FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

SD1

PROPOSAL P301

PRIMARY PRODUCTION AND PROCESSING STANDARD FOR EGGS & EGG PRODUCTS

RISK ASSESSMENT OF EGGS AND EGG PRODUCTS

September 2009

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ESAP Terms of Reference:

1. Provide comment and advice on the scientific assessments undertaken by FSANZ as part of the Eggs and Egg Products Primary Production and Processing Standard development process.

2. Provide guidance in identifying additional sources of data; and

3. Assist in addressing any uncertainty or variability in the information underpinning the scientific assessments - which may impact on the final output.

ABBREVIATIONS

ACMSF	Advisory Committee on the Microbiological Safety of Food
ADI	Acceptable Daily Intake
AECL	Australian Egg Corporation Limited
ANZFA	Australia New Zealand Food Authority
APVMA	Australian Pesticides and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
ATDS	Australian Total Diet Survey
a_w	Water activity
bw	Body weight
CAC	Codex Alimentarius Commission
CFIA	Canadian Food Inspection Agency
cfu	Colony forming units
Codex	Codex Alimentarius Commission
CoP	Code of Practice
DAFF	Department of Agriculture, Fisheries and Forestry
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FSANZ	Food Standards Australia New Zealand
FSIS	Food Safety and Inspection Service
g, ng, µg, mg, kg	Gram, nanogram, microgram, milligram, kilogram
GAP	Good Agricultural Practice
GMP	Good Manufacturing Practice
HACCP	Hazard Analysis Critical Control Point
ICMSF	International Commission on Microbiological Specifications for Foods
IRA	Import Risk Analysis
JECFA	Joint FAO/WHO Expert Committee on Food Additives
l, ml	Litres, millilitres
MCFA	Medium chain fatty acid
MOE	Margin of exposure
MRL	Maximum residue limit
NICNAS	National Industrial Chemicals Notification and Assessment Scheme
NRS	National Residue Survey
ppm, ppb	Parts per million, parts per billion
PTDI	Provisional tolerable daily intake
PTWI	Provisional tolerable weekly intake
RNA	Ribonucleic acid
SARDI	South Australian Research and Development Institute
SCFA	Short chain fatty acid
SE	*Salmonella* Enteritidis
WHO	World Health Organization
YMT	Yolk Mean Time

EXECUTIVE SUMMARY

This risk assessment brings together information on the microbiological and chemical hazards associated with the consumption of eggs and egg products in Australia.

The risk assessment was undertaken to answer the following questions:

1. What are the microbiological and chemical risks to food safety posed by the consumption of eggs and egg products and the use of eggs and egg products in food in Australia?

   For the microbiological assessment this includes:
   - The contribution eggs and egg products make towards foodborne illness.
   - The main pathogens that cause egg-related foodborne illness.
   - Determination of how conclusive is the epidemiological evidence.

   For the chemical assessment this includes:
   - Consideration of inputs (*e.g.* feed, water) into the production of eggs and egg products.
   - How chemicals used in the production of eggs and egg products might potentially impact on public health and safety.
   - Identification of areas in the current regulatory system which may require attention in relation to addressing potential public health and safety risks associated with chemicals in eggs and egg products

2. Where during the production and processing of eggs and egg products may hazards be introduced and/or their levels change, and which processing and production factors have the most significant impact on public health and safety?

3. What are the hazards and subsequent risks associated with emerging pathogens such as *Salmonella* Enteritidis and highly pathogenic avian influenza?

Background

The contents of the egg can become contaminated with microorganisms via two routes; trans-ovarian (vertically) and trans-shell (horizontally). Trans-ovarian transmission occurs via infection of the bird's reproductive tissues, primarily the ovaries and oviduct tissue. This may lead to direct contamination of the yolk, albumen, egg shell membranes or egg shell as the egg is being formed.

The egg shell and cuticle form the first line of defence against horizontal transmission of hazards into the egg contents. However, the shell is porous to enable exchange of respiratory gases and water vapour and may present a route for microorganisms to gain entry into the egg. In addition to the shell and cuticle, the egg has a number of membranes which limit the movement of microorganisms between the egg compartments (albumen and yolk).

The albumen (egg white) contains bacteriostatic compounds that inhibit, although not necessarily prevent, the growth of microorganisms. In contrast, the egg yolk provides an ideal growth medium for microorganisms if stored above minimal growth temperatures.

Internationally, risk assessments for eggs and egg products have focussed on *Salmonella* Enteritidis (SE) as this serovar has been responsible for the majority of foodborne illnesses associated with consumption of eggs and egg products. SE has an increased potential to colonise the reproductive tissue of hens and contaminate the contents of the egg as it is being formed compared with other *Salmonella* serovars. SE is not endemic in Australian poultry flocks, however other *Salmonella* serovars have been responsible for foodborne illness outbreaks associated with consumption of eggs.

Exposure of layer hens to chemicals such as agricultural and veterinary medicines, particulates and environmental contaminants may lead to the presence of chemicals in eggs and egg products.

Risk Assessment Approach

It was clear from epidemiological data and the literature that *Salmonella* spp. is the primary pathogen of concern in relation to foodborne illness associated with eggs. The risk assessment for *Salmonella* spp. in eggs and egg products follows the Codex Alimentarius Commission risk assessment framework. The hazard identification describes the properties of *Salmonella* spp. and its association with human illness. The hazard characterisation provides the dose-response relationship used to estimate the likelihood of illness following exposure to a given number of *Salmonella* spp.

The exposure assessment consists of a descriptive evaluation of on-farm and processing factors that may impact on the prevalence and levels of *Salmonella* spp. in or on eggs. This includes discussion on the main factors associated with introduction of *Salmonella* spp. into laying flocks. The Australian Egg Corporation Limited (AECL) commissioned development of a quantitative risk assessment model for *Salmonella* spp. in eggs and egg products. The model considered the fate of *Salmonella* spp. in eggs from the point of lay through to consumption and was developed to predict the effect of time and temperature of storage on the risk of illness. The AECL quantitative model formed the basis for this risk assessment.

The assessment of risk to public health and safety for eggs and egg products from the use, or presence, of chemicals in eggs has been undertaken in the form of a chemical risk assessment, which examines a broad range of chemicals either used in the production of, or present in, eggs and egg products.

Risk Assessment outputs

For microbiological hazards, there is limited epidemiological evidence in Australia implicating clean, intact eggs as a source of egg-associated foodborne illness outbreaks. However, it is important to recognise that outbreak data is not necessarily indicative of the incidence and causes of sporadic egg-associated cases of salmonellosis. Reported outbreaks of egg-associated salmonellosis were generally attributed to consumption of uncooked foods containing raw egg (*e.g.* raw egg-based sauces and deserts). A common risk factor identified in outbreaks was the use of eggs with visible surface faecal contamination (dirty eggs). Contributing factors included cross-contamination during food preparation and/or temperature abuse of the food containing raw egg.

Numerous factors during primary production have the potential to introduce *Salmonella* into a laying flock including feed, water, pests, the environment, personnel, new laying stock and equipment. Due to the multi-factorial nature of transmission of *Salmonella* spp. into laying flocks, and a lack of quantitative data, identification of those factors that have the greatest impact on flock contamination was not possible.

Factors that impact on the likelihood of horizontal transmission of *Salmonella* spp. into the egg contents includes the presence and load of external contamination (***e.g.*** faecal material), temperature differential between the egg and the environment, humidity, and condition of the shell (***e.g.*** cracks), cuticle and membranes. Practices during the production and processing of eggs and egg products that impact on these factors will affect the likelihood of transmission of *Salmonella* into the egg contents.

The output of the AECL quantitative model included an estimation of the number of cases of illnesses per million serves for eggs stored at various temperatures at retail and consumed uncooked, lightly cooked or well cooked. The following is a summary of the key outputs from the quantitative model:

- The model confirmed that the consumption of well-cooked eggs presented little risk of illness as the cooking step is high enough to inactivate *Salmonella* (>12-$\log_{10}$ reduction).
- The length of time until there is potential for rapid growth of *Salmonella* spp. in contaminated eggs is dependent on the temperature of the egg from point of lay through to consumption. It was predicted that for eggs produced and processed under median industry practices, growth of *Salmonella* could occur in contaminated eggs after approximately 10 days retail storage at 22°C. For eggs stored at 16°C during retail, the estimated time before growth of *Salmonella* in contaminated eggs would be 18 days. No growth of *Salmonella* was predicted if eggs were stored at 4°C.
- The predicted risk of illness is dependent on the prevalence of *Salmonella* contaminated eggs. The prevalence of *Salmonella* contaminated eggs was described in the model by a distribution based on results from a pilot microbiological survey of graded eggs in Australia (n=20,000) and data from large international surveys on the prevalence of non-SE contaminated eggs, with an overall mean prevalence of 0.004%.
- For eggs stored under conditions that would permit the growth of *Salmonella* (***i.e.*** yolk mean time has expired) the estimated number of cases of salmonellosis was 36 per one million serves of uncooked egg. Even if eggs were stored under conditions that do not permit the growth of *Salmonella* spp., the risk of illness if consumed raw was estimated to be approximately 4 cases per one million serves.
- The quantitative model did not consider the potential for cross-contamination during food preparation or multiple serves of uncooked food containing raw egg such as raw egg-containing sauces, desserts etc. These practices would increase the predicted number of salmonellosis cases.
- Raw egg pulp is often contaminated with *Salmonella* spp. and there is a potential for growth if stored at temperatures > 7°C.
- Current pasteurisation requirements for liquid whole egg resulted in a large predicted inactivation of *Salmonella* (>80-$\log_{10}$ reduction), with much less for liquid albumen and yolk (10.5-$\log_{10}$ and 4.1-$\log_{10}$ respectively).

Chemical risks associated with eggs and egg products are limited and can be summarised as follows:

- Although dioxins, PCBs and PBDEs have been detected in Australian eggs, an analysis of the consumption of eggs and egg products by the general population indicated that exposure to these contaminants in food is low. On the basis of the available data it can be concluded that the Australian public health risk arising from exposure to dioxins, PCBs and PBDE in food, including eggs, is low.
- Exposure of Australian consumers to heavy metal contaminants (*e.g.* cadmium, lead and mercury) through food is within safe levels and eggs are a minor contributor to this exposure. However it was identified that the routine consumption of eggs from a contaminated site may pose a risk to consumers, particularly in relation to children exposed to lead.
- There is a lack of data on both the total dietary exposure of Australian consumers to plant toxins, mycotoxins and bacterial toxins from all foods, and the presence or absence of these toxins in eggs and egg products. However the data, where available, indicates that exposure to these toxins by Australian consumers is generally low, and that eggs are a negligible source of exposure in most cases.
- Results from recent surveys of residues of agricultural and veterinary chemicals in eggs indicate that they are either absent or within safe levels and are unlikely to pose a risk to public health and safety.
- Some feed additives, such as the carotenoid pigments used to enhance yolk colour, appear to be unregulated, as they do not meet the definition of either a veterinary medicine or a food additive. However, this is not considered to pose a risk to public health and safety as these carotenoids are naturally found in eggs when laying hens are fed a diet containing particular plant foods (*e.g.* corn and lucerne). These same carotenoids are approved food colours in a range of foods in Australia and New Zealand. The use of sudan red dyes in duck feed to colour the eggs, which has been reported to occur overseas, would be of some concern, however the presence of sudan dyes in food in Australia is not permitted.
- The reported use of lead oxide as a processing aid or food additive in alkali-cured eggs is of concern. The use of lead compounds in food is not permitted in the Code, and enforcement action has been taken in New South Wales to eliminate this practice.
- The monitoring of chemical residues in eggs over recent years has demonstrated a high level of compliance with the regulations.

Conclusion

Consumption of well-cooked eggs (or cooked foods containing egg) presents little risk of salmonellosis. Results of the quantitative model, as well as epidemiological evidence, demonstrates that consumption of uncooked or lightly-cooked foods containing raw egg represents a potential risk for foodborne illness. A common risk factor identified in egg-associated outbreaks was the use eggs with visible surface faecal contamination.

For non-SE *Salmonella* serovars, the primary route of internal contamination of the egg is considered to be via transmission through the shell. The ability of *Salmonella* to migrate into the egg contents is influenced by many factors including the integrity of the shell, cuticle and membranes, the presence and load of external contamination, differences in temperature between the egg and the environment, and humidity.

Results from the quantitative model predicted that, for consumption of uncooked food containing raw eggs that were stored under conditions that permit growth of *Salmonella* spp., the estimated risk of illness is 36 cases per million serves. There is however, a lack of data on the actual exposure of consumers to foods containing uncooked or undercooked eggs or egg products.

Chemical residues in eggs and eggs products are either absent or low and of little public health and safety risk.

BACKGROUND

Food Standards Australia New Zealand (FSANZ) has responsibility for protecting the health and safety of consumers through the development of food standards.

This document seeks to assess the risks to public health and safety resulting from consumption of eggs and egg products in Australia. FSANZ uses a number of tools to assess risks to public health and safety, including risk profiling[1], quantitative and qualitative risk assessments[2] and scientific evaluations. The application of these tools to the assessment of the risks to public health and safety is dependent on the purpose of the assessment and on the availability, quality and quantity of relevant data.

FSANZ follows established international guidelines and incorporates elements of the Codex Alimentarius Commission risk assessment framework when undertaking risk profiles, risk assessments and other scientific evaluations. Guidance for undertaking risk assessments have been drafted internationally by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

In assessing risks to public health and safety, available scientific data concerning the safety of the commodity under consideration and the properties of the hazard are evaluated. This requires utilisation of relevant scientific data and includes procedures to address uncertainty and variability in the conclusions drawn from the data, *i.e.* consideration of the relevance and quality of data and the veracity of its source.

The outcome of any assessment of risks to public health and safety may include a statement on the probability and severity of an adverse health effect due to the consumption of a food containing a particular biological, chemical or physical agent. An assessment may also identify where in the production chain controls over hazards will have the greatest impact on minimising risk, *i.e.* informing risk managers where intervention will be most effective. The outcomes of assessing risks to public health and safety for eggs and egg products are used by FSANZ to inform risk management decisions.

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1 Risk profiling is defined by FAO/WHO as 'the process of describing a food safety problem and its context, in order to identify those elements of the hazard or risk relevant to various risk management decisions'.

2 Risk assessment is a scientific process undertaken to characterise the risk to public health and safety posed by foodborne hazards associated with a food commodity.

SCOPE AND PURPOSE

The objective of this risk assessment is to determine the public health and safety risks potentially associated with the consumption of eggs and egg products.

The assessment brings together available scientific and technical information on microbiological and chemical food safety hazards associated with eggs and egg products and aims to identify specific stages along the primary production, processing and retail chain where levels or prevalence of hazards may be altered.

The assessment considers the egg supply chain from laying flocks through to consumption, and was undertaken in the context of the current food safety management framework. It addresses shell eggs, processed egg products and specialty egg products such as salted, alkalised and fertilised eggs.

The risk assessment was undertaken following discussions with risk managers who sought the following information:

1. What are the microbiological and chemical risks to food safety posed by the consumption of eggs and egg products and the use of eggs and egg products in food in Australia?

