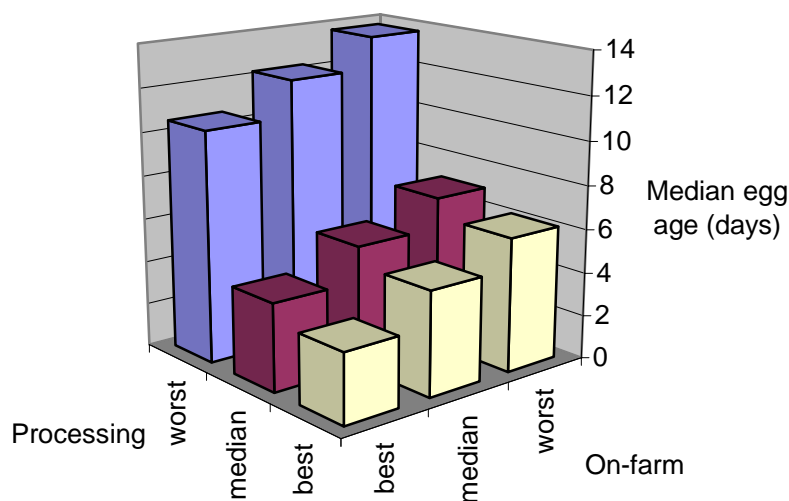
A summary of the model outputs for the proportion of the YMT expired by the beginning of retail storage is presented in Figure 5.2. Estimates of the proportion of YMT expired range from about 0.2 to nearly 0.83. As might be expected, the 'worst/worst' (worst on-farm and worst processing conditions) scenario results in the greatest proportion of YMT expired. This indicates that most eggs in this 'worst/worst' group are close to supporting the growth of *Salmonella* in the eggs at the start of retail storage. Interestingly, the other four scenarios that include a worst case input have values of median YMT in the range 0.48-0.55, despite the 'worst case' processing eggs being about twice as old as the 'worst case' on-farm eggs (for median and best processing conditions). This effect is a result of the high ambient on-farm storage and transportation temperatures[10] experienced by eggs subjected to 'worst' on-farm practices. The high storage temperatures for short periods (on-farm) are often matched by the long storage times at lower temperatures experienced during processing and storage. Thus good processing practices ameliorate the effects of poor on farm practices and *vice versa*. However our models indicated there is no substitute for 'best' practice both on farm and during processing. Importantly, Figure 5.2 highlights the importance of understanding the influence of both storage temperature and storage time on egg-handling and the implications for risk to consumers.

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[10] The on-farm storage temperatures used in the QRA are reflective of summer temperatures that might be experienced in Australia.

Figure 5.2: Predicted median Yolk Mean time (YMT) expired at the beginning of retail storage (days) for best, median and worst on-farm and processing scenarios.
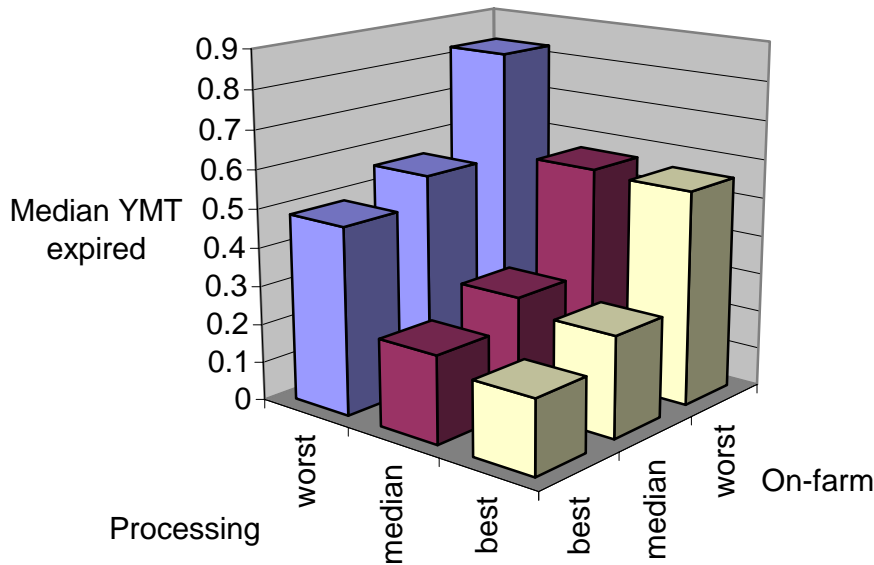
Of greater interest is the proportion of contaminated eggs that would have supported growth of *Salmonella* at the start of retail storage *i.e.* those eggs that have YMT≥1 (Figure 5.3a). No *Salmonella* growth would be expected to occur in contents for the majority of eggs (those subjected to 'median' and 'best' on-farm and processing conditions) prior to beginning retail storage. However, for the 'worst/worst' case over one third of eggs are predicted to have experienced a temperature history sufficient to exceed the YMT, with subsequent growth of *Salmonella* in contaminated eggs. For other 'worst' case scenarios, the significance of predictions shown in Figure 5.3a is less clear. One approach to assess the significance of these data is to examine the statistical bounds for YMT of eggs at the end of wholesale storage (see Figure 5.3b). This analysis clearly indicates that a significant proportion of eggs derived from 'median/worst', 'worst/best', 'worst/median' and 'worst/worst' on-farm – processing conditions will have YMT>1 and hence capable of supporting growth of *Salmonella* should those eggs be contaminated. All other eggs from other on-farm and processing scenarios are predicted to have YMT<1 at the end of wholesale storage.

This effect is also illustrated in Figure 5.4 where the impact of on-farm and processing practices on the predicted median time before growth of *Salmonella* in contaminated retail eggs stored at 16°C and 22°C are compared. Although the profile of median time before growth is the same for both retail temperatures, an increase in the retail storage from 16°C to 22°C halves the median predicted time before growth of *Salmonella* in contaminated eggs. The impact of on-farm and processing practices on median time before growth, for retail eggs stored at a range of temperatures is shown in Table 5.1. Data shown in this table clearly identify combinations of on-farm, processing and retail storage temperatures that impact on median time before growth of *Salmonella* in contaminated eggs.

Figure 5.3a: Predicted percentage (%) of eggs with YMT>1 at the end of wholesale storage that result from the best, median and worst on-farm and processing scenarios.
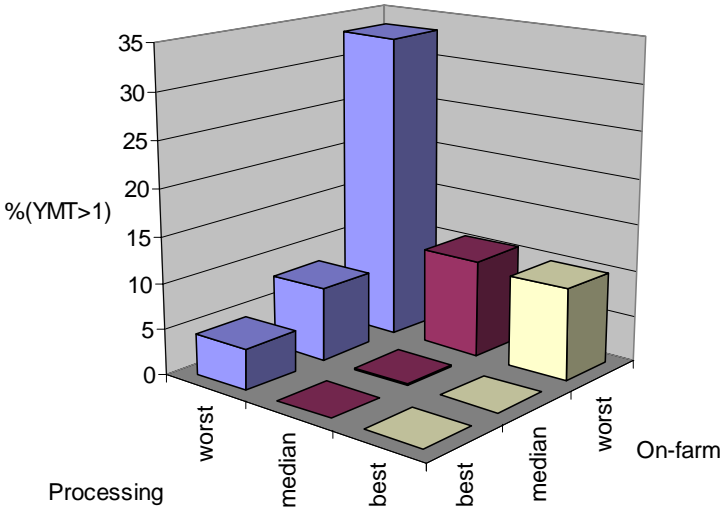
Figure 5.3b: Predicted range of YMT at the end of wholesale storage that result from the best (b), median (m) and worst (w) on-farm and processing scenarios. Data shown represents the median YMT and 5th and 95th percentiles. The horizontal line represents YMT expired. YMT>1 indicates a potential for growth of *Salmonella*.
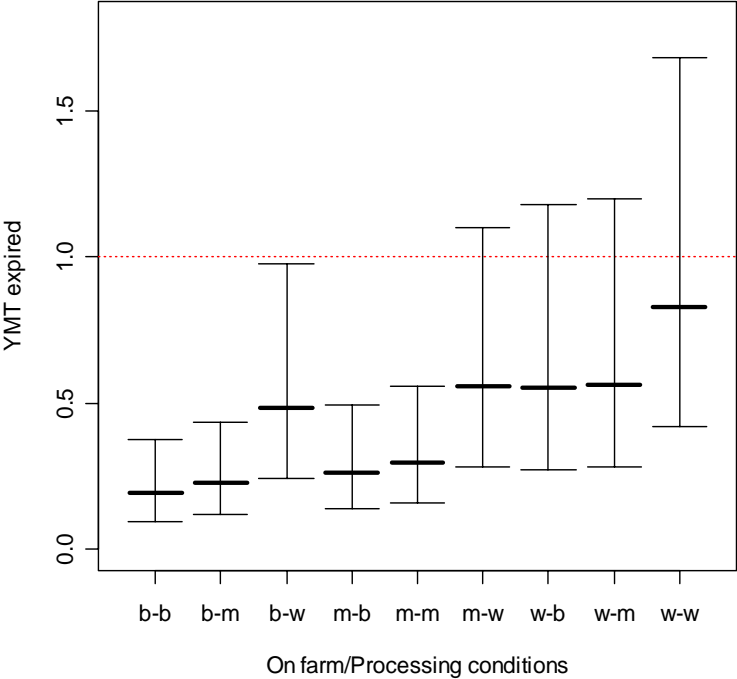
Figure 5.4: Predicted median time from end of wholesale storage before growth of *Salmonella,* for eggs stored at retail at 16°C and 22°C. Data is shown for eggs subjected to the 'best', 'median' and 'worst' on-farm and processing practice scenarios.
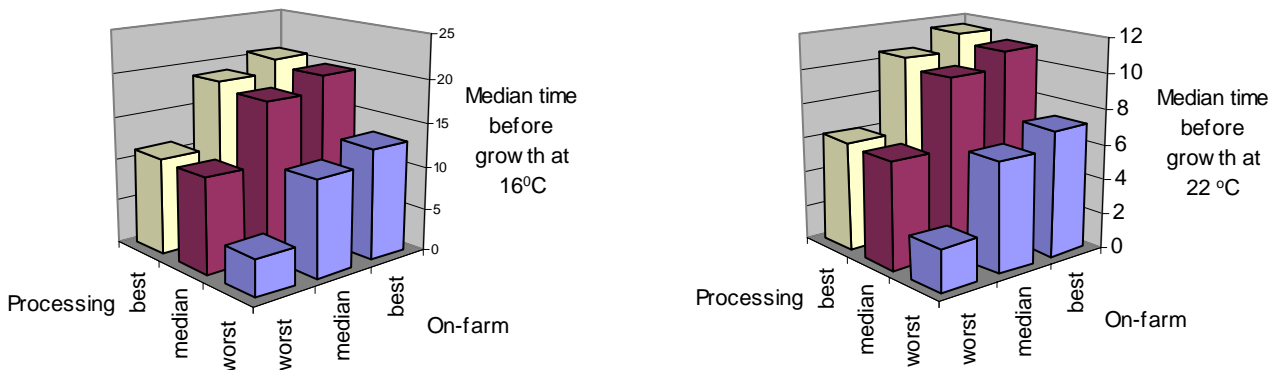
Table 5.1: Predicted median time before growth of *Salmonella,* for eggs from end of wholesale storage and stored at different retail temperatures. Data is shown for eggs subjected to the 'best', 'median' and 'worst' on-farm and processing practice scenarios.