   For the microbiological assessment this includes:
   - The contribution eggs and egg products make towards foodborne illness.
   - The main pathogens that cause egg-related foodborne illness.
   - Determination of how conclusive is the epidemiological evidence.

   For the chemical assessment this includes:
   - Consideration of inputs (*e.g.* feed, water) into the production of eggs and egg products.
   - How chemicals used in the production of eggs and egg product might potentially impact on public health and safety.
   - Identification of areas in the current regulatory system which may require attention in relation to addressing potential public health and safety risks associated with chemicals in eggs and egg products.

2. Where during the production and processing of eggs and egg products hazards may be introduced and/or their levels change, and which processing and production factors have the most significant impact on public health and safety?

3. What are the hazards and subsequent risks associated with emerging pathogens such as *Salmonella* Enteritidis and highly pathogenic avian influenza?

Eggs and egg products produced by all farmed avian species used for the production of eggs for human consumption including chicken, duck and quail have been included in the scope of the risk assessment. Eggs produced by ratites such as emus and ostriches were excluded from the assessment.

1 INTRODUCTION

This microbiological and chemical risk assessment provides an objective interpretation of available scientific data on the public health risks associated with the consumption of eggs and egg products in Australia. The assessment identifies key microbiological and chemical food safety hazards and assesses where in the primary production and processing supply chain these hazards may be introduced, increased, reduced or eliminated.

1.1 Previous risk assessments on eggs and egg products

A number of risk assessments (both qualitative and quantitative) have previously been undertaken to determine the food safety risk associated with consumption of eggs and egg products. Below is a brief summary of major risk assessments undertaken on egg and egg products.

1.1.1 International risk assessments

Todd (1996) undertook a risk assessment on the use of cracked eggs in Canada. The assessment examined the likelihood of cracked eggs being internally contaminated with *Salmonella* spp. compared with intact eggs, as well as analysing epidemiological data. From the epidemiological data it was determined that cracked eggs were 3 to 93 times more likely to be associated with outbreaks of foodborne illness than intact shell eggs. The probability of illness from consumption of cracked eggs, or foods made from cracked eggs, was estimated to be 1 in 3,800 for the general population. The number of cases of illness per year from consumption of cracked eggs was difficult to estimate due to large uncertainty associated with the potential for growth during storage as well as the frequency and amount of consumption of uncooked or lightly-cooked foods containing cracked eggs.

In 2002, the Food and Agriculture Organization and the World Health Organization (FAO/WHO) developed a quantitative risk assessment of *Salmonella* Enteritidis (SE) in eggs, drawing upon previous work by Morris (1990), Todd (1996), Whiting and Buchanan (1997) and FSIS (FSIS, 1998) (FAO/WHO, 2002). The exposure model considered egg production (prevalence of SE positive flocks and subsequent prevalence of SE contaminated eggs) through to consumption. A dose-response model for *Salmonella* spp. was also developed based on human feeding studies and epidemiological data, which is discussed in the hazard characterisation Section (Section 2.2) of this report.

A quantitative risk assessment of SE in egg and egg products undertaken by the US Food Safety and Inspection Service and the Food and Drug Administration (1998) was updated by the USDA-FSIS in 2005. The scope of the updated risk assessment was SE in shell eggs and *Salmonella* spp. in egg products (FSIS, 2005). For shell eggs, eight food preparation pathways were included, with each being modelled for domestic and institutional settings. Information was presented on times, temperatures and potential for contamination for each of the exposure pathways, through from point of lay, on-farm storage, grading, transport, retail and consumer storage to consumption. The data used in the model reflected US egg industry practices at the time.

Lake *et al*., (2004) conducted a risk profile on *Salmonella* in and on eggs in New Zealand. The authors concluded that SE and *S*. Typhimurium DT104 are not endemic to New Zealand poultry flocks and the majority of egg related illnesses in New Zealand are acquired overseas. The risk of exposure to *Salmonella* from domestic New Zealand eggs was found to be low.

1.1.2 Domestic risk assessments

1.1.2.1 Qualitative assessments

The Australian Egg Corporation Limited (AECL) recently funded development of a risk profile to determine the food safety risk associated with egg and egg products in Australia (Daughtry *et al*., 2005). The risk profile examined both microbiological and chemical hazards.

The assessment of chemical hazards concluded there was no evidence that residues of pesticides, veterinary medicines or other contaminants present a food safety or public health risk to Australian consumers.

A semi-quantitative methodology was used to rank microbiological risks associated with various egg exposure pathways. In total, 33 different exposure pathways were considered based on:

- Egg and egg product sales/supply chain pathways for commercial shell eggs and pulp products, and non-commercial eggs.
- Commercial egg handling and storage practices.
- Use in home, manufacturing and food service sectors.
- End use pathways for shell and processed commercial eggs (ingredient or egg-based meal).
- Effect of meal preparation (cooking).

For consumption of cooked or lightly-cooked egg meals using commercial eggs which had not undergone potential pathogen growth (*i.e.* stored at times/temperature where no growth of *Salmonella* would be expected), the risk rating was low. Use of eggs which had undergone pathogen growth in lightly-cooked meals resulted in a medium risk rating.

Those exposure pathways for which a high risk rating was obtained included use of non-commercial cracked eggs and unpasteurised egg pulp in uncooked foods. The risk of illness for cracked eggs was estimated to be 100 times higher than that for non-cracked eggs when used in meals subject to cooking that results in only a slight reduction of pathogens. For egg meals containing uncooked egg, the risk of illness was predicted to be 10 times higher for cracked eggs compared with intact eggs.

1.1.2.2 Quantitative risk assessments

In addition to the *National Food Safety Risk Profile of Eggs and Egg Products* the AECL commissioned a quantitative risk assessment of *Salmonella* spp. in eggs and egg products in Australia. The model was developed by researchers from Adelaide University and the South Australian Research and Development Institute (SARDI) (Thomas *et al*., 2006). The model was based on the USDA-FSIS exposure assessment but modified to reflect Australian egg production and consumption patterns. The exposure assessment was presented as a mathematical model describing the steps between point-of-lay and consumption. Where

possible, inputs to the model were described by probability distributions to reflect variation and uncertainty. Initially, it was proposed to develop an on-farm module to the model; however the quantity and quality of data during this stage of production was limited and prevented the construction of a complete through-chain exposure assessment model.

To gather data for the exposure model, a comprehensive survey of production practices undertaken by Australian egg producers and processors was undertaken. This included producers and processors from New South Wales, Queensland, South Australia and a small number from Victoria. Data gathered included flock size, egg collection frequency, storage, distribution and egg processing practices.

The quantitative risk assessment developed by Thomas *et al*. (2006) forms the basis for the quantitative exposure assessment in Section 2.3.5 of this document.

1.2 Risk Assessment Approach

The egg supply chain, from laying farms through to consumption, is complex with many factors that may impact on the potential to cause foodborne illness. This is particularly true for microbiological hazards where pathogens may be introduced and their levels change at numerous points along the production to consumption continuum.

Salmonella spp. has been identified as the primary microbiological hazard of concern in regards to consumption of eggs and egg products. Due to the quantity and quality of available data, previous risk assessments of microbiological hazards associated with eggs have focussed from point-of-lay through to consumption.

On-farm factors such as the layer environment, animal health and production practices have the potential to impact on the prevalence and/or levels of *Salmonella* spp in eggs. A substantial part of this risk assessment is the qualitative description of mechanisms whereby an egg can become contaminated with *Salmonella* spp. (external and internal) and the on-farm and processing factors that may impact on the prevalence and levels of organisms.

Very little information is available on the nature and extent of hazards associated with eggs from non-chicken species such as ducks, turkeys, geese, quails and pigeons. Although the focus of the assessment is on eggs from chickens (*Gallus gallus domesticus*), it is assumed that the hazards of concern for other poultry species are largely the same. Where appropriate data are available, microbiological and chemical risk factors specific for eggs obtained other from non-chicken poultry species are addressed.

The quantitative model developed for AECL was used as the basis for the risk assessment from the point of lay through to consumption. A summary of the approach taken for the microbiological risk assessment is provided in Figure 1.1.

The assessment of risk to public health and safety for eggs and egg products from the use, or presence, of chemicals in eggs has been undertaken in the form of a chemical risk assessment, which examines a broad range of chemicals either used in the production of, or present in, eggs and egg products.

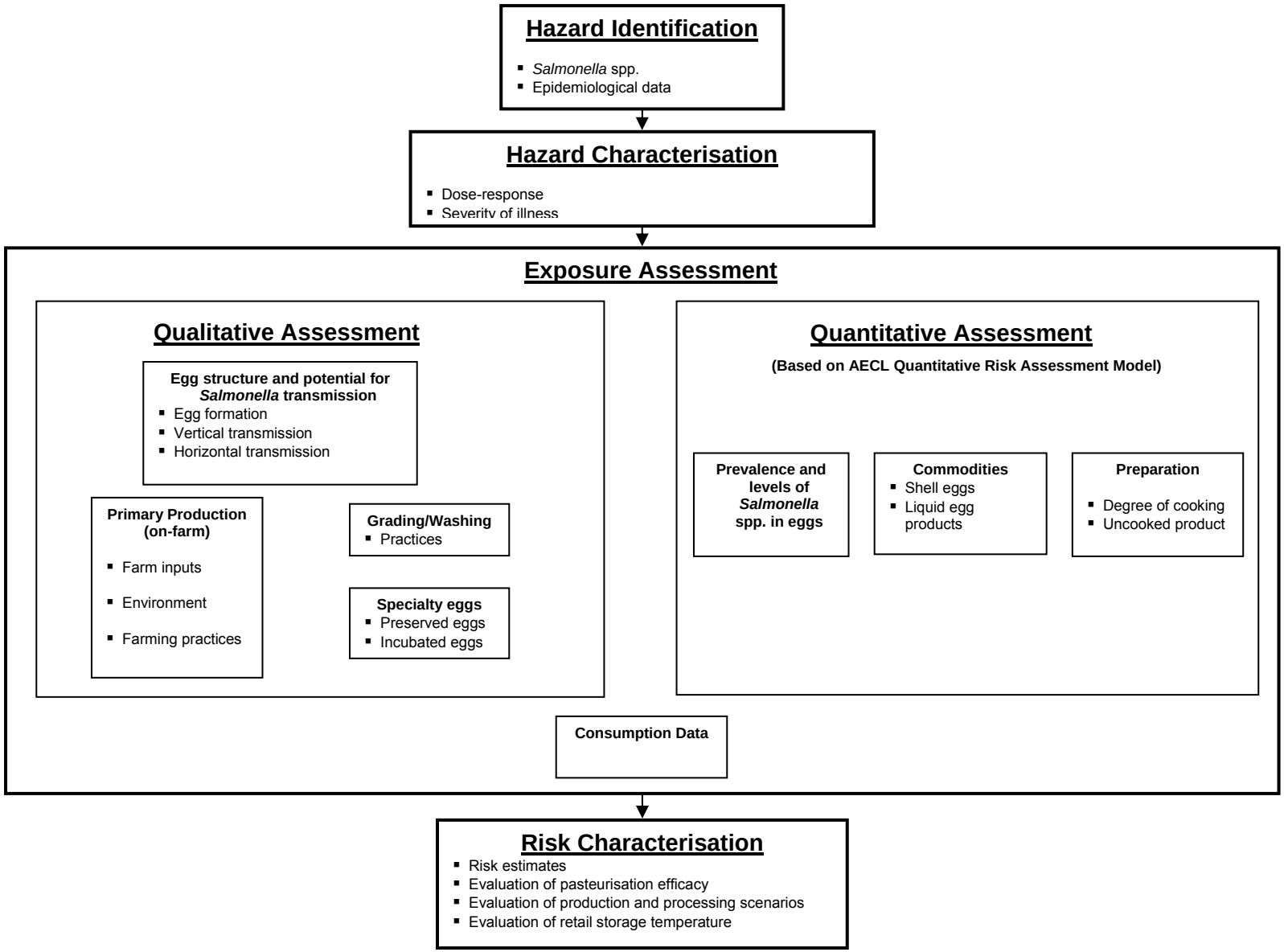


Figure 1.1 Main components of the microbiological risk assessment.

1.3 Description of Australian egg production

The Australian egg industry is largely based on eggs and egg products produced from chickens (*Gallus gallus domesticus*). Other egg-producing avian species, such as ducks, quails, geese, pigeons and guinea fowl form a minor part of the egg market. Unless specifically stated, further references in this report are to chicken eggs.

Eggs are produced in all States and Territories in Australia, with the greatest production occurring in New South Wales, Victoria and Queensland. In 2007, there are approximately 440 chicken egg producers in Australia with an annual production of approximately 236 million dozen eggs (ABS, 2008).

There are three main types of egg production systems in Australia: cage; barn; and free range systems. A description of these production systems is provided in Appendix 1. Egg production in Australia is predominantly caged-based systems (Table 1.1).

Table 1.1 Percentage of egg production types (AECL, 2008).

Egg production method	Approximate % of total egg production
Conventional cage	74.9
Free-range	20.0
Barn laid	5.1

AECL estimate that 65% of eggs produced in Australia are sold in shell from retail outlets, 20% sold in shell to the food service industry and 15% are diverted and processed into manufactured egg products *e.g.* pasteurised liquid, frozen and spray dried egg products.

1.3.1 Imported egg and egg products

Current quarantine requirements restrict the type of eggs and egg products permitted to be imported to Australia (administered by the Australian Quarantine Inspection Service; AQIS). Products containing greater than 10% egg are required to undergo assessment prior to importation. A summary of egg products permitted to be imported into Australia is provided in Appendix 2. In 2007, the amount of egg products imported into Australia was estimated to be 1,002 mt egg powder; 345 mt egg pulp; and 134 mt preserved/cooked eggs (AECL, 2008).

Under the *Imported Food Control Act (1992)*, imported egg products are referred to the AQIS Imported Food Programme for inspection at the "random surveillance" rate of 5% of all consignments. They are visually inspected and tested for compliance with the microbiological limits prescribed in Section 1.6.1 of the *Australian New Zealand Food Standards Code* (the Code).

Biosecurity Australia, an agency within the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), is responsible for the development of quarantine policies for the importation of animals and animal products. Biosecurity Australia is currently conducting an Import Risk Analysis (IRA) for edible eggs and egg products. The IRA will assess animal disease risks potentially associated with the importation of edible eggs and egg products from all countries. Import conditions may be amended and new conditions developed on completion of the IRA. Biosecurity Australia completed a *Policy Review for Taiwanese Preserved Duck Eggs* in 2007 (Biosecurity Australia, 2007).

1.4 Primary production and processing of eggs

In summary, fertilised eggs from breeder flocks are incubated and hatched in hatcheries, with the day-old chicks grown on pullet farms until they reach maturity at which time they are placed in layer farms. Once laid, eggs are collected, sorted, cleaned, candled, graded and packed either for the shell egg market or for processing. A generic description of the main steps during the primary production and processing of eggs is provided in Table 1.2.

Table 1.2 Summary of the main steps during primary production and processing of eggs.