On-farm Conditions	Processing Conditions	Retail Storage Temperature			
		4°C	**16°C**	**22°C**	**30°C**
Best	Best	66.2	20.5	11.5	5.3
Best	Median	63.4	19.7	11	5.1
Best	Worst	41.4	12.9	7.2	3.3
Median	Best	60.4	18.8	10.5	4.8
Median	Median	57.4	17.9	10	4.6
Median	Worst	35.7	11.1	6.2	2.9
Worst	Best	36.0	11.2	6.2	2.9
Worst	Median	35.0	10.9	6.1	2.8
Worst	Worst	13.9	4.3	2.4	1.1

5.2.3 Characterisation of Risk for Foods containing Eggs

The dose response model (developed in Section 3) and the exposure assessment (Section 4) can be combined to characterise the potential risk of Salmonellosis posed by consumption of foods containing eggs. In particular, this approach provides a capacity to assess the impact of single or multiple mitigations on consumer risk under current production and processing procedures.

To illustrate this approach we have modelled the effects of on-farm and processing practices and retail storage conditions on risk associated with consumption of uncooked foods containing eggs. The rationale for our choice is based on observation that uncooked food vehicles that are repeatedly incriminated in outbreaks include egg/milk drinks, mayonnaise and desserts (Section 2). When

uncooked foods are consumed by susceptible[11] individuals, this population sub-group are more likely to be at risk. However, for completeness, we also show the effect of cooking on risk.

Risk associated with eggs at end of wholesale storage
Figure 5.5 shows expected estimates of the number of illnesses per million servings of uncooked foods containing eggs used at the end of wholesale storage. This figure also shows the effect of different on-farm and processing practices on estimated illnesses. The estimates of illnesses range from <4 per million servings for median and best practice scenarios, to >6 per million servings for worst practice scenarios. It should be noted that a low number of illnesses are expected, even for servings containing contaminated eggs where limited growth of *Salmonella* has occurred[12].

Figure 5.5: Estimated illnesses per million servings of uncooked foods containing eggs from the end of wholesale storage. This figure is a pair with Figure 5.3a (Predicted percentage (%) of eggs with Yolk Mean time (YMT)>1). As the YMT expires and *Salmonella* begins to grow in contaminated eggs, the risk (= illnesses) increases.
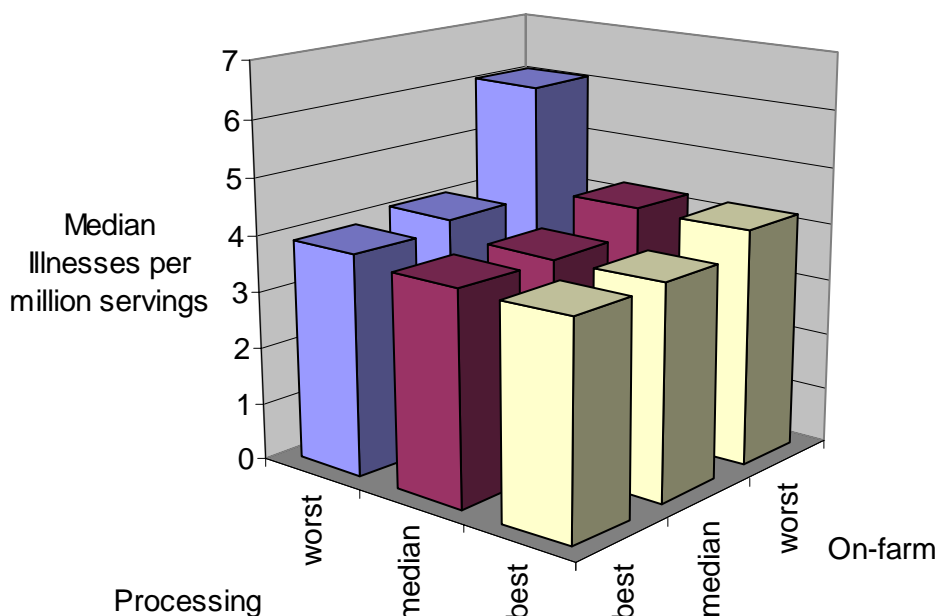
Whereas, Figures 5.1 to 5.5 present estimates of various indicators of risk for eggs from point of lay through to the end of wholesale storage, the scenarios presented in the following part of the report were chosen to highlight the capabilities of the QRA model and to give greater insight into how changes in handling practices influence risk through time. This approach represents a significant difference from other Risk Assessments (eg USDA-FSIS and FAO-WHO). A number of factors have been selected for detailed analysis: production and processing handling, cooking effect and retail storage temperature, level of contamination of eggs and changes in prevalence of contaminated eggs. The analyses presented are not exhaustive, but as examples they highlight the application and range of scenarios that may be assessed.

Impact of on-farm and processing practices on risk
The impact of on-farm and processing practices on risk are illustrated in Figure 5.6. This figure shows estimates of risk for uncooked foods containing eggs stored at 4, 16, 22 or 30ºC during retail. When

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[11] Susceptible individuals are defined as aged <5 years and >65 years and the immuno-compromised (Section 3).
[12] This effect is predicted by the dose response curves developed in Section 3, Table 3.3.

eggs subjected to median on-farm and processing practices are stored at 4ºC during retail and are used to prepare foods, no increase in risk is observed, even after extended retail storage. However, eggs prepared under the worst 10% of on-farm and processing practices, represent an increased risk. The increase in risk over the first 14 days of storage after point of lay are a direct outcome of those practices and correspond with the estimates shown in Figure 5.5. Subsequent retail storage of these eggs at 4ºC during retail limits risk.

As storage temperature is increased, the time required to exceed the YMT is decreased. Once the YMT is exceeded, growth of *Salmonella* is expected in contaminated eggs for scenarios where eggs are stored at retail at 16, 22 or 30ºC. Estimates of illness increase to a maximum of about 36 cases per million servings at the end of retail storage. This observation is due to the assumption that the prevalence of *Salmonella* contaminated eggs is the same. Thus once numbers of *Salmonella* in contaminated eggs have risen to levels required to deliver an infectious dose, all consumers of contaminated eggs will become ill.

Interestingly, the models indicated that eggs subjected to best practice on-farm and during processing resulted in greater numbers of illnesses than median or worst case practice scenarios when stored at 30ºC for up to 15 days after lay. This apparently paradoxical observation is expected since we have assumed that the prevalence of *Salmonella* in all eggs is the same and so eggs sourced from different operations have the same potential to cause illness. Since eggs from best practice operations reach retail sooner than worst practice operations (longer on-farm and processing storage), storage at 30°C leads to growth of *Salmonella* in contaminated eggs sooner than eggs sourced from other operations.

Effect of cooking on risk
Clearly uncooked foods containing raw egg will present the greatest risk to consumers. The level of risk will also be affected by:
- on-farm and processing practices, with the greatest risk involving eggs from a 'worst/worst' scenario;
- egg storage temperature; and
- the length of storage.

Figure 5.7 shows the effect of cooking on predicted illnesses per million food servings. The figures compare the outcomes for foods containing eggs (median on-farm and processing practices) stored at retail at 4, 16, 22 or 30°C and subjected to (1) no cooking, (2) light cooking and (3) substantial cooking (well cooked)[13]. Irrespective of the manner of cooking, risk associated with foods containing eggs is minimised by retail storage of eggs at 4°C. Risk increases by ~8 fold for uncooked and lightly cooked foods prepared from eggs stored at other temperatures once the YMT has been exceeded. However, well-cooked foods, such as scrambled eggs, are predicted to represent little or no risk because the cooking step inactivates all *Salmonella*.

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[13] The definitions of lightly cooked and well cooked are as described by Bates *et al,* (1995) and Humphrey *et al,* (1989). Light cooking: boiled 4 minutes, fried ('sunny side up') or microwaved; Well cooked: hard boiled or scrambled, cooking as for cakes and biscuits.

Figure 5.6: Comparison of production, processing (worst 10%, median 10% and best 10%) and retail storage temperature on estimated illnesses per million servings for eggs stored at different temperatures. Data shown is for uncooked foods.
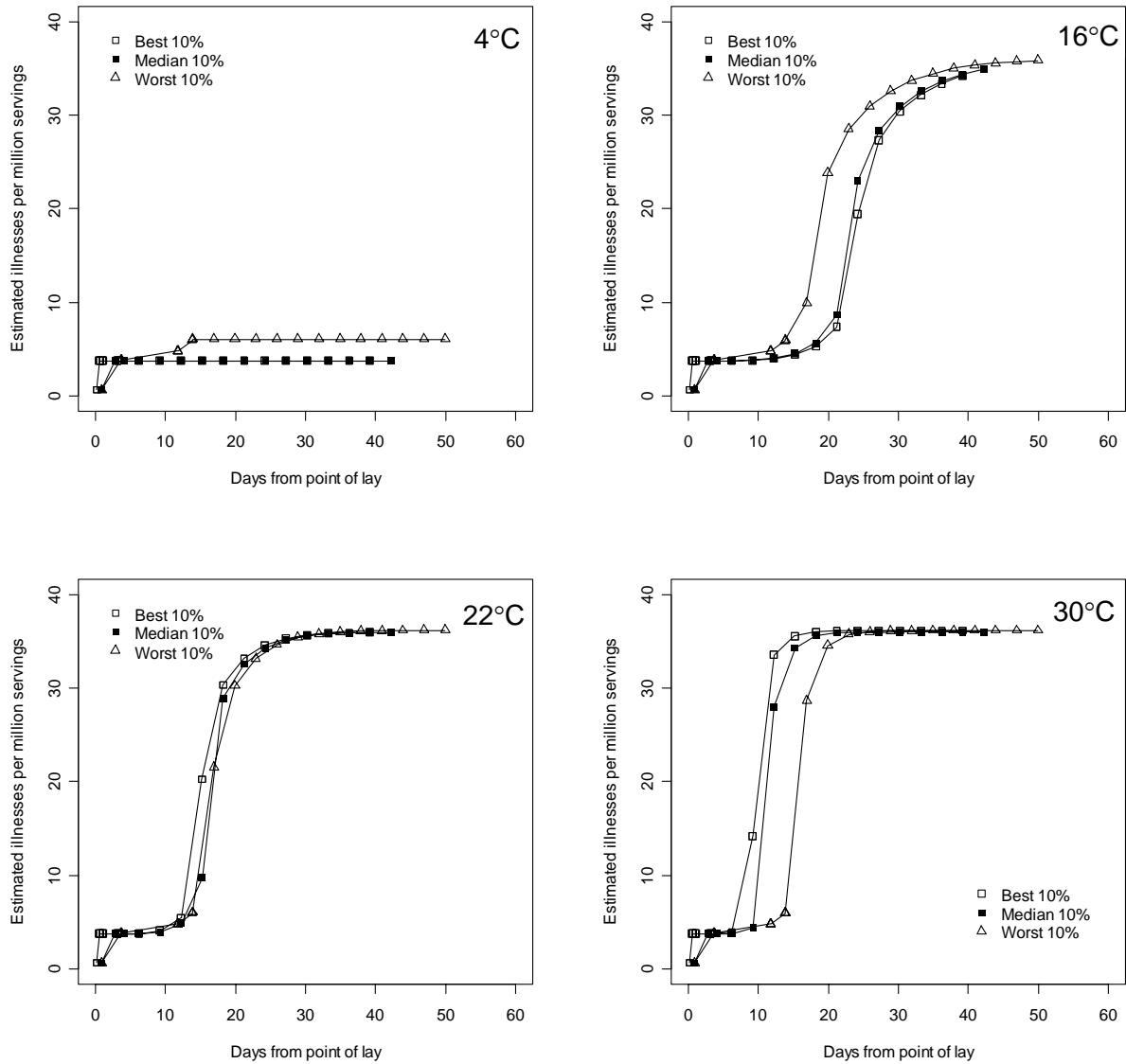
Figure 5.7: The effect of storage temperature of retail eggs on estimated illnesses per million servings for uncooked, lightly cooked and well cooked foods. Each panel represents risk associated with. Median on-farm and processing practices are assumed.
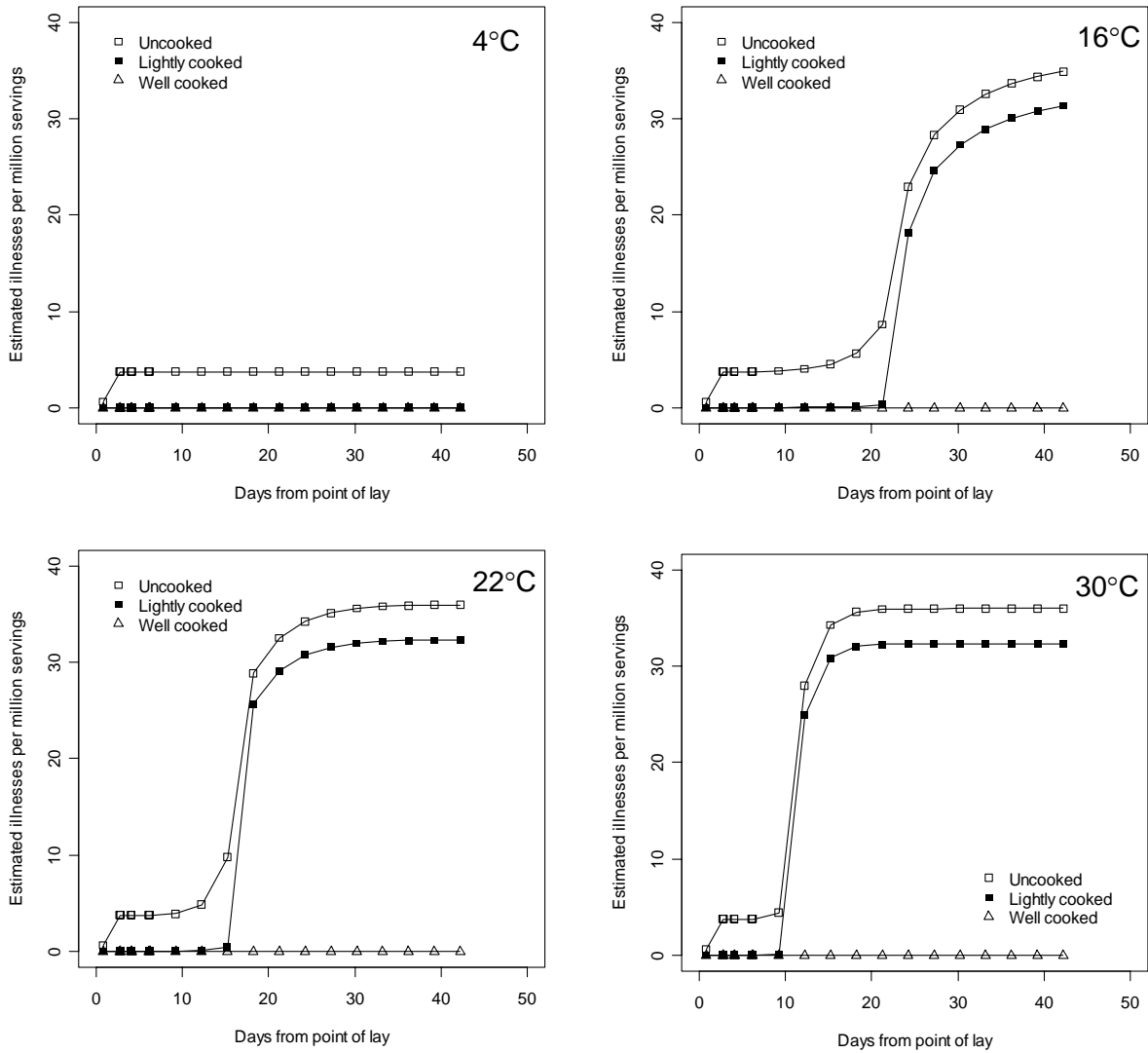
Variation associated with risk estimates

Figure 5.8 shows results obtained when estimates of illnesses are modelled for eggs stored in retail at 4, 16, 22 or 30ºC and used to prepare uncooked foods such as egg flips. These figures clearly illustrate the effects of storage temperature on risk as described previously. However, these figures also show the variation associated with the estimates of illness. Plots of the 5th and 95th percentiles for each temperature profiles are presented. As would be expected, under egg storage conditions that allow growth of *Salmonella*, considerable variation exists for risk estimates. At storage temperatures that do not allow growth of *Salmonella*, the precision of the estimates is higher.