Stage of production	Description
Parent breeder	Breeding farms house hens and roosters to produce fertilised eggs. The fertile eggs are collected daily and transported to the hatchery. Breeder stock are retained for approximately 12 months and then directed to meat processing.
Hatchery	Fertilised eggs are incubated in hatcheries. Day old chicks are screened and sexed before being vaccinated against avian diseases such as infectious bronchitis virus (IBV) and Marek's disease (MD). Day old chicks are then transported to farms for rearing.
Pullet rearing (immature layers)	Day old chicks are reared in either deep litter or cage rearing systems until approximately 17 weeks of age, after which they (pullets) are transferred to layer farms (either same farm or sold to other layer farms). Pullets are vaccinated against a number of endemic poultry diseases such as Fowl Cholera, avian encephalomyelitis (AE) and Newcastle disease (NDV).
Layer farm	Layers remain in production houses generally from approximately 18 until 74 weeks of age. Layer farms vary in size greatly, with small farms holding a few thousand birds through to larger operations with 500,000+ birds.
Egg collection	Eggs are generally collected daily, either manually or via conveyer belt, to an on-farm packing shed or to a centralised grading facility. Cracked or grossly dirty eggs are generally disposed of or collected for egg processing.
Egg cleaning	Can either involve wet or dry cleaning; wet washing involves a mechanised process of initial spray wetting, sanitising, rinsing and drying. Dry washing involves the use of a cloth or similar material to wipe away visible solids from the egg's surface. Alternative technologies such as UV sanitising may be used alone or together with washing in order to further decrease egg surface microbial loads.
Egg grading	Eggs are checked through a variety of mechanisms (*e.g.* visual inspections, candling, etc) for defects, cleanliness, and quality. Dirty, cracked and broken eggs are diverted for either disposal or further processing. Eggs are also graded by size for market specifications.
Packaging	Eggs are packaged to prevent damage and breakage occurring before reaching the consumer. Eggs are packaged for retail in clean moulded fibre or plastic cartons to prevent damage.
Storage and transportation	Eggs are stored between laying and grading / washing, after grading and during transportation to retail. Storage conditions (time, temperature and humidity) impact on the growth of microorganisms that may be present.
Further processing	Excess or second grade eggs (*e.g.* cracked or soiled) are often diverted to further processing steps for the manufacture of egg products such as liquid and dried egg.

Approximately 15% of shell eggs produced in Australia are used to produce egg products such as liquid whole egg, liquid albumen and liquid yolk (AECL, 2006b). A large range of value-added egg products are also produced, as illustrated in Table 1.3.

Table 1.3 Examples of different egg products.

Types of Egg Products	Examples
Refrigerated liquid egg products	Whole egg mix, egg yolk, egg albumen and mixes. These are used often in cakes, baked products, biscuit and pastry goods.
Frozen egg products	Frozen whole egg mix, egg yolk, egg albumen and mixes. These are often used in baked products, pastry goods, and food service industries.
Dried egg products (whole egg, yolk and egg white)	These are used in package cake mixes, packet crumb mixes, biscuits, dry diet meals, health drinks and protein drink additives
Value-added egg products	Whole hard-boiled peeled eggs, scrambled egg mix, omelette mix, chopped hard-boiled peeled eggs, pickled boiled eggs and yolk-free mix.
Consumer usage egg products	These egg products are sold through normal retail channels such as supermarkets and may include yolk-free low cholesterol mixes, crepes/pancake mixes, pavlova mixes, egg white and French toast
Shelled hard boiled eggs	Produced from hard boiled eggs and intended for sandwiches and salads. The eggs are steamed, shelled, packed in gas flushed bags and stored chilled.
Other speciality eggs	Mainly produced from duck or quail eggs, and includes salted, century or alkalised eggs and balut (embryonated) eggs.

1.5 Potential hazards associated with egg and egg products in Australia

1.5.1 Microbiological hazards

A wide range of microorganisms are associated with layer hens and the egg laying environment. During the production of eggs it is possible that microorganisms may be deposited on the surface of eggs, and in some situations the egg contents. Some of these microorganisms may be pathogenic to humans.

Salmonella is the most commonly reported aetiological agent for foodborne illness where eggs have been the implicated food vehicle. In Australia, *Salmonella* Typhimurium has been identified as the dominant serovar responsible for outbreaks of illness associated with egg consumption (see Section 2.1.6).

Salmonella Enteritidis phage type 4 is of major concern to the egg industry internationally. In the Northern Hemisphere and parts of Asia, this organism has become established in commercial egg laying flocks and has been the predominant serovar responsible for outbreaks of foodborne illness following the consumption of raw and undercooked eggs and egg products. More recently in the US, *S.* Heidelberg has also been responsible for a number of egg-associated outbreaks (Chittick *et al*., 2006). As discussed in Section 2.3.1.2, *S.* Enteritidis has an increased affinity to colonise the reproductive tissue of infected birds compared to other *Salmonella* serovars, enabling the direct internal contamination of eggs with *Salmonella* as they are being formed. *S.* Enteritidis phage type 4 is not endemic in Australian laying

flocks, with most human cases of this serovar being associated with travellers returning from overseas. This is discussed in further detail in Section 2.1.5.

Other human pathogens that may potentially be associated with eggs and egg products include *Campylobacter* spp, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Yersinia enterocolitica,* however there is very little epidemiological evidence associating these organisms with egg-related foodborne illness. Therefore these microorganisms have not been further considered in this assessment.

FSANZ has previously undertaken an assessment of the risk to consumers and handlers of poultry products, including eggs, from highly pathogenic avian influenza virus (H5N1) (Appendix 3 – Avian Influenza Risk Assessment). It was concluded that as the H5N1 avian influenza virus has not been detected in Australia there is a negligible risk of transmission of this virus via the handling and consumption of eggs and egg products in Australia. In addition, there is an absence of epidemiological data associating the handling or consumption of eggs or egg products with human cases of illness due of avian influenza (H5N1) infection. The full risk assessment is provided in Appendix 3

Occasionally parasites such as the nematode *Ascaridia galli* have been found in intact eggs (Martin-Pacho *et al*., 2005; Reid *et al*., 1973). Although uncommon, these organisms can colonise the oviduct and be incorporated into the egg during formation. *A. galli* and other helminthic parasites are readily detected by candling if performed. These organisms do not have the ability to infect humans and are therefore not considered a food safety hazard (Reid *et al*., 1973).

There is evidence of the growth of fungi on eggs, particularly in areas with high temperature and relative humidity (Davis and Stephenson, 1991). In a survey of eggs from supermarkets in Tropical Far North Queensland, Davis *et al.* (Davis *et al*., 1999) detected a variety of fungi, with *Cladosporium* and *Penicillum* being most commonly isolated. *Aspergillus flavus* was isolated from one egg which, under the right environmental conditions, may produce toxin. There is however very limited evidence of illness associated with exposure to fungi from consumption of egg and egg products, with fungal contamination typically associated with egg quality concerns.

1.5.2 Chemical hazards

As part of the assessment of eggs and egg products, FSANZ has evaluated the potential risks that may occur as a result of the use, or presence, of various chemicals at different points through the primary production and processing chain. This information has been used to identify areas where further data or additional controls may be necessary to ensure public health and safety concerns are addressed.

The major potential source of chemical contaminants in eggs is through the exposure of layer hens to chemicals at the primary production stage through ingestion, dermal contact or inhalation *e.g.* via feed and water, veterinary treatment, air, soil, or from housing materials. Further along the processing chain, additional chemical inputs may occur, including food additives and processing aids.

There are regulations currently in place that control the use or presence of chemicals in eggs; these are also discussed in this report where appropriate.

1.5.3 Physical hazards

Physical contaminants associated with egg and egg products include intrinsic contaminants (*e.g.* those introduced through malfunctioning of the shell glands) and extrinsic contaminants (*e.g.* material that is foreign to the nature of the food such as metal, glass, plastic). Extrinsic physical hazards are mainly a concern for egg products and may be introduced at any stage of the processing chain such as via raw materials, poorly maintained facilities and equipment, packaging materials and poor food safety practices.

Physical hazards would normally be addressed by adherence to Good Manufacturing Practices (GMP), a hazard analysis critical control point (HACCP) system and requirements relating to safe and suitable food in Chapter 3 of the Australia New Zealand Food Standards Code (the Code). Physical hazards associated with eggs and egg products are not covered by this risk assessment.

2 RISK ASSESSMENT – *SALMONELLA* SPP. IN EGGS AND EGG PRODUCTS

2.1 Hazard identification

Salmonellosis is a leading cause of enteric illness worldwide, with symptoms ranging from mild gastroenteritis to systemic illness such as septicaemia and other longer-term conditions. A wide range of foods has been implicated in foodborne salmonellosis. As the disease is primarily zoonotic, foods of animal origin have been consistently implicated as the main source of human salmonellosis (FAO/WHO 2002).

The genus *Salmonella* is divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori* (Table 2.1) (Brenner *et al*., 2000). The subspecies of most relevance in relation to food safety is *S. enterica* subsp. *enterica*, as over 99% of *Salmonella*s involved with human infection belong to this subspecies (Bell and Kyriakides, 2002).

Over 1,400 *Salmonella enterica* subsp. *enterica* serotypes are currently recognised and all are regarded as capable of causing illness in humans (Brenner *et al*., 2000). The formal names to describe *Salmonella* serotypes are rather cumbersome. For practical reasons, the names are commonly shortened, for example *S. enterica* subsp. *enterica* serotype Typhimurium (formerly *Salmonella typhimurium*) is shortened to *Salmonella* Typhimurium.

Some *Salmonella* serotypes are host-adapted to individual animal species and may differ vastly in the severity of the pathogenic infections they cause; others such as *S*. Typhimurium have the ability to infect a large variety of animals. For example *S*. Typhi and *S*. Paratyphi are specifically associated with infections and severe illness in humans (Bell and Kyriakides, 2002). Conversely, *S*. Gallinarum and *S*. Pullorum are host-adapted to poultry and associated with acute gastroenteritis and high mortality of birds but rarely associated with human illness (Lake *et al*., 2002).

Table 2.1 Species of the genus *Salmonella* (Brenner *et al*., 2000)**.**

***Salmonella* species/subspecies**	**No. of serotypes**	**Usual habitat**
S. enterica subsp. *enterica*	1,454	Warm-blooded animals
S. enterica subsp. *salamae*	489	Cold-blooded animals and environments[a]
S. enterica subsp. *arizonae*	94	Cold-blooded animals and environment
S. enterica subsp. *diarizonae*	324	Cold-blooded animals and environment
S. enterica subsp. *houtenae*	70	Cold-blooded animals and environment
S. enterica subsp. *indica*	12	Cold-blooded animals and environment
S. bongori	20	Cold-blooded animals and environment
Total	2,463	

[a] Isolates of all species and subspecies have occurred in humans.

Methods used to differentiate *Salmonella* serovars include both phenotypic methods (***e.g.*** phage typing, biotyping, antibiotic susceptibility) and genotypic methods ***e.g.*** DNA / RNA sampling, plasmid profiling, ribotyping, polymerase chain reactions (PCR), pulse field gel electrophoresis (PFGE), multiple-locus variable-number tandem repeat analysis (MLVA) (Cox and Fleet, 2003; Cox *et al*., 2002; Torpdahl *et al*., 2007). These techniques may be used for differentiating serovars in order to identify epidemiological links in the case of foodborne illness outbreaks.

2.1.1 Growth and survival

Salmonellae have relatively simple nutritional requirements and can survive for long periods of time in foods and other substrates (Jay *et al*., 2003). The rate of growth and extent of survival of the organism in a particular environment is influenced by the simultaneous effect of a number of factors such as nutrient availability, temperature, pH, and water activity (a_w). A summary of the growth limits for *Salmonella* spp. under various physical conditions is provided in Table 2.2. Being facultative aerobes, salmonellae have the ability to grow in the absence of oxygen. Growth and survival may also be influenced by the presence of inhibitors such as nitrite and short-chain fatty acids (Jay *et al*., 2003). Further discussion on the thermal inactivation of *Salmonella* spp. in egg and egg products is provided in the exposure assessment (Section 2.3.6).

Table 2.2 Limits for growth of *Salmonella* when physical conditions (***e.g.*** temperature, pH, a_w) are near optimum (ICMSF, 1996).

Condition	Minimum	Optimum	Maximum
Temperature (°C)	5.2*	35-43	46.2
pH	3.8	7.0-7.5	9.5
a_w	0.94	0.99	>0.99

* Most serotypes fail to grow at <7°C

2.1.1.1 Temperature

Growth of most salmonellae is substantially reduced at <15°C and prevented at <7°C (ICMSF, 1996; Jay *et al*., 2003). Growth generally does not occur at >46.2°C (ICMSF, 1998). The optimum temperature for growth is between 35 and 43°C (ICMSF, 1996). Freezing can be detrimental to salmonellae survival, although it does not guarantee destruction of the organism (ICMSF, 1996). There is an initial rapid decrease in the number of viable organisms at temperatures close to the freezing point as a result of the cellular damage. However, at lower temperatures (-17 to -20°C) there is a significantly less rapid decline in the number of viable organisms. *Salmonella* spp. have the ability to survive long periods of time at storage temperatures of < -20°C (Jay *et al*., 2003).

Heat resistance of salmonellae in foods is dependent on the composition, nature of solutes, pH, and water activity of the food and the conditions under which the food was exposed to before heating (Jay *et al*., 2003). In general, heat resistance increases as the water activity of the food, decreases. A reduction in pH results in a reduction of heat resistance (ICMSF, 1996). *Salmonella* organisms are considered sensitive to heat, with heat resistant stains being uncommon (Jay *et al*., 2003).

2.1.1.2 pH

The minimum pH at which *Salmonella* can grow is dependent on the temperature of incubation, the presence of salt and nitrite and the type of acid present. Growth has been reported to occur between pH 3.8 – 9.5 (Jay *et al*., 2003). The optimum pH range for growth is 7.0 – 7.5 (ICMSF, 1996). Volatile fatty acids are more bactericidal than acids such as lactic and citric acid.

2.1.1.3 Water activity (a_w)

Water activity has a significant effect on the growth of *Salmonella*, with the lower limit for growth being 0.94 (ICMSF, 1996). *Salmonella* can survive for long periods of time in foods with a low a_w (such as powdered egg products, black pepper, chocolate, gelatine). Exposure to low a_w environments can greatly increase the heat resistance of *Salmonella*.

2.1.2 Pathology of illness in humans

Outcomes of exposure to *Salmonella* can range from having no effect, to colonisation of the gastrointestinal tract without symptoms of illness (asymptomatic infection), or colonisation with the typical symptoms of acute gastroenteritis. Gastroenteritis symptoms may include abdominal pain, nausea, diarrhoea, mild fever, vomiting, headache and/or prostration, with clinical symptoms lasting 2–5 days (FAO/WHO 2002). Most symptoms of salmonellosis are mild, and only a small proportion of cases within the community tend to be reported to public health agencies (Mead *et al*., 1999). In a small number of cases, *Salmonella* infection can lead to more severe invasive diseases characterised by septicaemia and, sometimes, death.