Figure 5.8: Comparison of different retail storage temperatures on estimated illnesses per million servings of uncooked foods. Median on-farm and processing practices are assumed.
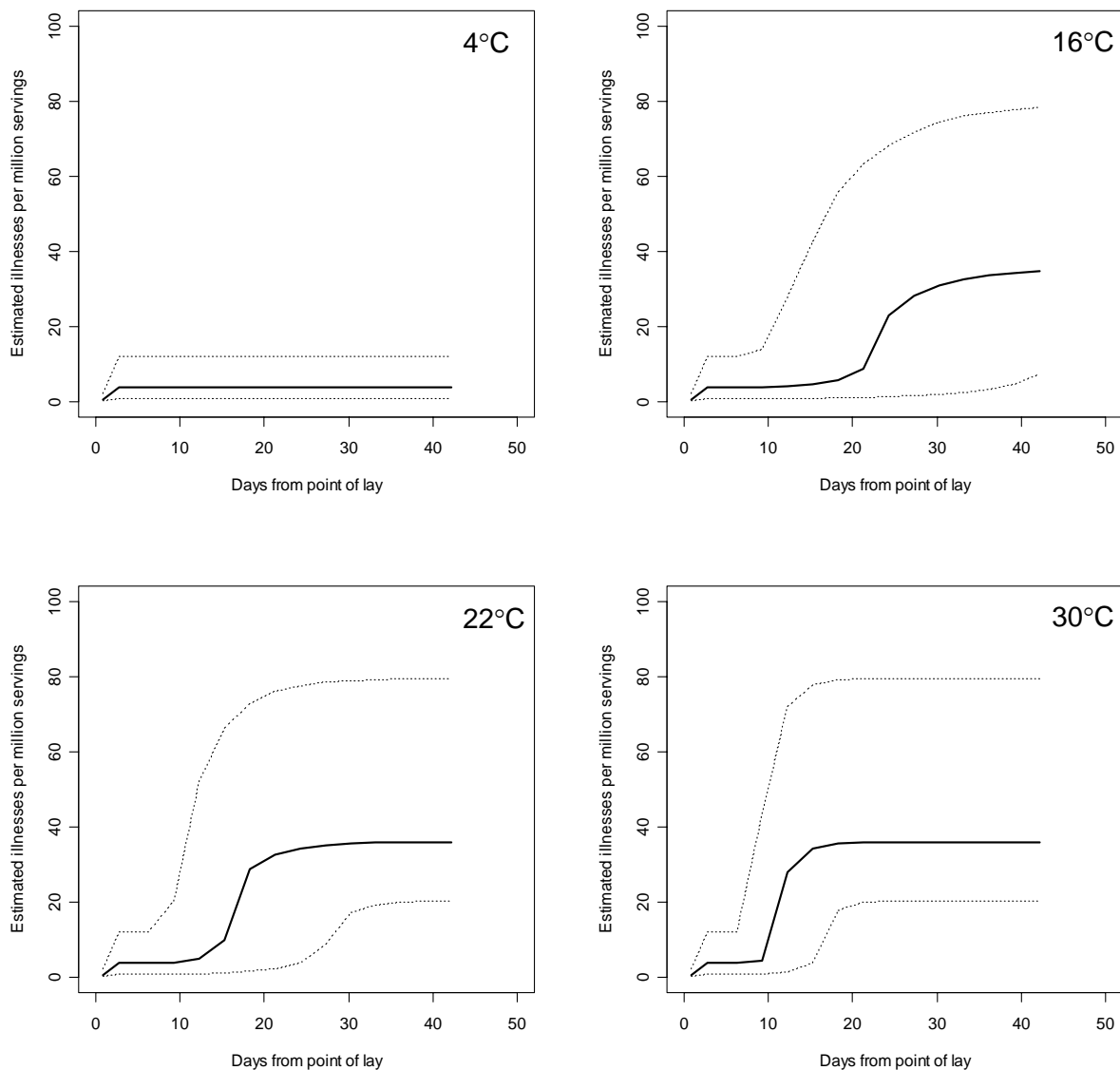
Impact of numbers of Salmonella per infected egg on risk

For well cooked foods containing eggs, the risk to consumers is negligible since the cooking effect is sufficient to greatly reduce the dose of *Salmonella* cells and hence the prevalence of contaminated servings consumed. Consequently, the numbers of *Salmonella* in infected eggs has little impact on risk for well cooked foods. The situation for uncooked (or lightly cooked foods) however, is quite different. Figure 5.9 shows the effect of numbers of *Salmonella* in shell eggs on risk estimates for uncooked foods containing eggs maintained at 16°C during retail storage. This figure illustrates that risk estimates for uncooked foods prepared using retail eggs, are dependent on numbers of *Salmonella* in contaminated retail eggs stored up to ~20 days (best observed when data is plotted on a log scale). At storage times greater than 20 days, the contribution of numbers of *Salmonella* per egg to risk is less clear since growth of *Salmonella* in infected eggs leads to illness in all individuals who consume the food. In other words, the number of illnesses per million servings approaches the prevalence of *Salmonella* contaminated eggs.

Figure 5.9: Effect of initial numbers of *Salmonella* in contaminated eggs immediately after lay, on estimated illnesses per million servings of lightly cooked foods. Eggs stored at 16°C. Median on-farm and processing factors assumed. Data plotted using arithmetic (left panel) and logarithmic (right panel) scales.
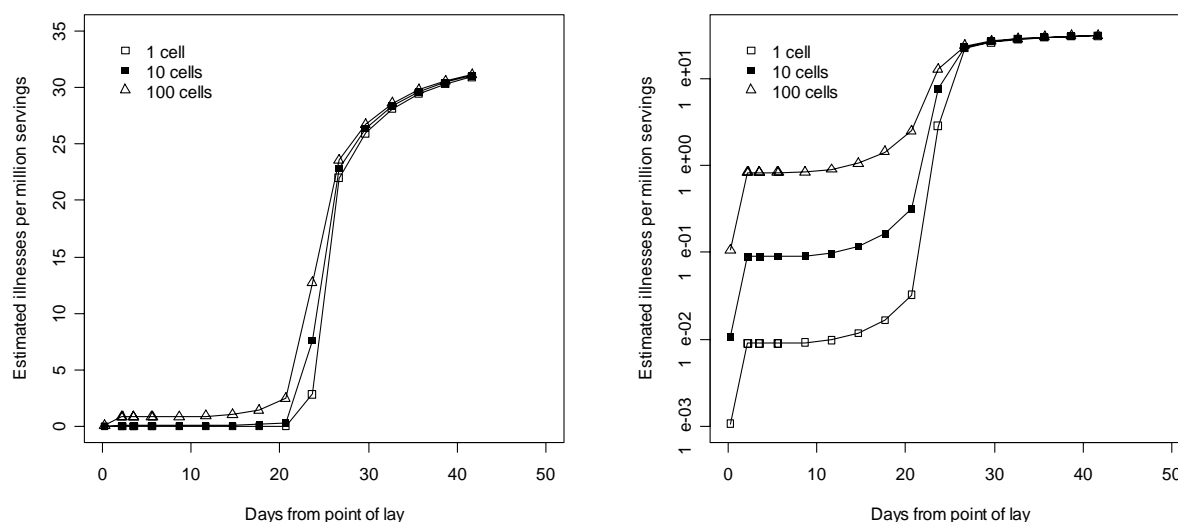
Association between prevalence of Salmonella contaminated eggs and risk

Figure 5.10 illustrates the association between prevalence and risk, for uncooked foods containing retail eggs. The solid line shows the median risk associated with eggs at different times during storage at 30°C. The horizontal bold dashed line represents the mean prevalence of *Salmonella* contaminated eggs. The close match between the prevalence of contaminated eggs and the rate of illness (for eggs stored longer than 15 days), indicates that nearly all contaminated eggs will result in illness if used in uncooked foods. The influence of on-farm and processing practices will have no influence on the risk to consumers for very old eggs.

Figure 5.10 also shows the variation (5th and 95th percentiles) associated with risk estimates and the estimate of prevalence. The range of these lines gives a visual indication of the uncertainty in the estimation of the prevalence of *Salmonella* contaminated eggs. The median estimated illnesses per million servings (and 5th and 95th percentiles) for the uncooked foods containing eggs from the end of the retail shelf life (taken to be 36 days) are: 36, 20 and 79, respectively. Similarly, the median prevalence of *Salmonella* contaminated eggs (and the 5th and 95th percentiles) are 40, 23 and 89 respectively.

From the perspective of factors under control by the egg production industry, risk is dependent on prevalence of *Salmonella* contaminated eggs. This relationship is illustrated in Figure 5.11. Irrespective of the duration of retail storage, a fold increase (or decrease) in prevalence will result in an approximately fold increase (or decrease) in risk (expressed as illnesses per million servings)[14]. Thus if the prevalence of *Salmonella* contaminated eggs is increased by a factor of 10, the risk will be increased by a similar amount. For eggs taken from the end of retail storage, the median illnesses per million servings will increase from about 36 to about 360. Similarly, if prevalence is decreased by a factor of 2, the number of illnesses will decrease from 36 to about 19.

Figure 5.10: Dependence of risk on prevalence of *Salmonella* contaminated eggs. Estimated illnesses per million servings are shown (solid continuous line together with the 5th and 95th estimation percentiles, thin dashed lines) for uncooked foods stored at 30°C in the retail environment. The horizontal lines are the estimated median prevalence of *Salmonella* contaminated eggs together with the 5th and 95th percentile estimates.
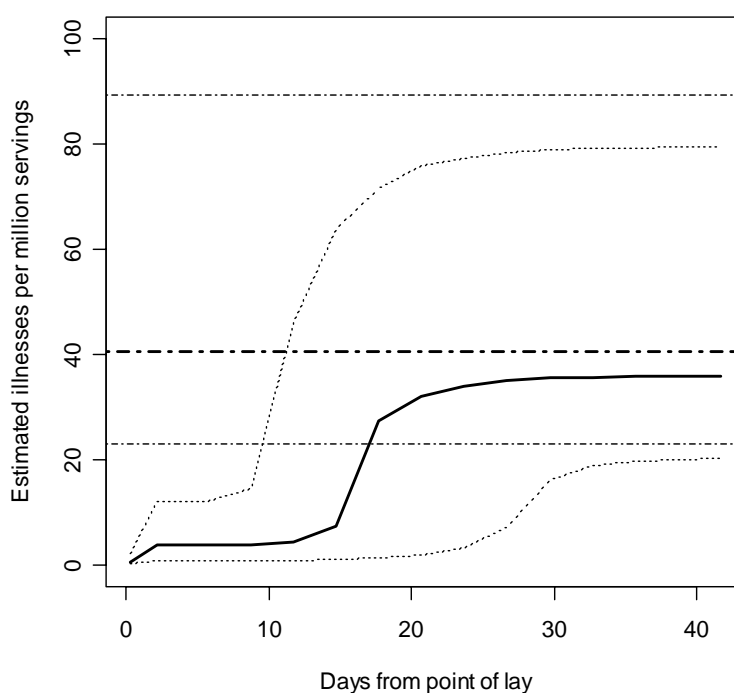
Relative risk assessment
Relative risk, the ratio of two risk estimates, is a simple measure of the difference in risk between scenarios. A value of relative risk < 1 indicates that the risk is reduced, a value > 1 indicates that the risk has increased, while a value of 1 indicates no change in risk between scenarios.

Figures 5.5-5.11 show the development of risk from the consumption of shell eggs during retail storage. Of particular interest is the change in risk for different food types made using eggs stored under different storage temperatures. The median risk estimates for eggs handled under the same on-farm and processing conditions at the start of retail storage scenarios will be the same; the relative risk of all scenarios is 1. As retail storage time increases it is apparent that temperature has an impact on risk for each of the food types considered in this report (Figure 5.7).

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[14] Fold increases (or decreases) in prevalence do not result in a 1:1 fold increase (or decrease) in risk. This is due to the probablistic modelling approach used in the development of this risk assessment.

Figure 5.11: Relationship between risk and prevalence of *Salmonella* contaminated eggs for uncooked foods containing eggs stored at 16°C assuming median on-farm and processing practices. Each of the plots are relative to baseline estimates of prevalence (median of 40 contaminated eggs per million).
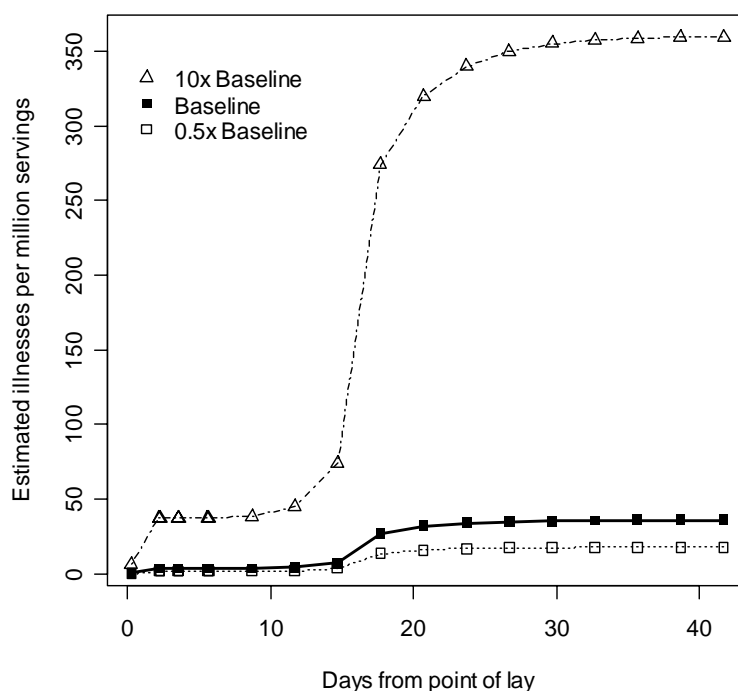
Relative risks for foods (=estimated risk at a specified storage temperature/estimated risk for storage at 22°C) for each of the three food groups made from eggs stored for 12 days and 36 days are presented in Tables 5.2 and 5.3, respectively. The relative risk for each food type at 22°C is 1, as expected.

It is important to note that the median estimates of risk for each of the three food types is not the same at 22°C. The actual risk estimates, in predicted illnesses per million servings after 36 days of retail storage, are 36.0, 32.3 and 2.1×10^{-4} for uncooked, lightly cooked and well cooked foods, respectively.

For each of the food types stored for 12 days at retail, the higher the storage temperature, the greater the relative risk. For uncooked foods, the relative risk between storage at 4 and 22°C is less than a factor of ten. By comparison the well cooked foods have a over a million-fold difference in relative risk. Interestingly, storage at 16°C only leads to a doubling of risk in comparison to refrigerated storage at 4°C.

Table 5.2: Relative risk estimates (comparison to risk at 22°C) for different food types for retail storage of shell eggs stored for 12 days at 4, 16, 22 and 30°C. Median on-farm and median processing conditions are assumed in each case.

Retail storage temperature (°C)	Uncooked food	Lightly cooked foods	Well cooked foods
4	0.14	2.4×10^{-3}	4.6×10^{-7}
16	0.19	4.5×10^{-3}	1.3×10^{-6}
22	1	1	1
30	1.3	1.3	7.8

By 36 days in retail storage there is little difference in the relative risk for eggs stored at 16, 22 or 30°C. This is due to the growth of *Salmonella* in the majority of contaminated eggs even at 16°C.

Only when eggs are stored at 4°C where the low temperature inhibits growth of *Salmonella,* is the relative risk similar to the values predicted after 12 days of storage.

Table 5.3: Relative risk estimates (comparison to risk at 22°C) for different food types for retail storage of shell eggs stored for 36 days at 4, 16, 22 and 30°C. Median on-farm and median processing conditions are assumed in each case.

Retail storage temperature (°C)	Uncooked food	Lightly cooked foods	Well cooked foods
4	0.10	1.8×10^{-3}	5.5×10^{-8}
16	0.97	0.96	0.76
22	1	1	1
30	1	1	1

5.3   Conclusions

In this section, risk to consumers of consuming foods containing shell eggs contaminated with *Salmonella,* has been evaluated by three separate processes.

Firstly, the evaluation of outbreaks potentially attributable to eggs indicated that there is little evidence of risk from consumption of commercially produced and graded eggs for consumers (Section 2). This is supported by the lack of concordance between the very low incidence of contaminated, commercial flock environments with *S*. Typhimurium versus the relatively high incidence of this serovar associated with eggs that are an ingredient of foods implicated in outbreaks (Section 2). In addition, uncooked foods, or foods containing raw egg/egg products seem to be an important contributing factor in egg-related outbreaks. Uncooked foods repeatedly implicated in outbreaks include egg/milk drinks, mayonnaise and desserts. For these reasons the estimation of the probability of illness focused on the consumption of contaminated egg/milk drinks, mayonnaise and uncooked desserts. Food histories recorded during investigations of outbreaks, indicated these foods are often subjected to temperature abuse. Thus poor catering practices could lead to outbreaks of illness amongst susceptible individuals in the population when foods containing raw egg are used.

Secondly, data from our pilot survey of the prevalence of *Salmonella* in and on eggs provided a substantive indication that the prevalence of *Salmonella* in commercial produced and graded eggs for *Salmonella* is low and reflects levels reported internationally.

Thirdly, we have prepared dose response and exposure assessment models to allow risk managers to examine impact of changes to process models on risk of Salmonellosis. In this section we have shown that risk estimates expressed as illnesses per million servings, are dependent on on-farm and processing practices, the temperature history of eggs during storage, method of food preparation and the susceptibility of the consumer population. Scenarios that minimise risk involve:
- Median production practices (or better);
- Minimisation of the prevalence of *Salmonella* contaminated eggs;
- Storage at refrigeration temperatures; and
- Where possible, a cooking step for foods containing eggs, particularly where egg containing foods are fed to at risk individuals.

5.4   Risk Management Options

Critical outcomes of this work that relate directly to options available to policy makers for managing risk include:
- Introduction of new mitigation strategies into commercial flocks producing graded eggs is unlikely to reduce the risk of human Salmonellosis. The biology of *Salmonella* in these

production systems, the evidence from human surveillance, and the evidence from animal surveillance suggest that hygiene measures in these circumstances are adequate.

- Refrigeration of eggs after processing and during wholesale and retail storage could substantially reduce the risk of human Salmonellosis. This option exerts the greatest impact on reduction of risk to the consumer and the industry, compared with other candidate mitigation strategies.

- A high risk of human Salmonellosis may occur where caterers service vulnerable individuals in our population. To reduce this risk it is essential for caterers to avoid using eggs that are more likely to be contaminated with *Salmonella*. Epidemiological evidence suggests that in these circumstances several types of eggs should be excluded from use. These include: cracked eggs, ungraded eggs and all eggs from flocks where an authoritative quality assurance program is not in place (eg backyard and some small commercial flocks) FSANZ food standards should be reviewed to reflect this need. Institutions that care for individuals susceptible to food-borne Salmonellosis should be advised of these measures.

- Uncooked foods fed to vulnerable individuals should not contain unpasterurised egg or egg products.

- General improvements to hygiene and food storage practices in catering operations. Special attention on the prevention of cross-contamination and temperature abuse of egg products is needed.

5.5 Uncertainty, Data Gaps and Future Research Needs

The systematic application of risk assessment has resulted in the identification of information gaps concerning the role of particular hazards as causes of food-borne illness. In particular, the level of certainty of attribution of product-hazard combinations to outbreaks, and uncertainty in relation to consumer exposure, is of concern.

Information (which is often unavailable) to assist in attribution of outbreaks where foods contain eggs include:
- Source and type of eggs, including grading status;
- The status of other ingredients used in the implicated food (eg herbs and spices); and
- Follow-up farm investigations.

However, through OzFoodNet, there is opportunity for improvement through the adoption of egg industry specific questions (eg Appendix 1) to be used when foods, in which eggs among other ingredients, are initially suspected as sources of *Salmonella*.

In only a few publicly available reports of food-borne outbreaks of Salmonellosis, was the case rate or the level of contamination determined. If useful insight into the dose-response relationship required for risk assessment is to be obtained, investigators should be encouraged to place greater emphasis on these epidemiological criteria whenever possible.

Salmonella serovars commonly found in stockfeed ingredients appear to cause infrequent and transient flock infections, but are implicated in a minority of food-borne outbreaks in which eggs are implicated. Given this background, it is not understood why *S*. Typhimurium which occurs relatively infrequently on-farm, can be responsible for the majority of outbreaks unless foods, in which eggs are an ingredient, are contaminated by other ingredients or cross-contaminated during preparation. Alternatively, backyard and non-commercially produced eggs may be more likely to be contaminated with *S*. Typhimurium.