Illness is usually self-limiting, with patients fully recovering within a week, although in some severe cases of diarrhoea, significant dehydration can ensue which may require medical intervention such as intravenous fluid replacement. Septicaemia is caused when *Salmonella* enters the bloodstream, with symptoms including high fever, pain in the thorax, chills, malaise and anorexia (FAO/WHO 2002). Although uncommon, long-term effects or sequelae may occur including arthritis, appendicitis, cholecystitis, endocarditis, local abscesses, meningitis, osteomyelitis, osteoarthritis, pericarditis, peritonitis, pleurisy, pneumonia and urinary tract infection (ICMSF, 1996).

At the onset of illness, large numbers of *Salmonella* are excreted in the faeces. Numbers decrease with time, but the median duration of excretion after acute non-typhoid salmonellosis has been estimated at five weeks, with approximately 1% of patients becoming chronic carriers (Jay *et al*., 2003). Due to the general self-limiting nature of the disease, antibiotics are not usually prescribed for healthy individuals suffering from mild to moderate *Salmonella* gastroenteritis (Hohmann, 2001). Antibiotics are considered necessary, however, for those who are severely ill and for patients with risk factors for extra intestinal spread of infection, after appropriate blood and faecal cultures are obtained and the presence of the organism is confirmed.

Of recent concern worldwide is the emergence of multiple antibiotic resistant strains of *Salmonella*, an example being *S*. Typhimurium definitive phage type 104 (DT104). Multi-drug resistant *S*. Typhimurium DT104 is a significant human and animal pathogen, with high morbidity observed in cattle and poultry (Crerar *et al*., 1999). To date, this organism is not endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay *et al*., 2003). *S*. Typhimurium DT104 carries resistance to multiple antibiotics including ampicillin, chloramphenicol, trimethoprim/sulphamethazol, streptomycin and tetracycline (Blumer *et al*., 2003). *S*. Typhimurium DT104 constitutes around 8–9% of human *Salmonella* isolates in the USA, while sporadic human cases are reported in Australia, these are most commonly acquired overseas (Blumer *et al*., 2003).

2.1.3 Mode of transmission

Salmonella spp. are transmitted by the faecal-oral route. Sources of transmission include person-to-person, foodborne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals (Jay *et al*., 2003).

2.1.4 Occurrence of *Salmonella* in food

The primary reservoir of *Salmonella* is the intestinal tract of warm and cold-blooded vertebrates. *Salmonella* has been isolated from a wide range of foods, particularly those of animal origin and those foods that have been subject to faecal contamination (ICMSF, 1996).

Raw meat products, in particular poultry, have frequently been associated with the presence of *Salmonella* spp. (Bryan and Doyle, 1995). *Salmonella* positive animals at the time of slaughter may have high numbers of organisms in their intestines as well as on external surfaces (faecal contamination of hides, fleece, skin or feathers). Cross contamination during processing may also lead to increased prevalence of *Salmonella* in finished products (Bryan and Doyle, 1995).

As discussed in Section 2.3.1, eggs can be contaminated with *Salmonella* either externally or internally. Contamination may occur directly from infected hens (*i.e*. as the egg is being laid) or from the surrounding environment (exposure to faecal material, contaminated litter etc). A summary of the reported prevalence of *Salmonella* spp. in egg and egg products in Australia and internationally is provided in Appendix 4 and discussed further in Section 2.3.5.

2.1.5 Incidence and outbreak data

Salmonellosis is one of the most commonly reported enteric illnesses worldwide (FAO/WHO 2002). In Australia, approximately 7,000-8,000 cases of salmonellosis are formally notified to health authorities annually. Taking into account under-reporting, it has been estimated that around 80,000 cases of foodborne salmonellosis may occur in Australia annually (Hall *et al*., 2005).

The salmonellosis notification rate in Australia for 2007 was 45.3 cases per 100,000 population (Figure 2.1), with rate of infections varying depending on location, ranging from 26.8 cases per 100,000 population in Victoria to 187.0 cases per 100,000 population in the Northern Territory (NNDSS, 2008). Children less than five years of age have by far the highest notification rate, with a rate of 202.3 cases per 100,000 population reported for 2007

(NNDSS 2008). The higher rate of notified salmonellosis cases in this age group may reflect an increased susceptibility upon first exposure, but may also be a result of other factors such as an increased likelihood of exposure and increased likelihood to access medical care and having samples tested.

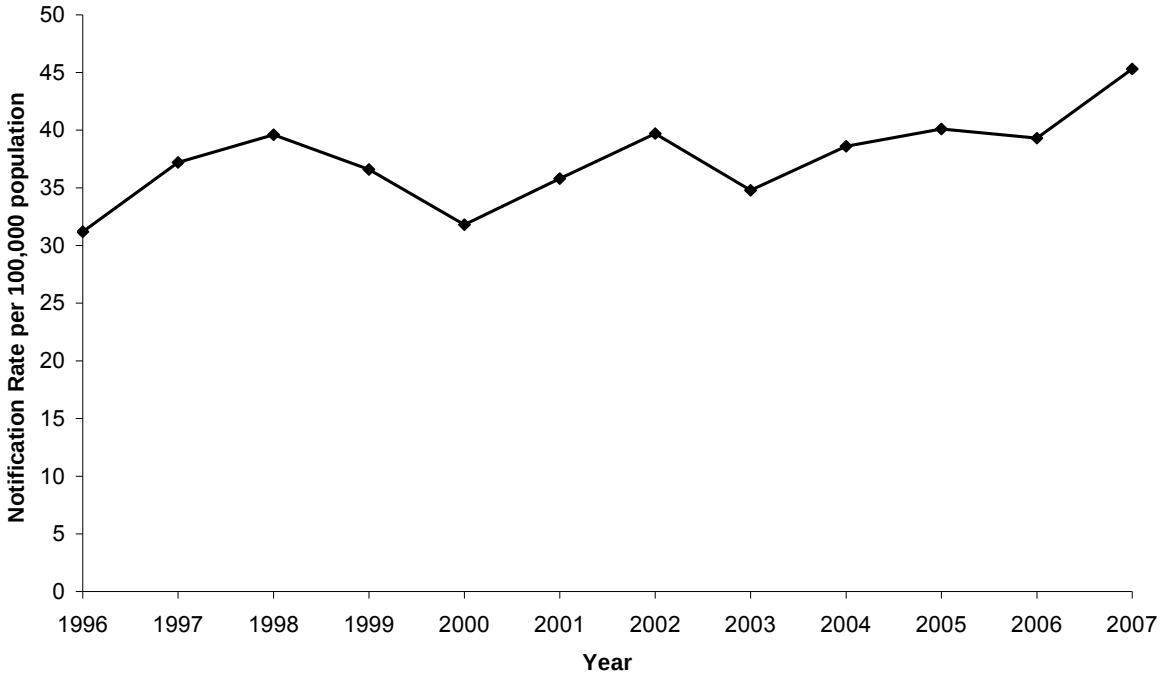


Figure 2.1 Salmonellosis notification rates in Australia by year -1996 to 2007 (NNDSS 2008).

The distribution of *Salmonella* serovars varies geographically, however *S*. Typhimurium is the most commonly reported serovar in all Australia States and Territories except Queensland and Northern Territory, whereby *S*. Saintpaul is the most common (Owen *et al*., 2007).

It has been estimated that the proportion of salmonellosis that is foodborne in Australia is 87% (Ashbolt *et al*., 2005). In the United States (Mead *et al*., 1999) and England and Wales (Adak *et al*., 2002), it has been estimated that the proportion of salmonellosis that is foodborne is 95% and 91.6% respectively. Other potential sources of infection are contaminated water, person-to-person transmission and direct contact with infected animals.

Based on national and international epidemiological data (primarily outbreak investigations) a wide range of foods have been implicated in human salmonellosis. However foods of animal origin (*e.g.* meat, eggs, and dairy products) are identified as the most important sources of human salmonellosis.

Internationally, epidemiological investigations have identified raw or undercooked egg and egg products as foods most commonly associated with human cases of salmonellosis, in particular due to SE (Parker *et al*., 2001). A summary of salmonellosis outbreaks associated with consumption of eggs and egg products is provided in Appendix 5.

Fortunately SE (in particular phage 4) is not endemic in Australia, with most human cases reported from travellers returning from overseas. Of the 396 cases of SE infections reported in 2007, 25 cases were locally acquired with no one phage type predominating (Fullerton, 2008). In previous years (2003-2006), the most common phage type associated with locally acquired cases has been SE phage type 26 (OzFoodNet, 2004; OzFoodNet, 2007a). While this phage type has been isolated from Queensland egg production environments (n=4, 2004-2006) (NEPSS, 2005; NEPSS, 2006) the pathway of exposure for human cases has not been fully elucidated.

2.1.6 Outbreaks of Salmonellosis associated with eggs and egg products in Australia

In a review of foodborne illness outbreaks in Australia from 2001 to June 2005 undertaken by OzFoodNet, a total of 31 out of 441 (7%) all outbreaks investigated were attributed to eggs or egg products. All of these outbreaks were due to *Salmonella* spp. A summary of these outbreaks and retrospective analysis of the level of evidence attributing illness to eggs is provided in Appendix 6. The majority of outbreaks were epidemiologically linked to mixed dishes suspected to contain raw or under-cooked eggs. It is important to recognise that there are considerably more sporadic cases of salmonellosis than those associated with outbreaks, which makes it difficult to generalise about the causes of community-acquired *Salmonella* infections.

In 2006 and 2007, the number of reported egg-associated foodborne illness outbreaks (confirmed and suspected) increased, representing 14% (16/115) and 16% (24/149) of total foodborne outbreaks respectively (OzFoodNet, 2007b; OzFoodNet, 2008). Attribution of foodborne illness to eggs is often difficult due to the retrospective and non-point source circumstances of most outbreaks; the low level of *Salmonella* contamination of eggs; and the low level of reporting of foodborne illness in the community. It is further complicated by the potential for cross-contamination, temperature abuse of the implicated food, and the lack of an available regional database on *Salmonella* serovars found in commercial layer environments.

A large outbreak of foodborne illness (125 laboratory confirmed cases) involving *S*. Typhimurium PT 135a (a local variant of PT 135) cases occurred in Tasmania between June and December 2005 and was linked to the consumption of products containing raw egg (Stephens *et al*., 2007). A number of food businesses were identified during the investigation as point sources, each of which was supplied eggs from a single farm. Eggs supplied to the foods businesses were unwashed, with visible external faecal contamination of eggs and packaging being observed during the environmental investigation. During the investigations, inadequate food handling and storage conditions were identified in the food businesses, which clearly contributed to the outbreak. This included the potential for cross contamination and/or storage under conditions that permitted growth of *Salmonella*. Although *Salmonella* was not isolated from the surface of eggs sampled, *S*. Typhimurium 135a was isolated from the farm that supplied the eggs (from samples of faeces, spilled feed, an egg conveyer belt and the surface of pulp-grade eggs) and a number of on-farm practices were identified that may have increased the risk of egg contamination.

These outbreaks in Tasmania highlight the multi-factorial nature of foodborne disease outbreaks, in particular those associated with eggs. A review of the epidemiological data suggests the use of dirty and/or cracked eggs in uncooked foods is a significant risk factor for human salmonellosis.

There is very little epidemiological data linking the consumption of clean, intact eggs with foodborne outbreaks of salmonellosis in Australia. However, the difficulty in attributing sporadic cases of foodborne illness to specific food needs to be recognised, and the true incidence of egg-associated cases of illness is uncertain.

2.2 Hazard Characterisation

2.2.1 Virulence and infectivity

Once ingested, *Salmonella* must be able to overcome the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defence mechanisms to enable infection (Jay *et al*., 2003).

Salmonella possesses a number of structural and physiological virulence factors enabling it to cause acute and chronic disease in humans. The virulence of *Salmonella* varies with the length and structure of the O side chains of lipopolysaccharide (LPS) molecules at the surface of the cell. Resistance of *Salmonella* to the lytic action of complement is directly related to the length of the O side chain (Jay *et al*., 2003). Other important virulence factors include the presence and type of fimbriae, which is related to the ability of *Salmonella* to attach to epithelium cells, as well as the expression of genes responsible for invasion into cells (Jones, 2005). These include *Salmonella* pathogenicity island I (SPI-1) which is required for invasion of the microorganism into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* are dependent on the function of SPI-2 (Valle and Guiney, 2005).

The presence of virulence plasmids has been associated with the ability to spread rapidly after colonisation and overwhelm the host immune response (D'Aoust, 1997). These virulence plasmids are large cytoplasmic DNA structures that replicate independently of the chromosomal DNA. Virulence plasmids are present in a limited number of *Salmonella* serovars and have been confirmed in *S.* Typhimurium, *S.* Dublin, *S.* Gallinarum, *S.* Pullorum, *S.* Enteritidis, *S.* Choleraesuis and *S.* Abortusovis. It is notable, however, that virulence plasmids are absent from *S*. Typhi, which is host-adapted and highly infectious.

Once attached to small intestine epithelial cells, the organism is drawn into the host cell in a vesicle (endosome) where it can multiply in the mildly acidic environment. Heat labile enterotoxin may be released during *Salmonella* growth, resulting in the loss of intestinal fluids. This enterotoxin is closely related functionally, immunologically and genetically to cholera toxin and the heat labile toxin (LT) of pathogenic *E. coli* (Jay *et al*., 2003). Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage to the intestinal mucosal surface and results in general enteric symptoms and inflammation. For non-typhoidal *Salmonella*, infection is generally limited to a localised intestinal event.

Australian data suggests that *S*. Virchow has a greater propensity to cause invasive salmonellosis (isolated from the bloodstream) compared with other *Salmonella* serovars, particularly in the young with the median age of cases of 9 years in 2005 (NNDSS, 2007). The median age of invasive salmonellosis cases due to *S*. Typhimurium was 65 years.

2.2.2 Dose response

Human feeding trials for a range of *Salmonella* serovars were undertaken during the 1950's to determine the relationship between the dose of pathogen ingested and the response of the individual (McCullough and Eisele, 1951a; McCullough and Eisele, 1951b; McCullough and Eisele, 1951c; McCullough and Eisele, 1951d). The study population consisted of healthy males confined in an institutional setting who were fed known doses of an individual *Salmonella* serovar. Infection was confirmed by recovering the administered *Salmonella* serovar from faecal samples.

Fazil (1996) combined all the data from the feeding trials and found that a single beta-Poisson relationship could adequately describe the dose-response for all serovars. However, a number of limitations exist on the use of such feeding trial data. Firstly the use of healthy adult male volunteers could underestimate the pathogenicity to the overall population. In addition, volunteers were exposed to high doses of *Salmonella*, with the minimum dose being 10^4 cells.

In dose-response analysis, the critical region is the lower-dose region, as these are the doses that are most likely to exist in real food contamination events. This requires extrapolation of the model to doses much lower than those used in the human feeding trials. It must also be noted that the dose-response models are based on the risk of infection as an endpoint rather than illness, and therefore may introduce a level of conservatism into the dose-response relationship.

It has been shown, through salmonellosis outbreak investigations, that doses resulting in illnesses (gastroenteritis) were often several orders of magnitude lower than the doses reported in the feeding trials (D'Aoust, 1994). Using a reasonably large data set, the WHO/FAO in 2002 developed a dose-response model based on actual outbreak data. Again, a beta-Poisson model was used to describe the dose-response relationship (Figure 2.2).