Other important sources of uncertainty include the effectiveness of food preparation at the retail, catering and domestic level on reduction in levels of contamination. For example, there is considerable uncertainty in circumstances where large volumes of unpasteurised egg pulp are used in baking by caterers.

Further work to assess the role of single or multiple mitigations is possible through the use of the QRA models, but would be the subject of future industry consultation that take into account technical feasibility, cost-effectiveness and ability to verify controls. The models provide a tool to assess the relative impact of control procedures under different production and processing conditions. The risk assessment approach identifies data gaps and uncertainty that in turn can be used to define research and development. This methodology provides a food safety risk-basis to the prioritisation of research and development for industry to consider along with other criteria such as market access, cost, production/processing efficiency and regulatory obligations.

Model Uncertainty
Key uncertainties not included in the development of Exposure Assessment models include for example, the effect of climate or management practices on the flock and egg prevalence.

Additional factors such as growth and survival behaviour of different *Salmonella* strains, may be different. As a result the risk estimates may be higher than what is actually the case. This may have important impacts on the risk to consumers.

However, in some areas of the risk assessment, uncertainties may have been reduced by the inclusion of producer and processor surveys. These surveys gathered information that became inputs to the quantitative modelling.

5.6   Summary

1. Exposure Assessment and Dose response models have been used to assess the impact of on-farm, processing and retail storage on risk of consuming foods contaminated by *Salmonella*.

2. Overall, our analysis data indicate that the risk of illness associated with commercially produced eggs is low provided best practice on-farm and processing methods are used.

3. There are, however, indications that inappropriate storage after egg grading (ie during wholesale and retail) can substantially increase risk. Analysis of production process models indicated that growth of *Salmonella* in eggs can be avoided by refrigeration of eggs at 4°C after they leave on-farm storage.

4. Storage at 16°C in the retail environment is predicted to allow growth of *Salmonella* in contaminated eggs in as little as 18 days after the end of on-farm storage under median industry practices (Section 5, Table 5.1). This estimate is reduced to 10 days if eggs are stored at 22°C and 4.6 days if stored at 30°C. Continued storage beyond this point is predicted to result in up to 36 cases of illness per million serves of uncooked or lightly cooked foods containing eggs. Given that commercially produced and graded eggs are given a shelf-life of up to 37 days, there is a risk to consumers that eggs will contain substantial numbers of *Salmonella*. Thus the commonly used '37 day shelf-life at 20ºC' may result in potential risk to consumers.

6. Exposure Assessment and Risk Characterisation for Egg Products

6.1 Introduction

The purpose of this section is to develop an EA model to simulate the efficacy of FSANZ processing requirements (Section 1.6.2) for *Salmonella* in liquid egg products, namely whole egg, yolk and albumen. Model inputs include the prevalence and concentration of *Salmonella* in unpasteurised pulp, and the thermal inactivation rate for *Salmonella* in each of the three products. The EA model outputs include prediction of the efficacy of the current requirements, concentration of *Salmonella* in pasteurised product and the percent of pasteurised product that would fail the FSANZ sampling requirement (*Standard 1.6.1 Microbiological Limits for Food)* of no *Salmonella* detected in 5×25 g samples. This model is an extension of that described in Section 4, and is underpinned by data obtained from the egg production and processing surveys.

6. 2 Model Background

Production of liquid eggs products (whole egg, yolk and albumen, plus the addition of sugar and salt) comprises approximately 9% of the eggs produced in Australia. Egg products are used by food processing industries to manufacture a diverse range of foods. In comparison to overseas countries, the use of processed egg products is low. For example, about 25% of shell eggs produced in the USA are processed.

Pasteurised liquid egg products are sold to commercial users in the form of large volume pallecons and smaller 5 and 25 kg containers. Sugar and salt may also be included as additives. The retail sale of unpasteurised egg products is not permitted under the FSANZ standards. However, wholesale sale is permitted for use in products where a 'kill' step, such as adequate cooking, will be applied before retail sale.

Dried products will not be considered in this risk assessment.

6.2.1 Liquid Egg Processing Standards (Australia and US)

Table 6.1 shows the current Australian (FSANZ) and USA standards for pasteurisation of whole egg pulp, albumen and yolk. The FSANZ processing standards for egg products require that product be retained at temperatures and times not less than those indicated in Table 6.1 followed by rapid cooling to a temperature not greater than 7°C. US processing conditions are provided for comparison only. The main differences in the two standards are that the US process for whole egg will result in a more severe process than that specified by FSANZ.

6.2.2 Performance of Pasteurisation Processes used by Australian Egg Processors

As part of this risk assessment project, eight egg processors from around the country were surveyed on their pasteurisation processing standards. Production/Technical Managers from each company were surveyed for the minimum, maximum and most likely values of the pasteurisation temperature, holding time and the temperature after pasteurisation for each of the egg product categories. A summary of the survey results is presented in Table 6.2.

Based on survey responses, it is apparent that some pasteurisation conditions reported were outside (lower pasteurisation temperatures and shorter times) those required under the FSANZ processing requirements. As such, there is a possibility that pasteurised egg products may not be sufficiently heat treated to ensure the elimination of *Salmonella*. NEPSS report (Appendix 2, Table 2.6) that pasteurised product has tested positive for *Salmonella*, though there is no indication of how often this occurs. The conclusion drawn from these observations is that pasteurised *Salmonella* positive samples may be due either to inadequate pasteurisation or post-processing contamination.

Table 6.1: Australian and US standards for the pasteurisation of whole egg, yolk and albumen. (Source FSANZ, 2003, USDA-FSIS, 1998)

Product	Australia		USA	
	Temperature (°C)	Time (minutes)	Temperature (°C)	Time (minutes)
Whole egg	64	2.5	60	3.5
Yolk	60	3.5	60	6.2
			61.1	3.5
Albumen	55	9.5	55.6	6.2
			56.7	3.5

Table 6.2: Egg product pasteurisation conditions for whole egg, yolk and albumen based on survey responses. Values in brackets represent the minimum and maximum values reported by all processors who responded to the survey.

Product	Survey respondents	Mean Temperature and (range) (°C)	Mean holding time and (range) (minutes)	Mean storage temperature and (range) (°C)
Whole egg	8	64 (60 – 64)	2.8 (2.5 – 5)	4 (1 – 9)
Yolk	3	61 (60 – 62)	3.5 (3.5 – 3.6)	7 (5 – 10)
Albumen	3	55 (55 – 55.5)	9 (3.5 – 10)	4 (1 – 9)

Figures 6.1, 6.2 and 6.3 illustrate industry performance for processing of whole egg, egg yolk and egg albumen as a means of eliminating *Salmonella*. These figures allow processors to assess their pasteurisation practices relative to current FSANZ standards. The data shown reflect that obtained from the processing survey. Of the processors surveyed, the majority employ practices that are as good, or better than that specified by the standard. However, this analysis does not indicate how well the processing procedures deal with contents from a small number of contaminated eggs that have exceeded the YMT and consequently carry a large load of *Salmonella*. This event is considered in the following part of this section.

6.3 Thermal Inactivation

A necessary first step in evaluating the rigor of the FSANZ processing requirements is to gather information on the inactivation kinetics of *Salmonella* in egg products.

A preliminary assumption for the thermal inactivation of microorganisms is that first order inactivation is applicable. Mathematically, this is presented as:

$$\ln\frac{N}{N_0} = -kt$$

Where N_0 is the number of cells at time, $t = 0$, and k is the rate of inactivation. A related term is the decimal reduction time, D (the time required for the number of microorganisms to be reduced by a factor of 10). The relationship between the decimal reduction time and the rate of inactivation is:

$$D = \ln(10)/k$$

Figure 6.1: Comparison of reported industry holding conditions (squares) for the pasteurisation of whole egg pulp. The circle represents FSANZ processing requirements. The solid line shows temperature and holding times of equivalent severity (z=4°C) to the FSANZ standard. Combinations of holding times and temperatures to the left of the solid line represent under-processing.
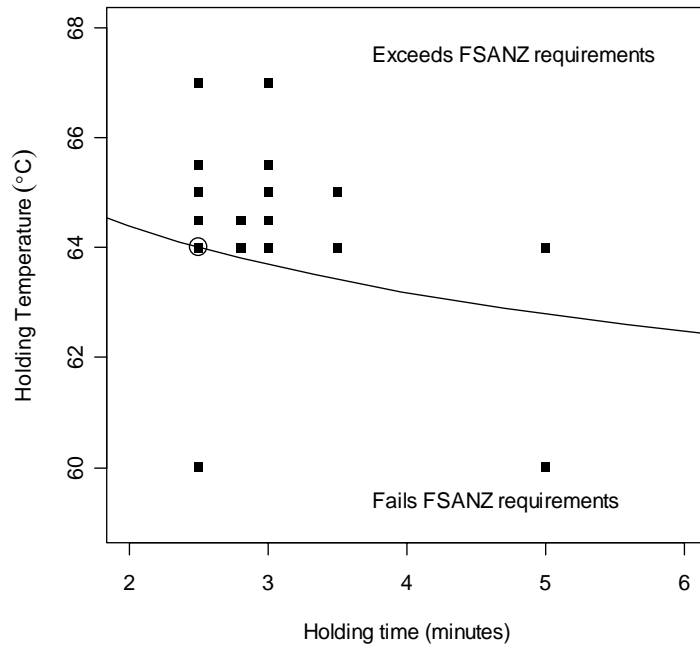