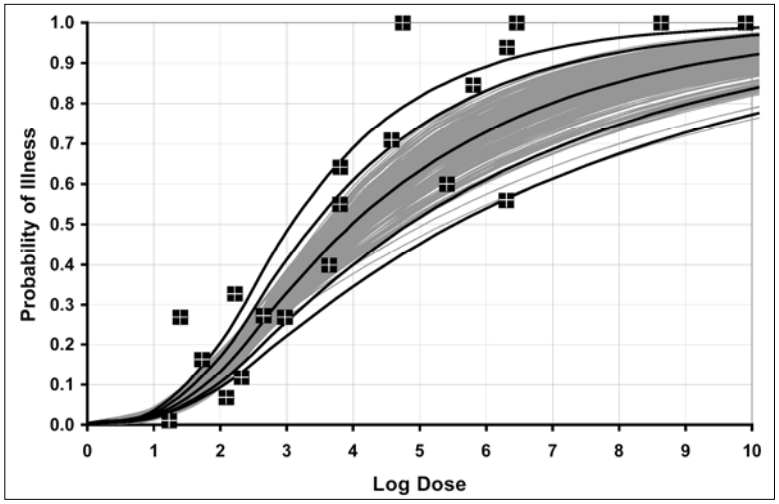


Figure 2.2 Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO 2002) .

Although not subject to some of the inherent flaws associated with using purely experimental data, data used in this model have a certain degree of uncertainty, which required assumptions to be made. This uncertainty is primarily due to the uncontrolled settings under which the information and data were collected. It is often difficult to determine the actual dose ingested (based on the level of the organism in the food at the time of consumption and the amount of food consumed), as well as determining the actual number of people exposed or ill during the outbreak.

In developing the Australian quantitative risk assessment model for *Salmonella* in eggs, Thomas *et al.* (2006) re-evaluated the WHO/FAO outbreak data and estimated the values for α and β (Table 2.3) and using an alternative Beta-Poisson dose-response equation (Equation 1). At low doses (< 100 cells) and at high doses (>10^6 cells), the predicted probability of illness was similar between the WHO/FAO model and the alternative dose-response model. At intermediate doses, estimated probabilities of illness from the alternative dose-response model were less variable than the WHO/FAO model.

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

Table 2.3 Predicted $\log_{10}\alpha$ and $\log_{10}\beta$ values for the alternative (D2TAP) dose-response model (Thomas *et al*., 2006).

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: α = 0.1346; β = 53.33

2.2.3 Host factors

Individual susceptibility to *Salmonella* infection and/or disease can vary significantly, depending on host factors such as pre-existing immunity, nutrition, age, ability to elicit an immune response, structural and functional anomalies of the intestinal tract, or pre-existing disease (Gerba *et al*., 1996; Jay *et al*., 2003). Individuals who are generally at greater risk of infection and/or risk of developing more severe outcomes from exposure to *Salmonella* include the very young, the elderly, pregnant women and the immune-compromised (organ transplant patients, cancer patients, AIDS patients) (Gerba *et al*., 1996).

2.3 Exposure Assessment

2.3.1 Egg function, structure and potential for microbiological contamination

The egg provides a complex series of physical and chemical barriers to microbiological invasion and growth. While maintaining integrity from bacterial invasion, the egg possesses numerous pores for exchange of respiratory gases and water vapour during growth of the embryo. However, these pores also present a potential route for microorganisms to penetrate the egg (Bruce and Drysdale, 1994).

The initial physical barrier to microbial penetration of the egg is a fine hydrophobic proteinaceous layer called the cuticle (Haigh and Betts, 1991). The cuticle covers the egg which, when dry, forms "plugs" within the pores providing enhanced protection from microbial penetration (Bruce and Drysdale, 1994; Haigh and Betts, 1991).

In addition to the external barriers of the shell and cuticle, inner shell membranes (separating the internal surface of the shell and the albumen) and the vitelline membrane (separating the albumen and the yolk) provide further barriers to microbial penetration. In addition to providing a physical barrier to microbial invasion, these semipermeable membranes are involved in the diffusion of gases to and from the egg compartments (Bruce and Drysdale, 1994).

The albumen contains a number of compounds that are inhibitory to bacterial survival and/or growth. Approximately 15% of the albumin consists of ovotransferrin which chelates metal ions required for microorganisms to grow (Board *et al*., 1994; Schoeni *et al*., 1995). Freshly laid eggs have a pH in the range of 7.6-7.8, however after 1-3 days storage at room temperature the pH rises to 9.1-9.6, at which ovotransferrin has an enhanced ability to chelate metal ions (ICMSF, 1998; Li-Chan *et al*., 1995). Studies have demonstrated that adding ferric ammonium citrate overcomes the bactericidal properties of the albumen for a range of *Salmonella* spp. when incubated at 20°C and 30°C (Lock and Board, 1992). Without adding ferric ions (Fe^{3+}), the majority of 26 *Salmonella* serovars tested remained viable, but did not increase in numbers, when incubated at 20°C and 30°C. At 4°C however, a decrease in viable *Salmonella* spp. was observed. A study by Guan *et al*. (2006) also found that *S*. Typhimurium DT104 and SE were able to survive in albumen for >120 hours when incubated at 37°C.

Another important antimicrobial compound present in the albumen is lysozyme which lyses bacterial cells. Gram-positive bacteria are more sensitive to lysozyme compared with Gram-negative bacteria (ICMSF, 1998). However, studies have shown that a number of Gram-negative bacteria, including *S*. Typhimurium and *Y. enterocolitica* can grow in media containing lysozyme (Hughey and Johnson, 1987). When directly inoculated into albumen and incubated at 4 and 10°C, Schoeni *et al*. (1995) found numbers of *S.* Enteritidis, *S*. Typhimurium and *S*. Heidelberg to remain stable or increase slightly. However, growth of *Salmonella* in albumen was observed when incubated at 25°C.

In a study by Cogan *et al*. (2001), growth of SE was not observed when either 2 or 25 cells were inoculated into separated albumen and stored at 20°C for 8 days. When the inoculum was increased to 250 – 2500 cells, 32.5 and 65.0% of albumen sampled contained high levels of contamination. This suggests that the presence >25 cells may overcome the bacteriostatic

properties of the albumen. Growth of *Salmonella* in albumen at temperatures >20°C has also been reported Chen *et al*. (Chen *et al*., 2005).

In contrast, egg yolk provides an ideal medium for bacterial growth (Board *et al*., 1994). The pH of the yolk at time of lay is approximately 6.0 and can rise up to 6.9 during storage (Li-Chan *et al*., 1995). Once in the yolk, *Salmonella* spp. may grow to levels up to 10^{11} cells, especially at temperatures >20°C (Braun and Fehlhaber, 1995; Cogan *et al*., 2001; Guan *et al*., 2006; Humphrey, 1994). Generation times of < 2 hours have been reported when stored at 25°C (Daughtry *et al*., 2005).

2.3.1.1 Egg formation

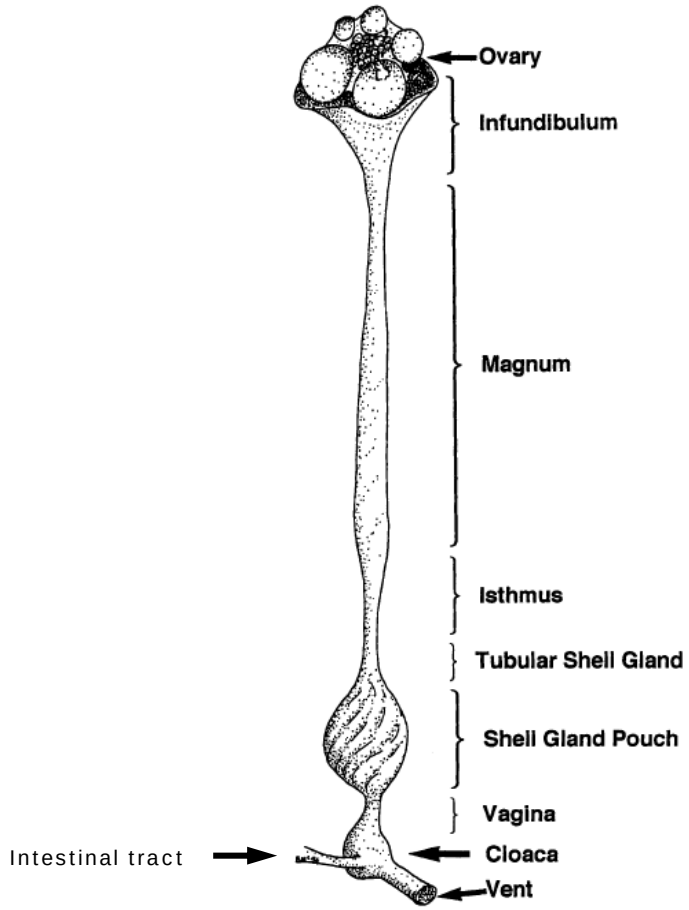


Figure 2.3 Diagram of the oviduct (from Roberts, 2004)

The first step in the production of an egg is ovulation of the yolk from the ovary. The yolk is captured by the infundibulum where it remains for approximately 15 min (Roberts, 2004). It is at this stage that the vitelline membrane and chalazae are formed. The egg then passes through the magnum where it remains for 2.5 - 3 hours while the albumen is produced (Board *et al*., 1994; Roberts, 2004). Following this, the egg moves through the isthmus, where shell membranes are produced (approximately 1 hour). The egg then enters the tubular shell gland where water and electrolytes enter the albumen (termed "plumping"). The egg spends approximately 15 – 20 hours in the shell gland pouch where the egg shell is formed and the

process of "plumping" is completed. Once completed, the egg is laid via the vagina, cloaca and vent. Overall, the time required for the formation of an egg is approximately 26 hours (Keller *et al*., 1995).

The contents of the egg can become contaminated with *Salmonella* via two routes, trans-ovarian (vertically) or trans-shell (horizontally). Trans-ovarian transmission occurs via infection of the bird's reproductive system, mainly the ovaries and oviduct tissue. This may lead to direct contamination of the yolk, albumen, egg shell membranes or egg shell as the egg is being formed (Bruce and Drysdale, 1994).

2.3.1.2 Vertical transmission of Salmonella *(trans-ovarian)*

Where SE is endemic in layer flocks, trans-ovarian transmission is considered the most important route of internal *Salmonella* contamination (FAO/WHO 2002). *S*. Enteritidis has an enhanced ability over other *Salmonella* serovars to colonise and infect the reproductive tissues in poultry (Okamura *et al*., 2001a).

It was previously thought that infection of the oviduct was solely a result of ascending infection from the cloaca (De Buck *et al*., 2004b). It is now accepted that prior to deposition of eggshell, forming eggs can be subject to descending infection from colonised ovarian tissue, ascending infections from colonised vaginal and/or cloacal tissue, and lateral infections from colonised upper oviduct tissues (Keller *et al*., 1995).

Timoney *et al*. (1989) exposed hens to SE via inoculation of the crop with 10^6 cfu and showed SE to be highly invasive, causing bacteraemia with infection in many parts of the body including the peritoneum, ovules and oviduct. Persistent infection was observed in the peritoneum, caeca and liver. Infected birds only showed signs of mild diarrhoea, with the exception of four hens (out of 42) that died during the experiment. In addition, egg production was not significantly affected.

Results from in-vitro studies, using cultures of isthmus and magnum tubular gland cells, have suggested SE preferably invades tubular glandular cells of the isthmus in comparison with the magnum (De Buck *et al*., 2004a). Using an in-vivo method to confirm results of the in-vitro analyses, De Buck *et al*. (2004a) studied invasion of oviductal tissue in laying hens. The authors stated that SE cells were commonly associated with tubular gland cells, with few cells observed being attached to the surface epithelial cells. It was suggested that intermittent production of infected eggs could be explained by the ability of SE to colonise intra-cellularly in the oviduct for long periods, and under a yet undefined stimuli, exit the cells and colonise the egg as it forms.

Okamura *et al*. (2001b) investigated the ability of *S*. Enteritidis, *S*. Typhimurium, *S*. Infantis, *S*. Hadar, *S*. Heidelberg and *S*. Montevideo to colonise the reproductive organs of laying hens and their ability to contaminate eggs. Hens were inoculated intravenously with 10^6 cfu. Following inoculation, the ovary and preovulatory follicles were colonised significantly higher with *S*. Enteritidis compared with other serovars. The cloaca and vagina were colonised in hens infected with all serovars, whereas eggshell contamination was only observed for *S*. Enteritidis (3.7%) and *S*. Hadar (3.0%). The authors stated that fact that hens with contaminated vaginas and cloacae did not lay shell-contaminated eggs suggests that under these conditions egg contamination occurred via contact with colonised reproductive organs rather than by eggshell penetration.

An earlier study by Barnhart *et al*. (1991) undertaken in the US investigated the prevalence of SE and other *Salmonella* serovars in the ovaries of layer hens at the time of slaughter. Of 42 flocks sampled, 32 (76%) were positive for *Salmonella* infection in the ovaries, with a number of different *Salmonella* serovars being detected. Only one flock was positive for SE (PT 23), with the predominant serovar being *S*. Heidelberg. Other serovars detected in the ovaries included *S*. Agona, *S*. Oranienburg, *S*. Mbandaka, *S*. Kentucky, *S*. Montevideo, *S*. London, *S*. Typhimurium, *S*. Infantis, *S*. Schwarzengrund, *S*. Ohio, *S*. Cerro, *S*. Anatum plus other un-typed serovars. The authors stated that the public health significance of this data is unclear as a direct link between ovarian infection and internal contamination of eggs was not investigated. Based on epidemiological data, SE appears to be more readily transmitted to the egg during formation than other *Salmonella* serovars.

Mizumoto *et al*. (2005) also identified the increased ability of SE to colonise the vaginal epithelium compared with other *Salmonella* serovars. Results indicated that the affinity of *Salmonella* serovars to colonise the epithelium was related to their lipopolysaccharide (LPS) type, with SE producing high molecular mass LPS compared with other *Salmonella* serovars. Other factors such as flagella, fimbriae and outer membrane proteins have also been reported as important determinants of attachment of SE to epithelium cells (Allen-Vercoe and Woodward, 1999; Guard-Petter *et al*., 1996; Woodward *et al*., 2000).

Brown and Brand (1978) exposed point-of-lay and in-lay pullets to 10^{10} *S*. Typhimurium via oral inoculation and measured the infection status of birds and eggs over a three week period. Systemic infection was observed in exposed hens, including recovery of *Salmonella* spp. from the ovaries of birds. Despite the systemic infection, *Salmonella* spp. was not isolated from the surface or contents of 257 eggs sampled. Similar studies by Cox *et al*. (1973), Baker *et al*. (1980a) and Brown *et al*. (1976) have also found no evidence of vertical transmission of *S*. Typhimurium (or *S*. Infantis, *S*. Senftenberg or *S*. Thompson) in experimentally infected hens.