Figure 6.2: Comparison of reported industry holding conditions (squares) for the pasteurisation of yolk. The circle represents the FSANZ processing requirements. The solid line shows temperature and time conditions of equivalent severity (z=5.11°C) to the FSANZ standard. Combinations of holding times and temperatures to the left of the solid line represent under-processing.
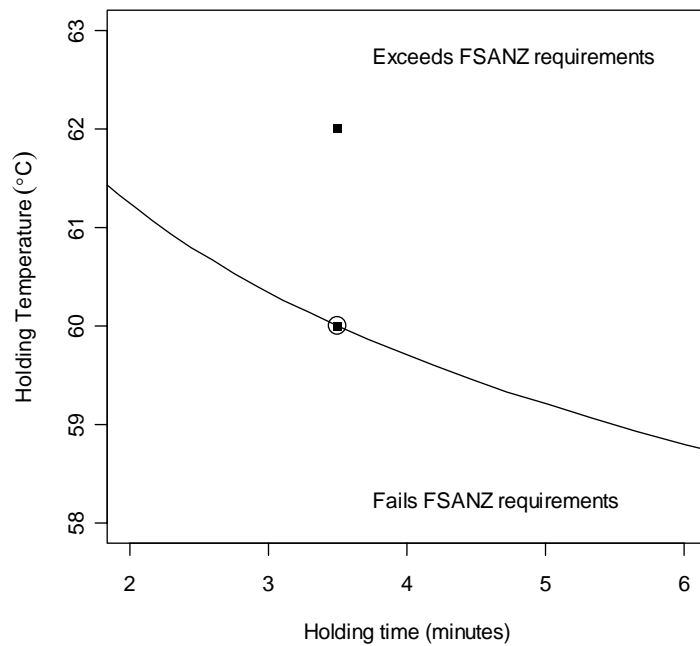
Figure 6.3: Comparison of reported industry holding conditions (squares) for the pasteurisation of albumen. The circle represents the FSANZ processing requirements. The solid line shows temperature and time conditions of equivalent severity (z=4.34°C) to the FSANZ standard. Combinations of holding times and temperatures to the left of the solid line represent under-processing.
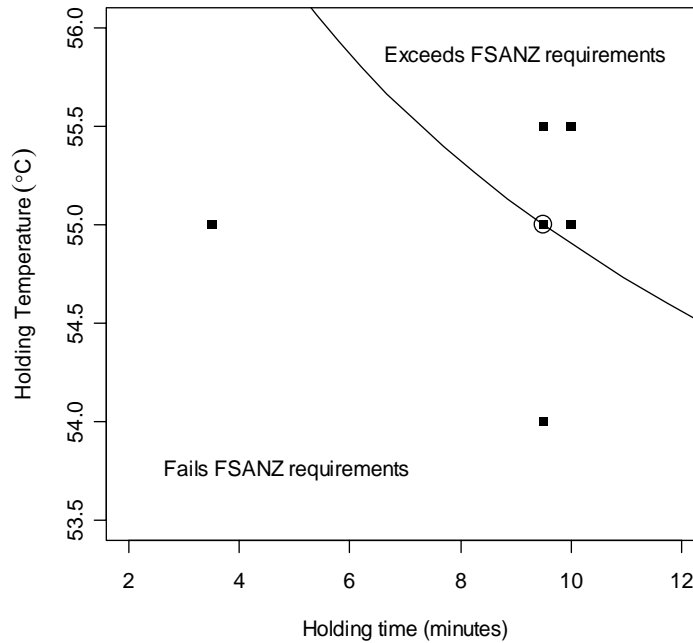
In prior exposure assessments of pasteurised egg products (USDA-FSIS, 1998; Whiting and Buchanan, 1997; and FAO-WHO, 2002) equations have been developed for predicting the effect of temperature on the inactivation rate of SE in whole egg. The model of Whiting and Buchanan (1997) was based on SE inactivation data from Shah, Bradshaw and Peeler (1991). The later USDA-FSIS model included additional studies by Humphrey *et al* (1990). The USDA-FSIS exposure assessment also included inactivation equations for both yolk and albumen.

Both exposure assessments proposed equations of the form:

$$\log_{10} D = \beta_0 + \beta_1 T$$

Where D is the decimal reduction time and T is the temperature in °C. The equation coefficients β_1 and β_2 are determined by linear regression.

As SE has not been found in Australian egg pulp samples (NEPSS and IMVS) the equations developed in the earlier exposure assessments may not be applicable. New predictive equations for thermal inactivation of *Salmonella* were developed using published decimal reduction time data for non-SE *Salmonella* serovars in whole egg, yolk and albumen. The least squares regression coefficients are presented in the Table 6.3. The coefficients represent the predictions for the data that has the longest decimal reduction time at the required processing temperature. The predictions for the thermal inactivation of *Salmonella* in whole egg, egg yolk and albumen are presented in Figure 6.4.

Table 6.3 Summary of the thermal inactivation models for *Salmonella* in egg products.

Egg Product	Intercept β_0	Slope β_1	Residual standard error	Reference
Whole egg	14.46931	-0.24966	0.08177	Humphrey *et al*, 1990
Egg yolk	11.65951	-0.19552	0.05313	Humphrey *et al*, 1990
Albumen	12.61814	-0.23018	0.08832	Cotterill 1973

Figure 6.4: Effect of temperature on the logarithm of the decimal reduction time (minutes) for *Salmonella* in whole egg, egg yolk and albumen.
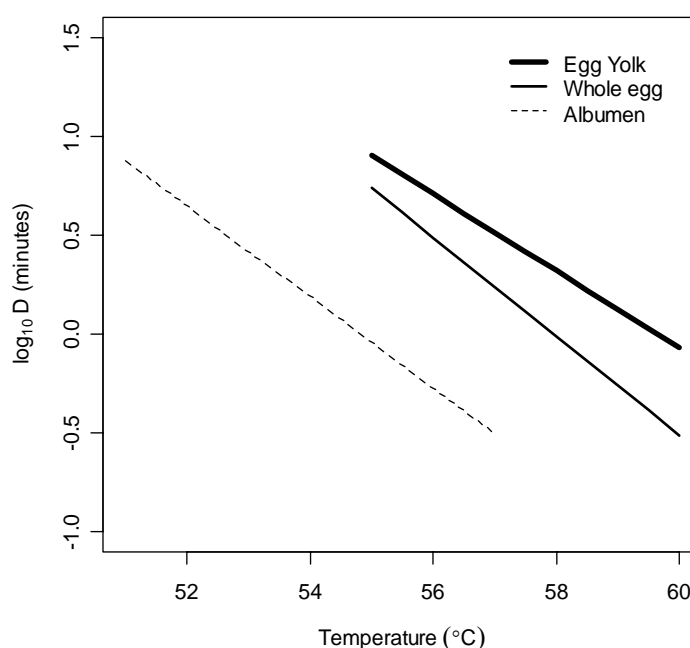
6.4 Evaluation of the FSANZ Processing Requirements for *Salmonella* in Egg Products

The following discussion examines the FSANZ standards for the pasteurisation of egg products and uses the EA model and predictive models for the thermal inactivation of *Salmonella* in whole egg, yolk and albumen to test adequacy of pasteurisation processes in use in Australia.

The flowchart for the pasteurisation model is presented in Figure 6.5. The model allows investigation of the importance of eggs contaminated internally with *Salmonella* under processing conditions identified in the survey of Australian egg producers and processors. The on-farm storage, transportation and processor handling temperatures and times are the same as for the Exposure Assessment of shell eggs. The approach of modelling the number of *Salmonella* in egg pulp using shell egg prevalences is different to other risk assessments for *Salmonella* in eggs. For example, Whiting and Buchanan used estimated concentrations, while the USDA-FSIS and FAO-WHO risk assessments were based on results for raw pulp from US processing plants from the 1960's.

Unlike the USDA-FSIS (1998) and FAO-WHO (2002) Exposure Assessments the contribution of *Salmonella* from shells is not considered. In evaluating the FSANZ processing conditions for eggs the contribution of *Salmonella* from shells of (especially washed) eggs would be small compared to the

numbers in eggs where internal growth had occurred. Experimental data indicated that the survival of *Salmonella* on the external surface of an eggshell is poor.

Current FSANZ egg processing requirements were evaluated by simulating the potential for recovery of *Salmonella* from any of five x 25 g samples of egg product following pasteurisation. This simulation assumed that the *Salmonella* in the pasteurised product was randomly distributed and followed a Poisson distribution. In this case, the mean concentration of *Salmonella* cells is Y per gram, and if there are 25 grams per sample, the count per sample has a mean of 25×Y. More importantly, the probability of a positive result for a sample of 25 grams is then: 1 - exp(-25×Y).

The median predicted degree of thermal inactivation (in base 10 logarithms) for whole egg, egg yolk and albumen were 80.7 (58.6, 109.8), 4.12 (3.37, 5.03) and 10.46 (7.42, 14.6), respectively. The values in the brackets are the 5th and 95th percentiles of the degree of thermal inactivation. These results indicate that the minimal processing temperature and times suggested in the Australian standards do not give the same degree of inactivation for each of the egg products.

The predicted probability of pasteurisation failure (detection of *Salmonella* spp. in at least 1 of 5 x 25 g samples) for whole egg, egg yolk and albumen are presented in Table 6.4. The nine scenarios representing eggs from three (best, median and worst) combinations of on-farm and processing conditions are presented.