2.3.1.3 Horizontal transmission of Salmonella *(trans-shell)*

For non-SE *Salmonella* serovars, trans-shell transmission is the major route of internal egg contamination (ICMSF, 1998). As previously discussed, the egg provides various physical and chemical barriers to penetration from microorganisms, however a number of factors impact on the efficacy of these defence mechanisms. As SE is not endemic in Australian poultry flocks, horizontal transmission is considered the primary route of internal egg contamination.

<u>External contamination</u>

For trans-shell contamination to occur there must be initial contamination of the surface of the egg shell. Reproductive tissue is rarely contaminated with microorganisms of faecal origin (Board *et al*., 1994). Therefore contamination of the egg shell surface usually occurs at, or after, the point of lay. *Salmonella* may be transmitted via contact with the vent of an infected bird as the egg is laid or via contact with faeces in the surrounding environment (***e.g.*** faeces-contaminated nest boxes, conveyer belts, floor).

Schoeni *et al*. (1995) demonstrated the level of faecal contamination on the egg shell impacts on the extent of trans-shell transmission. For example, when egg surfaces were experimentally contaminated with faeces containing levels of 10^4 cfu/g, *S*. Heidelberg was found in the contents and *S*. Enteritidis was found in the membrane by day one. *S*. Typhimurium penetrated the shell at a slower rate, not being detected in the membrane until day three. However, when the level of *Salmonella* in faeces was 10^6 cfu/g, all *Salmonella* serovars tested were found in the egg contents by day one.

Temperature differential

Differences in temperature between the contents of the egg and the surrounding environment can lead to pressure differentials (Messens *et al*., 2005b; Schoeni *et al*., 1995). This is of particular concern when the temperature of the egg is greater than the environment. As the egg cools, a negative pressure is created which may result in contamination being drawn in through the pores of the shell (Bruce and Drysdale, 1994). As discussed in Section 2.3.3.1, exposure of eggs to water of lower temperature has been found to enhance penetration of *Salmonella* into egg contents during the washing process. Eggs are also particularly vulnerable to trans-shell contamination at the point of lay where the egg cools after leaving the warm internal temperature of the bird (approximately 40°C). However, studies have shown that for eggs with intact shells, cooling in air to refrigeration temperatures does not result in increased penetration of *Salmonella* into the egg contents (Curtis *et al*., 1995; Thompson *et al*., 2000; Zeidler *et al*., 1999).

Moisture

Moisture is considered an important factor for the penetration of microorganisms through the shell (Bruce and Drysdale, 1994; Messens *et al*., 2005b). Moisture can be generated when eggs are moved from refrigeration temperature to room temperature, whereby condensation results in water droplets accumulating on the shell surface (particularly under high humidity conditions). This process is often referred to as “sweating”.

Studies have demonstrated an increased rate of penetration of *S*. Enteritidis through the egg shell when stored at 24 hours at 6°C and then 20 days at 20°C (inducing condensation) compared with eggs stored for 21 days at a constant 20°C and 60% relative humidity (62% and 48% penetration respectively, $p<0.01$) (de Reu *et al*., 2006a). This experiment used eggs whose contents had been aseptically removed and replaced with nutrient agar, retaining the shell and shell membranes. When the experiment was replicated using whole intact eggs, there was no observable difference between contamination rates for both condensate and control eggs. The authors suggest this may reflect either the increased nutrients available from the agar and/or conditions did not simulate the antimicrobial components of the egg contents.

A study by Padron *et al*. (1990) investigated the penetration of *S*. Typhimurium through egg shells by spraying the eggs with the bacterial suspension or by contact with dry contaminated nest litter. *S*. Typhimurium was found in 83% of those eggs sprayed, and 59% of eggs that were in contact with dry contaminated nest litter. The authors suggest that although the presence of water on the shell enhances bacterial penetration, its presence is not essential for penetration to occur.

Egg porosity

For an intact egg the pores are the only route available for bacteria to penetrate the egg shell (Bruce and Drysdale, 1994). It is estimated that a hen's egg shell contains 7,000-17,000 pores, most of which are "plugged" with organic material (the cuticle), reducing the potential for microorganisms to gain entry (Messens *et al*., 2004).

A number of studies have found that penetration of egg shell by *Salmonella* spp. is independent of the number of pores (de Reu *et al*., 2006c; Nascimento *et al*., 1992). In addition to pores being blocked by the cuticle, some pores do not extend entirely through the thickness of the shell (Bruce and Drysdale, 1994).

Condition of the shell and cuticle

As previously discussed, the cuticle is the initial physical barrier to bacterial penetration. The thickness of the cuticle ranges from 0.5-12.8 μm (average of 10μm), although it is not evenly distributed across the egg shell (Bruce and Drysdale, 1994; Nascimento *et al*., 1992).

The cuticle takes between 1 – 3 minutes to dry after lay, during which time the egg is particularly vulnerable to penetration from microorganisms (Sparks and Board, 1985). The thickness of cuticle is variable and often large areas of the shell are devoid of cuticular cover (Bruce and Drysdale, 1994). Many factors impact on the degree of cuticle deposition on the egg including the age and strain of the laying bird, as well as environmental factors (Solomon *et al*., 1994). Ageing of the egg after lay leads to drying out and shrinking of the cuticle, which may leave pores exposed (Messens *et al*., 2005b). The cuticle is considered to be one of the most important structures preventing the migration of microorganisms through the shell (Baker and Bruce, 1994; Keum-Il *et al*., 1999).

The strength of the eggshell as a whole depends on geometric variables such as the shape and thickness of the shell combined with the shell's microstructure and chemical composition (Bain *et al*., 2006). Studies have shown the thickness of the shell has a variable impact on bacterial penetration. For example De Reu *et al*. (2006c), Jones and Musgrove (2005), Messens *et al*. (2005a) and Williams *et al.* (1968) reported shell thickness did not influence the rate of bacterial penetration, however Sauter and Peterson (1974) found penetration to be significantly related to shell thickness.

Shell thickness is influenced by numerous factors. As the bird ages, egg size increases at a greater proportional rate than the weight of the shell (*i.e.* the ratio of shell weight to egg weight decreases) (Roberts, 2004). Diet also plays an important role in determining eggshell strength. Each eggshell contains approximately 3 g of calcium which needs to be supplied to the bird in a form that can be utilised efficiently (Roberts, 2004). In addition to the availability of calcium in the diet, the ratio of calcium to phosphorous is important as high levels of phosphorous can reduce the adsorption of calcium from the gut, which may result in reduced eggshell quality (Boorman and Gunaratne, 2001). In addition to maintaining general bird health, specific vitamins such as vitamin D are necessary for calcium metabolism (Roberts, 2004).

Regardless of the thickness of the shell, any crack or fracture of the shell greatly enhances the likelihood of bacterial penetration (Bruce and Drysdale, 1994; ICMSF, 1998; Messens *et al*., 2005b). Although *Salmonella* would still need to penetrate the vitelline membrane to gain access to the yolk, any organisms lodged under the shell or on the membranes may have the potential to grow if storage conditions are inadequate (Todd, 1996).

Membranes

Underneath the eggshell are the shell membranes, which consist of three distinct layers: the inner and outer-shell membranes which consist of a network of randomly orientated fibres and a homogeneous third layer of electron-dense material called the limiting membrane (Bruce and Drysdale, 1994; Solomon *et al*., 1994).

These membranes provide a physical barrier to prevent the movement of bacteria into the albumen as well as allow the diffusion of respiratory gases to and from the egg, and the movement of calcium from the shell (Bruce and Drysdale, 1994).

The outermost of the shell membranes is porous and does not provide a significant barrier to microorganisms (ICMSF, 1998; Solomon *et al*., 1994). The inner shell membrane is considered the most important internal barrier to bacterial penetration (Bruce and Drysdale, 1994; Mayes and Takeballi, 1983). Berrang *et al*. (1999) investigated the role of the physical structure of the inner shell membrane and the potential penetration of *S*. Typhimurium; however no relationship between the two were identified. The authors suggested other factors such as charge or chemical structure of the membrane may play a more important role than physical hindrance or entrapment in the fibrous network of the inner shell membrane.

The vitelline membrane that surrounds the yolk is made up of glycoproteins (Kirunda and Mckee, 2000). The strength of this membrane decreases with egg age (Jones *et al*., 2002; Romanoff and Romanoff, 1949) and temperature (Romanoff and Romanoff, 1949). The main process leading to the decrease in the strength of the vitelline membrane is diffusion of water from the albumen into the yolk due to an osmotic gradient. This movement of water results in an enlargement of the yolk that stretches and consequently weakens the membrane. As the diffusion of water into the yolk is temperature dependent, higher temperatures result in a more rapid weakening of the vitelline membrane (Conner *et al*., 2003; Humphrey and Whitehead, 1993; Romanoff and Romanoff, 1949). Deterioration of the vitelline membrane results in yolk being introduced into the albumen, reducing its antimicrobial properties and allowing growth of *Salmonella* (Conner *et al*., 2003; Humphrey, 1994; Whiting *et al*., 2000) .

Humphrey and Whitehead (1993) investigated the growth of SE PT4 in albumen and yolk in experimentally infected eggs stored at 20°C. Rapid growth of *Salmonella* was not observed until eggs had been stored for 3 weeks and was associated with a deterioration of the vitelline membrane surrounding the yolk.

2.3.2 On-farm factors

Many factors during the on-farm production of eggs have the ability to introduce *Salmonella* spp. into the laying environment and/or laying flock, and impact on the extent of faecal contamination of the egg itself. No systematic data is available on the prevalence of *Salmonella* in layer flocks in Australia, however data from the New South Wales SE monitoring program reported in Thomas *et al*. (2006) showed that of 2252 drag swabs taken from layer sheds between 2000-2002, 3.1 % (2.4%-3.9%, 95% CI) were positive for *Salmonella* spp.

Below is a description of the main on-farm factors that may ultimately impact on *Salmonella* contamination of eggs.

2.3.2.1 Production systems and practices

Bacterial pathogens may be introduced to laying flocks through numerous environmental sources. The production method under which eggs are produced is an important factor to consider in regards to the potential for eggs to become contaminated. Differences between eggs produced from cage, barn, and free range systems have previously been reviewed by Quarles *et al*. (1970) and Dawson *et al*. (2001). A brief description of the different production systems is provided in Appendix 1.

Conditions under which laying hens are kept have the potential to influence disease presence, susceptibility and/or pathogen transmission, with factors such as the environment around the layer shed, animal husbandry practices, stocking density, diet, water supply, hygiene conditions and stress all having a potential impact (Durant *et al*., 1999; Holt, 2003; Humphrey, 2006).

Apart from specific serovars such as *S*. Enteritidis, which have the ability to be vertically transmitted to eggs via the reproductive tissue of infected hens, eggs may become externally contaminated with *Salmonella* via exposure to contaminated faeces, litter, nest boxes and handling equipment (Chen *et al*., 2002).

It has been reported that eggs produced from hens housed in caged systems have a reduced potential for contamination compared with those from floor-based systems, due to the reduced exposure to litter and faeces (Cox, 2001; Dawson *et al*., 2001). Quarles *et al.* (1970) identified that laying houses with litter floors (barn/free range) averaged nine times more bacteria in the air than did caged laying houses (in Dawson *et al*., 2001). Studies have found a positive correlation between the concentration of total bacteria in the air of poultry houses and the initial concentration on the shell (de Reu K. *et al*., 2005; de Reu *et al*., 2006b).

Due to the birds' increased access to the environment outside the confines of the shed, free range systems tend to have lower level biosecurity controls in place to prevent the introduction and spread of diseases amongst layer flocks (Humphrey, 2006). Free range flocks have also be found to be subject to a higher risk of poultry diseases than flocks housed in sheds (Dawson *et al*., 2001). Other hazards specific to the production of free-range eggs include the direct exposure of laying hens to wild birds and other animals, potential access to un-treated water and other miscellaneous environmental contaminants in the environment (Dawson *et al*., 2001).

Results presented in a recent report from the European Food Safety Authority (EFSA) showed a higher prevalence of *Salmonella* in flocks housed in cage-based systems than that for barn or free-range (EFSA, 2007). However, compared to alternative production systems, cage farms were also characterised by larger flock sizes. It is therefore difficult to identify the true risk factor for *Salmonella* positive eggs in this study as either due to the production system or to flock size.

Egg collection practices play an important role in the potential for eggs to become exposed to faecal contamination, and vary between production systems. For both free-range and barn production systems there is an increased potential for eggs to be exposed directly to litter and faecal matter, in particular for eggs laid outside of the nest box (often referred to as floor eggs). This is discussed further in Section 2.3.2.10.

2.3.2.2 Supply of day-old chicks and replacement pullets

Regardless of production system, supply of *Salmonella* infected laying hens (either day-old chicks or replacement pullets) is a source of flock contamination. Chicks may become infected either during the hatching period or via exposure to *Salmonella* in the hatching environment (Cox *et al*., 2000). A study by Cason *et al*. (1994) demonstrated chicks were able to hatch from inoculated eggs with high numbers of *S*. Typhimurium.

2.3.2.3 Feed

Contaminated feed is considered an important avenue by which *Salmonella* spp. can be introduced into poultry flocks, potentially leading to endemic infections (Bisgaard, 1992; Crump *et al*., 2002). However, although *Salmonella* spp. has often been isolated from poultry feed, there have been few published cases where a direct link has been identified between the presence of the pathogen in feed and subsequent infection in poultry and/or ultimately humans (Sapkota *et al*., 2007). The true extent of contaminated feed being a source of flock contamination is unclear due to the absence of a systematic surveillance system that monitors *Salmonella* spp. in feed, poultry and humans.

Poultry are fed primarily a mixture of cereal grain (***e.g.*** wheat, oats, barley, triticale, maize/corn, millet, sorghum, rye, pollard, bran, millrun), protein meal (***e.g.*** oilseed meals, peanut meal, soya bean meal) and other ingredients (***e.g.*** pulses/legumes, oilseeds, fruits and fruit by-products). Feeds may also include added vitamins and minerals supplements (APVMA, 2002). Meat meal which may be included is generated from rendering plants, which process meats, meat trimmings and other animal by-products (Sapkota *et al*., 2007).

The inclusion of unprocessed animal proteins, or animal proteins that have been contaminated post-processing, are thought to be a major source of *Salmonella* contamination in feed (Dawson *et al*., 2001; Williams, 1981). However the grain component of feed has also been shown to be a source of *Salmonella* spp. (Bains and MacKenzie, 1974; Dawson *et al*., 2001).

The environmental condition under which feed is stored and transported is also an important factor in determining the likelihood of contamination. Storing feed at low temperatures and low moisture levels will limit the potential for growth of *Salmonella* spp. if present (Dawson *et al*., 2001). Contamination by insects, rodents and wild birds has been identified as a major source of *Salmonella* contamination in feed (Davies *et al*., 1997; Maciorowski *et al*., 2006; Sapkota *et al*., 2007).

Layer hens are generally fed non-pelleted (mash) feed which has not undergone heat-treatment. The use of non heat-treated feed has been recognised as an increased biosecurity risk to animal health than is the use of heat-treated feed (East, 2007). Research by Zindel and Bennett (1968) failed to isolate any *Salmonella* spp. from heat-treated feed but found 1.6% of feed ingredients to be contaminated (from Dawson *et al*., 2001). More recently Jones *et al.* (1991) isolated viable *Salmonella* spp. from 35% of unprocessed feeds and only 6.5% of processed feeds, indicating a significant reduction in contamination due to processing.

Young birds (less than one week of age) are particularly susceptible to colonisation with *Salmonella* spp., thought to be as a result of an immature immune system and un-established gut microflora (Beal *et al*., 2004; Cox *et al*., 1990). Hinton (1988) undertook a study whereby day-old chicks were fed feed artificially contaminated with *S*. Kedougou or *S.* Livingston over a period of three weeks. When levels of *Salmonella* were 0.1 cfu/g of feed, up to 6% of birds become infected, while all birds became infected when levels in feed were above 100 cfu/g. It is common practice in the Australian egg industry to start pullets on heat-treated commercial rations and move to mash rations as the birds mature and are less susceptible to colonisation with *Salmonella* spp. (P. Scott, personal communication).

Australian data shows that poultry feed can be contaminated with *Salmonella* at a rate of between 3.5- 25.6% (Personal Communication, WA Department of Health). The Stock-Feed Manufacturers' Council of Australia (SFMCA) operates under a code of practice and accreditation system for the production of stock feeds which includes testing for *Salmonella* spp. These guidelines, however, do not specify a frequency for microbiological testing of feeds (FeedSafe, 2007).

In Japan during the period of 1993 – 1998, 10,418 samples of layer feed were tested for *Salmonella* contamination, with 53 (0.5%) of these testing positive which included serovars such as *S*. Enteritidis, *S*. Orion, *S*. Amersonfoort, *S*. Derby, *S.* Infantis and *S*. Tennessee (Shirota *et al*., 2001). McChesney *et al*. (1995) found that 56% (n=101) of animal-protein based feed samples and 36% (n=50) of vegetable-protein based feed samples contained *Salmonella* spp. however these feeds were not necessary designated for poultry use in the US. Hofacre *et al*. (2001) found that 14% (n=165) of a meat and bone meal obtained from poultry feed mills was contaminated with *Salmonella* spp.

The practice of adding organic acids to feed mash has been proposed to reduce the levels of *Salmonella* in feed and subsequently reduce the likelihood of infection in laying hens. The use of acid mixes has also been claimed to reduce mould growth in feeds potentially reducing the risk of mycotoxin production (Hinton and Linton, 1988; Hinton *et al*., 1985; Van Immerseel *et al*., 2006). Examples of these organic acids include short chain fatty acids (SCFA) (*e.g.* formic acid, acetic acid, propionic acid and butyric acid) and medium chain fatty acids (MCFA) (*e.g.* caproic acid, caprylic acid, capric acid and lauric acid). Studies have reported that MCFA have a greater antimicrobial action than SCFA (Van Immerseel *et al*., 2006). In-vitro studies have shown acidification of feed to be a possible intervention for the control of *Salmonella* in the feed samples, although in-vivo studies in hens have shown limited protective effects (Heres *et al*., 2004).

The composition of feed can also impact on the natural production of SCFAs via organisms in the intestinal system of laying hens. This can also be achieved via the addition of prebiotics and/or probiotics such as certain species of *Lactobacillus* (Van Coillie *et al*., 2007). Increased production of organic acids has been demonstrated to decrease *Salmonella* levels (and other pathogenic bacteria) in the gut whilst stimulating growth of "beneficial" probiotic bacteria (Van Immerseel *et al*., 2006).

2.3.2.4 Water

Drinking water has previously been identified as a source of *Salmonella* transmission in poultry flocks (Van Immerseel *et al*., 2006). In a study by Poppe *et al.* (1985), chlorination of drinking water at the level of 10ppm available chlorine resulted in a decreased of total plate count, faecal coliform count and an absence of *Salmonella* spp. compared with untreated water. The efficacy of chlorination, however, is highly dependent on the pH, temperature and amount of organic matter present in the water (ICMSF, 1998; Poppe *et al*., 1985). The method of delivering the water to the flocks is also important, as devices which limit or prevent environmental contact with the water, such as nipple drinkers have been shown to maintain sufficient available chlorine levels whereas open sources, such as troughs are not as effective (Poppe *et al*., 1985).

Open troughs used for drinking water can also potentially be a source of *Salmonella* through contamination with litter particles, feed, faecal material, dust and other foreign matter (Mayes and Takeballi, 1983; Poppe *et al*., 1985).

The addition of organic acids, such as SCFAs to water has also been used as a method for sanitising the drinking water available to poultry flocks (Van Immerseel *et al*., 2006). Al Chalaby *et al* (1985) conducted a study using a commercial product containing a propionic acid which was shown to prevent *Salmonella* contamination of drinking water, with control groups of environmentally contaminated water samples showing greater than 80% contamination. However whilst the acid addition was shown to eliminate *Salmonella* in the water samples it did not alter *Salmonella* carriage by the hens involved in the study with significant *Salmonella* shedding still occurring after water treatment (Al-Chalaby *et al*., 1985).

Acidification of drinking water available during feed withdrawal of poultry meat birds has been shown to be effective in reducing the prevalence of *Salmonella* in the crop of birds, with the addition of lactic acid (at the level of 0.44%) to drinking water during a 10 hour feed withdrawal periods significantly reducing *Salmonella* prevalence in broiler crops at slaughter (Byrd *et al*., 2001). However a study by Kubena *et al*., (2005) showed that addition of acetic acid or lactic acid to drinking water did not significantly reduce artificial crop or caecal *Salmonella* contamination rates during forced periods of feed withdrawal. This led the authors to conclude that during times of high stress, water acidification is shown to be less effective in reducing *Salmonella* transmission than it is under normal circumstances (Van Immerseel *et al*., 2006).

Another potential hazard is the practice of 'fogging', or releasing a fine aerosol mist of water over laying hens kept in hot climates. If untreated contaminated water is used, there is a potential for the introduction of *Salmonella* spp. into the layer house environment. This water can also potentially pool and may be accessible to the birds (Dawson *et al*., 2001).

2.3.2.5 Pests

Pests such as rodents and insects may be responsible for introducing *Salmonella* into laying flocks, as well as being a source of continual re-infection.

Salmonella has frequently been isolated from rodents found on laying farms, particularly mice (Davies and Breslin, 2001; Guard-Petter *et al*., 1997; Henzler and Opitz, 1992; Liebana *et al*., 2003; Pocock *et al*., 2001). Faeces from rodents have the potential to contaminate feed, drinking water, egg belts and other egg handling equipment (Dawson *et al*., 2001; Henzler and Opitz, 1992; Williams, 1981). The ability of rodents to spread *Salmonella* between birds and between flocks depends on the individual farm layout and the rodent species involved. It has been suggested that mice tend to stay localised, where sufficient food, water and shelter is present, whilst rats may tend to travel long distances which may more easily transmit pathogens across the farm (Dawson *et al*., 2001; Henzler and Opitz, 1992).

Internationally, Henzler and Opitz (1992) surveyed *Salmonella* Enteritidis contamination of 10 poultry farms with mice infestations and found that out of those farms with SE present, SE was isolated from 7.5% of environmental samples and 24% of mice samples tested. From farms that were certified as SE free, no mice samples tested positive to SE. This organism has also been found to persist for 10 months in infected mice populations (Henzler and Opitz, 1992). In this study it was shown that one faecal pellet out of the estimated 100 excreted per mouse per day contained an average of 2.3×10^5 viable SE organisms. Additional *Salmonella* serovars isolated from rodents in the same study included *S.* Heidelberg, *S.* Hadar, *S.* Typhimurium, *S.* Anatum, *S.* Mbandaka, *S.* Cerro and *S.* Schwarzengrund.

Flies, foxes, cats, dogs, beetles (*e.g.* lesser mealworm beetle), cockroaches and wild birds have also been shown to harbour *Salmonella* organisms in laying farm environments (Davies and Wray, 1995; Henzler and Opitz, 1992; Jay *et al*., 2003; Liebana *et al*., 2003; Olsen and Hammack, 2000).

The ability of *Salmonella* to persist in adverse conditions in the layer environment is important for the potential of spread amongst birds and on the surface of eggs. *Salmonella* can survive for extended periods of time in laying house environments and on the surface of egg handling equipment (Liebana *et al*., 2003).

2.3.2.6 Biosecurity practices

The term biosecurity is used to describe a set of management practices which, when followed collectively, reduce the potential for the introduction or spread of disease-causing organisms into and between laying farms.

A positive relationship has been found between low levels of viral and bacterial infections in birds and high biosecurity control within poultry farms (East, 2007; Gibbens *et al*., 2001). Risk factors include wild bird entry into housing facilities, poor on farm hygiene practices, use of fibre egg trays, inadequate sanitisation of the water supply, inadequate cleaning and disinfection of housing prior to restocking birds, inadequate treatment of feed supply, housing of multiple avian species on the same farm and in the same area, and disposal methods for dead birds (East, 2007; Gibbens *et al*., 2001).

Biosecurity is a key component in minimising the potential for entry of avian influenza H5N1, *S*. Enteritidis as well as other exotic avian diseases into laying flocks. The egg industry has developed a *Code of Practice for Biosecurity in the Egg Industry* (Grimes and Jackson, 2001) however adoption of this management strategy is voluntary, and the actual level of implementation by egg producers is unknown (East, 2007).

2.3.2.7 Airborne contamination

Infections due to airborne contaminants have been suggested as a potential route of *Salmonella* contamination of laying flocks, with infections resulting from the inhalation of airborne particles, which may be circulating as droplets or dust particles (Baskerville *et al*., 1992; Dawson *et al*., 2001). Baskerville (1992) found that only very low does of SE (PT 4) were required to infect poultry via the respiratory tract and consequently transmitted to other bodily tissues including the ovary and oviduct.

2.3.2.8 Stress

Laying flocks can be affected by physical and/or psychological stress. Stress may be introduced into flocks by allowing excess human movement within the housing areas, the provision of poor housing facilities, social disruptions in the flock and via production practices such as induced moulting, thinning and feed withdrawal. Hens naturally experience stress when they enter sexual maturity (Humphrey, 2006).

El-Lethey *et al.* (2003) investigated the effects of stress caused by a lack of foraging material being provided to layers hens and found that immune function was adversely affected, impairing both humoral and cell- mediated immunity. Reduced immune function has also been reported in hens exposed to heat stress, with reduced white blood cell counts and antibody production being observed (Mashaly *et al*., 2004; Zulkifli *et al*., 2000). Inhibition of the immune system may lead to the increased susceptibility of birds to infection with microorganisms (Dohms and Metz, 1991; El-Lethey *et al*., 2003; Holt, 2003). Burkholder *et al*. (2008) also demonstrated that exposure of poultry to heat stress causes changes in the normal intestinal microbiota and epithelial structure of ileal tissue, leading to an increased attachment of SE.

The physiological response of animals to stress includes the increased circulation (and intestinal levels) of neurotransmitters and corticosteroids (Humphrey, 2006). In-vitro studies have shown that the presence of neurotransmitters can result in increased growth and expression of virulence factors in many bacteria including *Salmonella* spp., *Eschericia coli* and *Listeria* spp. (Bailey *et al*., 1999; Belay and Sonnenfeld, 2002).

Studies have also investigated the influence of stress on shell quality and appearance and have found that the proportion of shell abnormalities increases with levels of stress whilst the total production of eggs declines (Humphrey, 2006).

<u>Induced moulting</u>
Induced moulting is used internationally in poultry flocks to force a resting period in laying hens for the purpose of improving shell quality and quantity of egg production, and to extend the laying life of hens (Murase *et al*., 2006a; Murase *et al*., 2006b; Webster, 2003). Moulting induces the regression and rejuvenation of the oviduct and ovary tissue.

Induction of moulting can be achieved by reducing light (photoperiod reduction) and restricting feed (Hurwitz *et al*., 1995). It has been suggested that moulting hens through feed deprivation may increase the risk of *Salmonella* susceptibility and faecal shedding due to immunosuppression caused by the stress involved (Holt and Porter, Jr., 1992; Murase *et al*., 2006b; Murase *et al*., 2001).

Induced moulting of hens though removal of feed sources may increase the severity of *Salmonella* colonisation and increases the likelihood of cross-contamination amongst the flock. Feed withdrawal alters the microenvironment of the intestinal tract, potentially changing the commensal flora, lactate and short chain fatty acid concentrations and increasing the pH level of the intestinal environment (Humphrey, 2006). This can in turn lead to increased colonisation by intestinal pathogens such as SE and is thought to be a factor in infections involving *Salmonella* by increasing potential invasion of the crop, caeca, spleen and liver of the bird (Durant *et al*., 1999; Humphrey, 2006).

Using non-feed withdrawal methods such as feeding hens wheat middlings or wheat bran diet have been identified as better methods of inducing the moulting process (Biggs *et al*., 2003; Murase *et al*., 2006b; Seo *et al*., 2001). Research has shown a comparable production increase when using non feed reduction methods and additionally, *Salmonella* shedding has been shown to be higher in experimentally infected SE flocks using feed removal methods than in flocks using wheat middling feeds (Seo *et al*., 2001). Murase (2006b), demonstrated that using wheat bran feeds as an alternative to feed withdrawl methods resulted in a successful moulting process without increasing the overall *Salmonella* infection of the flock.

2.3.2.9 Vaccination

Vaccination of laying hens has been used as a means of controlling rates of *Salmonella* infection and the subsequent potential contamination of eggs. Many studies have been undertaken to determine the efficacy of vaccines against *Salmonella* spp. in poultry. However, the outcome of infection under experimental conditions can vary greatly depending the study design, such as the *Salmonella* strain used for challenge, the route and dose of exposure and the age of the bird at the time of vaccination and challenge (Zhang-Barber *et al*., 1999).

Commercially, two types of vaccines have been used against *Salmonella* spp; inactivated and live (attenuated) vaccines. Inactivated vaccines have been found to elicit an immune response, however protection has been variable (Barrow, 2007; Zhang-Barber *et al*., 1999). A study by Clifton-Hadley *et al*. (2002) found that poultry vaccinated using an inactivated *S*. Typhimurium and *S*. Enteritidis vaccine had reduced faecal shedding of the challenge strain compared with non-vaccinated poultry; however no difference was observed in the rate of intestinal colonisation. Live vaccines generate both cell-mediated and humoral immune responses and generally result in more rapid and consistent protection (Barrow, 2007).

Vaccination of poultry flocks against specific strains of *Salmonella* is often difficult, with *Salmonella* pathogenicity being highly serovar- and host-dependent, as well as a general lack of knowledge on the factors involved in the immune response of poultry to *Salmonella* (Barrow, 2007; Beal *et al*., 2004). In contrast, vaccination against poultry-specific *Salmonella* serovars, such as *S*. Gallinarum, has proved very effective in poultry flocks (Barrow, 2007).

Research is being undertaken nationally and internationally to further develop effective vaccines against zoonotic *Salmonella* spp. Although vaccination has been identified as a tool to reduce *Salmonella* infection in poultry, it will not guarantee protection of poultry flocks from *Salmonella* and is generally recommended it be used in conjunction with other control measures (Davies and Breslin, 2003a).