Figure 6.5: Flowchart showing the connectivity for the Pasteurisation model.
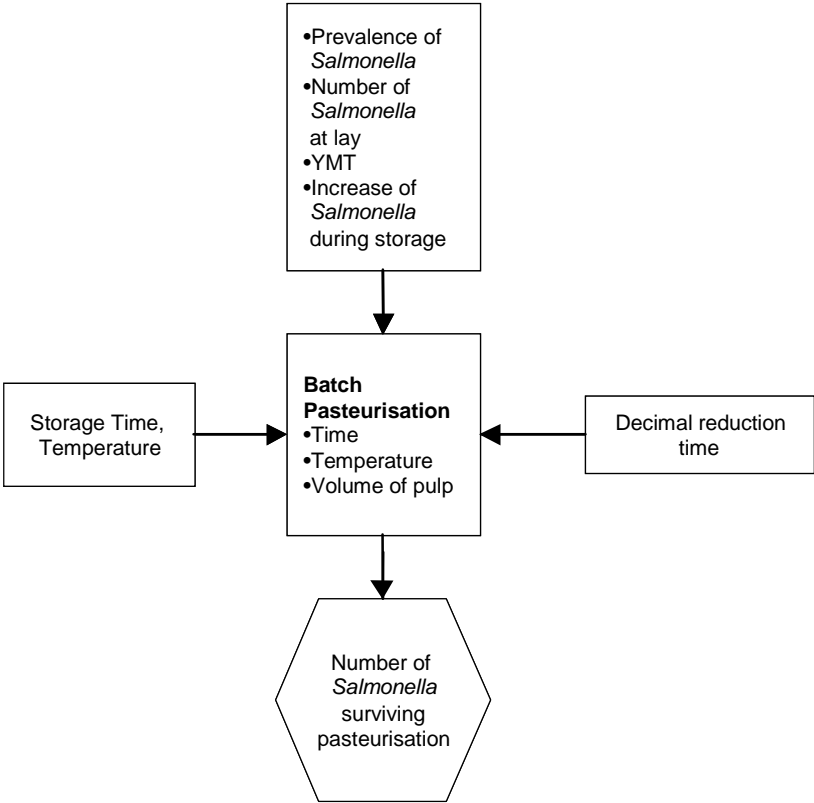
None of the nine scenarios evaluated were predicted to result in pasteurisation failures for whole egg pulp (Table 6.4). The 80.7 log inactivation achieved by the FSANZ recommended pasteurisation process is more than sufficient to inactivate any *Salmonella* cells likely to be present in raw egg. By contrast, a small percentage of pasteurised albumen batches (<0.1%) and yolk (up to 13%) were predicted to contain detectable levels of viable *Salmonella* after heat treatment. Pasteurisation failures for pasteurised yolk were all associated with the worst-case of on-farm handling prior to pasteurisation. When best processing practices were evaluated, 1.8% of pasteurised samples were

predicted to fail. This figure increased to 13% when eggs were stored for extended periods prior to pasteurisation.

Table 6.4: Predicted probability of pasteurisation failure for whole egg, egg yolk and albumen using minimum Australian processing conditions (see Table 6.1).

		Percentage pasteurization failure (%)		
On-farm Conditions	**Processing Conditions**	**Whole egg**	**Egg yolk**	**Albumen**
Best	Best	0	0	0
Best	Median	0	0	0
Best	Worst	0	0	0.0019
Median	Best	0	0	0
Median	Median	0	0	0
Median	Worst	0	0	0.0050
Worst	Best	0	1.8	0.0050
Worst	Median	0	2.5	0.010
Worst	Worst	0	13	0.045

Typical outcomes obtained using the pasteurisation model are presented for whole egg pulp, egg yolk and albumen (Table 6.5). The data shows the impact of storage of eggs destined for egg product processing assuming a storage temperature of 20°C and that no cross contamination of eggs during storage occurs. Thus the longer the storage time, the greater the number of *Salmonella* present in eggs due to growth following invasion of the yolk.

Table 6.5: Effect of egg age on predictions for efficacy (pass/fail*) of the FSANZ processing conditions of pasteurisation of whole egg, egg yolk and albumen.

Days from point of lay	**Egg product**		
	Whole egg	**Yolk**	**Albumen**
0	Pass	Pass	Pass
7	Pass	Pass	Pass
14	Pass	Fail	Pass
21	Pass	Fail	Fail
28	Pass	Fail	Fail

*Salmonella detected from at least 1 of 5 x 25g samples.

The model simulations revealed that for *Salmonella* at least, the FSANZ standards for egg yolk and albumen may not be satisfactory. Depending on the extent of growth, *Salmonella* may survive pasteurisation and depending on the use of the egg product, may lead to illness in consumers. Based on the FSANZ standards, the predicted log inactivation for yolk is only of the order of four magnitudes (4 log reductions in the concentration of *Salmonella*). By comparison, the standard for whole egg is severe, with a potential excess of 80 decimal reductions with the standard for albumen being 10 decimal reductions. These smaller decimal reductions are the reason failures are predicted for yolk and albumen with high microbial load caused by long storage times.

6.5   Risk Characterisation

To provide some estimate of risk to consumers from the consumption of foods containing uncooked pasteurised egg products, the probability of illness associated with uncooked foods containing this egg product was evaluated. Uncooked foods were selected because they represent a worst-case scenario. By comparison, cooked foods containing pasteurised egg product are subjected to an additional kill-step that will reduce numbers of any surviving *Salmonella* cells present in the pasteurised product.

Estimates of risk were calculated as follows. A dietary exposure model was developed along similar assumptions as described in Chapter 5. The probability of consuming a contaminated serving was based on the assumption that the *Salmonella* cells are homogeneously distributed with the batch. The serving size for whole egg was 100g (~2 eggs, as used in Chapter 5), while egg yolk and albumen the serving size was 50g.

The median probability of illness for the three egg products is presented in Table 6.6. In all three cases the median probability of illness is exceptionally low viz. $<10^{-25}$, 1.4×10^{-17} and $<10^{-25}$ for whole egg, egg yolk and albumen, respectively. However, there is a probability that 5% of samples with worst/worst on-farm and processing will lead to a significant probability of illness ($p_{III} > 0.02$)

As noted previously these simulations were determined for uncooked foods. The probability of illness for cooked (lightly and well cooked) foods will be reduced in comparison to the values shown in Table 6.6.

Table 6.6: Predicted median probability of illness per serving from the consumption of pasteurised whole egg, egg yolk and albumen.

On-farm Conditions	Processing Conditions	Probability of illness per serving		
		Whole egg	Egg yolk	Albumen
Best	Best	$<10^{-25}$	6.0×10^{-18}	$<10^{-25}$
Best	Median	$<10^{-25}$	5.9×10^{-18}	$<10^{-25}$
Best	Worst	$<10^{-25}$	5.7×10^{-18}	$<10^{-25}$
Median	Best	$<10^{-25}$	6.0×10^{-18}	$<10^{-25}$
Median	Median	$<10^{-25}$	5.8×10^{-18}	$<10^{-25}$
Median	Worst	$<10^{-25}$	5.8×10^{-18}	$<10^{-25}$
Worst	Best	$<10^{-25}$	6.6×10^{-18}	$<10^{-25}$
Worst	Median	$<10^{-25}$	6.8×10^{-18}	$<10^{-25}$
Worst	Worst	$<10^{-25}$	$1.4 \times 10^{-17*}$	$<10^{-25}$

* 95th percentile = 0.026

6.6   Conclusions

The Exposure Assessment models developed in this section allowed an examination of the efficacy of current pasteurisation processes for elimination of *Salmonella* from whole egg pulp, yolk and albumen

Our investigation into the differences between published experimental data that describes the thermal inactivation of *Salmonella* in liquid egg products highlights significant variation in D-values. The between experiment variation in D-values could lead to enormous differences in the predicted level of inactivation for different products. Our analysis of the data also highlights differences between the Australian and US standards for liquid egg products.

The pasteurisation model predictions were based on the 'worst case' scenarios by using the largest decimal reduction times. Using the smaller decimal reduction times will reduce the predicted failure rate, as the predictions will be many orders of magnitude less than if the largest D value was used. Experimental methodology used appears to be the major factor for the observed differences in D-values. For example for whole egg, several different methods were used: capillary tubes, quartz tubes, glass test tubes, three neck flasks. Some methods have fast heat-up times, while others have slow heat-up times and the impact of each method on the D-value determined must be accounted for. Additional factors such as differences in media and methods for counting *Salmonella*, recovery of injured cells etc may also influence the reported D-values. The use of dummy variables allows for these differences to be allowed for in the prediction of inactivation. To account for differences

between serovars and strains within each experiment, the use of dummy variables becomes cumbersome. Alternative statistical modelling methods (eg mixed effects models) may be useful in further investigation of these nested factors.

The implications of the Exposure Assessment for egg processing industry are as follows:

1. We have produced graphical models that allow processors to assess their pasteurisation practices relative to current FSANZ standards.

2. The egg processing EA model indicated that pasteurisation practices used for egg yolk and egg albumen may not adequately result in elimination of viable *Salmonella* for worst case scenarios, especially for eggs stored for extended times pre processing (Table 6.4). If processors need to keep eggs for extended periods of time, we recommend that these eggs should only be used for production of whole egg. Only the holding times and temperatures used to pasteurise whole egg are sufficient to ensure complete inactivation of all *Salmonella* present in egg contents.

6.7   Summary

The processing of liquid egg products (whole egg, yolk and albumen) has been modelled and the adequacy of existing standards for pasteurisation of these products and elimination of *Salmonella* has been assessed.

1. Tools to assist processors to evaluate their processing practices against current FSANZ standards have been prepared.

2. Pasteurisation practices currently used for whole egg can be confidently used to eliminate *Salmonella* from whole egg pulp from commercially produced and graded eggs.

3. The mathematical model developed in this report indicated that FSANZ recommended pasteurisation practices used for yolk and albumen may be insufficient to guarantee elimination of *Salmonella* from these products, especially if eggs are stored at high temperatures for long periods before processing. Although current FSANZ standards for albumen pasteurisation are in excess of current USDA-FSIS approved standards, the survey data indicated some producers under-process albumen and whole egg. Consequently it is recommended that the Australian industry review current practice and the resulting risk associated with whole egg and albumen pasteurisation. Risk reduction could be brought about by reducing the storage time for eggs destined for pasteurisation.

4. The Exposure Assessment indicated that the standards for pasteurisation of yolk and albumen should be re-evaluated and new standards designed, if required, which provide assurance that *Salmonella* can be reliably eliminated in these products.

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