2.3.2.10 Egg collection systems

The environmental conditions under which eggs are exposed after lay can influence the ultimate quality and safety of the shell egg. Bacterial contamination of eggs can occur as the egg exits the vent, or from contact with faecal material and other contaminated material in the environment. As previously discussed, eggs that are cracked during the collection process will have an increased potential for *Salmonella* to penetrate into the egg contents.

Egg collection practices vary considerably between farms and may be undertaken manually or by using an automated process. Automated collection systems, utilises gravity to allow the egg to roll away from the hen onto a conveyer belt and be transferred to a grading area, or moved on to further processing (Lake *et al*., 2004). Automated systems such as these are reported to reduce the potential for surface contamination of eggs compared with eggs collected manually from nests (ICMSF, 1998). There is, however, a potential for contamination of automated collection systems with material from broken/leaking eggs (Dawson *et al*., 2001).

2.3.2.11 Personal / handling hygiene

Handling of eggs, such as via manual collection from nests, may allow for cross contamination to occur between the hands of the workers and the egg surface (Cox, 2001). Environmental conditions, health and hygiene of personnel, and the presence of cracked and/or dirty eggs may contribute to a higher risk for cross contamination to occur.

2.3.2.12 Storage and transportation of eggs

How eggs are stored can affect the integrity of the shell and membranes, likelihood of cross-contamination, and the potential for growth of microorganisms that may be present. The effect of storage time and temperature on egg safety is discussed in detail Section 2.3.5.

2.3.2.13 Summary of on-farm factors

The introduction of *Salmonella* spp. on-farm and subsequent contamination of eggs is a multifactorial process. The *Salmonella* status of the laying flock, as well as on-farm practices, impact on the likelihood of egg contamination.

There are many potential transmission routes whereby flocks may become exposed to *Salmonella* spp. Studies have demonstrated the role of contaminated feed and water as a source of infection in poultry flocks. Faeces from infected birds contain high numbers of *Salmonella* which contaminates the laying environment and may lead to transmission to other birds.

Of primary concern in Australia is the external contamination of eggs, either as the egg is laid and/or from contact with contaminated faecal material, litter etc in the production environment. These factors can be influenced by the type and design of production systems, animal health, egg collection systems, and handling and hygiene practices.

2.3.3 Egg washing, grading and packing

Following collection, eggs are transferred automatically, or by hand, to reusable trays to continue along the processing chain and and may include steps such as sorting, washing, candling, grading and packing. This may occur on-farm or at a centralised grading facility.

Sorting of eggs involves the diversion of grossly dirty, cracked or misformed eggs from the grading process. These eggs are generally collected and sent to a processing plant for manufacture of liquid egg products. In some circumstances cracked and leaking eggs may be pulped at the grading facility prior to transportation to a processing plant. Storage of these products above refrigeration temperatures can result in rapid growth of microorganisms, including *Salmonella* spp (refer to Section 2.3.4).

2.3.3.1 Washing

Washing of eggs is undertaken to remove faecal material and other particles such as dirt, litter and debris from the egg surface (Hutchison *et al*., 2003). It is also undertaken to improve the aesthetic appearance, overall hygienic status of the egg, and reduce the risk of cross contamination (EFSA, 2005c; Northcutt *et al*., 2005). Commercial egg washing has been found to significantly reduce the surface concentration of aerobic bacteria, Enterobacteriaceae, yeasts and fungi potentially found on the surface of shell eggs (Curtis, 2007).

There is a difference of opinions on the benefits or otherwise of washing eggs. Those in favour, suggest that wet washing removes faecal material and reduces microbial populations on the egg shell surface therefore reducing the likelihood of horizontal transmission occurring as well as reducing the potential for cross contamination during food handling/preparation. Critics, however, suggest that wet washing removes the protective cuticle layer thereby leaving pores exposed and increasing the risk of potential bacterial penetration (EFSA, 2005c; Sparks, 1994).

Egg washing is widely used in many countries including Australia, the US and Japan (EFSA, 2005c; Hutchison *et al*., 2004). The European Union, however, prohibits washing grade A table eggs due to a historical view that the wetting of eggs increases the likelihood of spoilage and may potentially increase moisture loss from the egg's contents due to destruction of the protective outer cuticle layer (Baker and Bruce, 1994; Hutchison *et al*., 2004). If performed incorrectly, egg washing can indeed increase the likelihood of bacterial penetration through the shell and subsequent contamination of the egg's contents (EFSA, 2005c).

Jones *et al*. (2004) conducted a study comparing the microbial quality (total aerobic bacteria counts, *Enterobacteriaceae* and pseudomonads) of washed and unwashed eggs stored for a period of 10 weeks under refrigeration temperatures (4°C). The surface contamination of washed eggs was significantly less than for unwashed eggs. No significant difference was found between the internal contamination of washed verses unwashed eggs.

Within Australia, studies have highlighted the importance of removing faecal material from the surface of egg shells. Cox *et al.* (2002) observed that, under experimental conditions, *S.* Infantis inoculated onto the surface of the egg was able to penetrate the egg shell and had the potential to grow within the contents of the egg. In this experiment however, freshly laid eggs were washed in 70% ethanol prior to external inoculation which may have damaged the cuticle, reducing its protective properties. However, as discussed in section 2.3.1.3, there is an increased likelihood of internal contamination of eggs if there is surface contamination with faecal material.

Egg washing techniques

Modern egg washing techniques usually involve eggs moving through an automated washing system on conveyor belts, constantly rotating throughout the process to maintain uniform exposure of the egg to the wash water via spray nozzles whilst brushes move across the egg shell surface. Detergents such as alkaline solutions are generally used to aid in removal of proteinaceous material from the egg shell. After washing, eggs are typically sprayed with a sanitising chemical such as a chlorine-based solution (EFSA, 2005c; Northcutt *et al*., 2005).

Alternatives to wet washing include dry cleaning, such as wiping with a clean dry cloth or more abrasive materials such as a scouring pad or steel wool. These methods remove debris such as feathers, faecal material, bloodstains or environmental particles without introducing the risks associated with wet washing; however these practices still damage of the cuticle. This practice may also allow cross contamination between eggs.

During the washing process, if wash water is recirculated, the water may become contaminated by a build up of egg contents and shell from eggs broken/cracked during the physical process, faecal material and dirt (Hamm *et al*., 1974; Harris and Moats, 1975). This will increase the level of organic matter in the wash water, which will impact on the effectiveness of sanitisers (*e.g.* chlorine) used in the egg washing process (Knape *et al*., 2001).

It was discovered in the 1950's that eggs that were spray washed had much lower spoilage rates than those washed by immersion methods (Lorenz and Starr, 1952). Spray washing reduces the likelihood of a temperature dependent negative pressure gradient developing, therefore reducing the potential for microorganisms being drawn into the egg contents (Hutchison *et al*., 2003). High pressure jets/sprays are also used to achieve physical removal of solids, however the strength of these actions needs to be balanced with the potential for damage of both cuticle and egg shell (EFSA, 2005c; Hutchison *et al*., 2004; Sparks, 1994).

The longer the amount of time faecal material remains on the surface of an egg, the more opportunity there is for horizontal transmission into the egg content. Therefore, eggs should be washed as soon as practicable following lay (EFSA, 2005c).

Many factors associated with egg washing have the potential to impact on food safety. These can be summarised as follows:

- Water condition - quality, mineral content, pH, temperature
- Use of chemicals/detergents/sanitisers
- Physical cleaning mechanisms - brushes, sprays, jets
- Duration of washing and contact time with chemicals
- Drying
- Storage of product before/after washing process (time and temperatures)

- Recycling of wash water
- Condition of the eggs entering the system
- Cleanliness and hygiene of the washing equipment

Temperature and pH of wash water

The temperature of the wash water is considered one of the most important factors that affects the safety of egg washing (EFSA, 2005c; Hutchison *et al*., 2004). If eggs are placed in water which is cooler than the egg, the egg contents may contract and allow for water, and any microorganisms which may be present, to be drawn into the egg via a pressure differential (Baker and Bruce, 1994; Curtis, 2000). Utilising wash water at a higher temperature to that of the egg is used to prevent this transmission occurring. Additionally the temperatures of water used along the chain of washing should continually increase at each step, *i.e.* rinse water temperature should be above that of wash water temperature (EFSA, 2005c).

A study by Hutchison *et al*. (2004) investigated the impact of different washing conditions, in particularly the temperatures at which the wash cycle and the rinsing was performed, on eggs that had been artificially contaminated with SE (PT4) and *S.* Typhimurium (DT104). For the laboratory study, eggs were washed in a chlorine-based detergent followed by rinsing in a quaternary ammonium and non-ionic sanitiser at various temperatures. When eggs were washed and sanitised at 44°C and 48°C, a 5 – 6 $\log_{10}$ reduction in external *Salmonella* contamination was observed. Additionally, *Salmonella* was not isolated from egg contents. Contamination of egg contents was observed when the temperature of wash and sanitising water was reduced to 25°C and 27°C respectively. However commercial scale studies have demonstrated that when the pH of the wash water is maintained above 10, low wash temperatures (15.5°C) did not result in increased penetration of *Salmonella* (Lucore *et al*., 1997). A benefit of washing at lower temperatures is that it reduces the time required to cool eggs under refrigeration (Jones *et al*., 2006; Jones *et al*., 2005; Lucore *et al*., 1997).

Minimum temperature requirements for egg wash water were first proposed by Brant and Starr (1962), who recommended that wash water temperature be above that of the temperature of the egg, later specifying that the temperature differential should be greater than 11°C (Brant *et al*., 1966). More recently, the USDA recommend that the temperature of wash water be kept in excess of 32°C with a minimum differential of 12°C, whilst in Sweden recommendations state that the egg wash water temperature needs to exceed egg temperature by at least 15°C in addition to requiring that rinse water must not be sourced from recycled water (EFSA, 2005c).

The Australian Egg Corporation Limited (AECL) *Code of Practice for Shell Egg Production, Grading, Packaging and Distribution* recommends a temperature of wash water between 41 – 44°C for a three stage shell egg washing and sanitising process (AECL, 2005). It also recommends that washing should take place at 42°C, sanitising at 45°C and rinsing at 47°C, although this may vary between facilities based on their own experience and the equipment used (Table 2.4).

Table 2.4 AECL Code of Practice - Recommended washing procedures (AECL, 2005).

Machine Type	Washing	Sanitising	Rinsing
Single Stage		Water temperature 41-44°C. A sanitiser such as a chlorine based sanitiser specifically for use on eggs shall be used. Eggs air dried/mechanically dried.	
Two Stage		Water temperature 41-44°C. A sanitiser such as a chlorine based sanitiser specifically for use on eggs shall be used.	Pathogen free water 2- 3°C higher than sanitising water. Eggs air dried/ mechanically dried.
Three Stage	Water temperature 41-44°C. Egg detergent.	Water temperature 3-4°C higher than wash water. A sanitiser such as a chlorine based sanitiser specifically for use on eggs shall be used.	Pathogen free water 2- 3°C higher than sanitising water. Eggs air dried/ mechanically dried.

Kinner and Moats (1981) suggested that temperature of wash water alone is not as lethal to microorganisms as is the combination of factors such as increased pH via the addition of an alkaline detergent to the wash water. A study by Leclair *et al.* (1994) showed that the concentration of *S.* Typhimurium and *L. monocytogenes* were reduced by 4-$\log_{10}$ when wash water temperature was increased above 42°C at a pH of 10.5..

Reductions in surface populations of *Salmonella* Heidelberg (Jones *et al*., 1995), *E. coli* and *Salmonella* spp. (Pearson *et al*., 1987), *S.* Typhimurium and *L. monocytogenes* (Hutchison *et al*., 2003) have also been observed when eggs have been washed in water with pH greater than 10.5 (Bartlett *et al*., 1993). Teo *et al.* (1996) also found a synergistic effect between a high pH in combination with high temperature for the destruction of *Salmonella* and *E. coli*.

A study by Holley and Proulx (1986) showed that using wash water with a pH greater than 10 and a temperature greater than 38°C prevented growth and survival of *Salmonella* in the wash water. A study by Catalano and Knabel (1994) reported a 4 $\log_{10}$ reduction of SE when using wash water with a pH of >11 and temperatures > 37.7°C. However, Bartlett *et al.* (1993) showed high temperatures and pH of wash water alone was not sufficient for reduction of microorganisms and recommended that a minimum of 0.45mg/L total available chlorine should also be used in the wash water, although the bactericidal effects of chlorine at this high pH would be reduced (Dychdala, 2001).

Wash water high in iron solutes may increase the risk of bacterial contamination. If wash water was to enter the contents of the egg, the increased availability of free iron may interact with the conalbumin (ovotransferrin) in the egg albumen, reducing its antimicrobial properties. A correlation between the iron content of the wash water and an elevated number of spoiled eggs has been noted by Baker and Bruce (1994). Internationally it is generally recommended that wash water should not exceed two parts per million iron content, this is however not specified in the AECL Code of Practice (AECL, 2005; EFSA, 2005c). Concerns have also been raised that the presence of iron may reduce the effectiveness of some chemical sanitisers used in the washing process (Moats, 1978).

Any detergents utilised during washing should be compatible with other chemicals used in the process. Silicone based antifoaming agents are often used in the process to prevent excessive detergent foaming occurring (EFSA, 2005c; Knape *et al*., 2001).

Sanitising agents

Sanitising agents such as chlorine, quaternary ammonium solution or acid solutions (*e.g.* peracetic acid) are used to reduce the bacterial load on the egg surface and prevent the potential build up of microorganisms in rinse water (Moats, 1978).

The bactericidal activity of chlorine is positively correlated with the concentration of undissociated hypochlorous acid (HOCl) (Dychdala, 2001). Levels of hypochlorous acid increase with decreasing pH (<7.5). At high pH, the predominant form of chlorine is hypochlorite (OCl^-) which has reduced bactericidal activity. The typical level of free available chlorine used for the sanitising of eggs is 100 – 200 ppm (Srikaeo and Hourigan, 2002).

A study by Soljour *et al.* (2004) examined the efficacy of sodium carbonate, sodium hypochlorite and potassium hydroxide –based sanitising solutions in inactivating *S.* Enteritidis on artificially contaminated eggs. At the higher levels of contamination (10^4 and 10^6 cfu/ml) the manufacture's recommended chemical concentrations were insufficient to inactivate all SE present; however at the lower contamination level of 10^2 cfu/ml all three chemicals were effective. Additionally it was found that the higher the pH of the wash water, the greater the inactivation of SE was observed.

The chemicals used throughout egg washing process may have the potential to damage the cuticle and/or shell surface (Favier *et al*., 2000; Jeong-Weon and Slavik, 1996; Wang and Slavik, 1998). Wang and Slavik (1998) demonstrated that washing with heated water only (*i.e.* no chemical additives) the cuticle appeared unaffected by the washing process. Addition of a quaternary ammonium compound (pH 7.5) or a sodium hypochlorite solution (pH 7.5) was also found to have minimal impact on the structure of egg surface, however using a sodium carbonate solution (pH 12) resulted in damage to the shell surface, and subsequent increased penetration of *Salmonella* spp. A study by Favier (2000) showed that the addition of sodium carbonate, cetylpyridinium chloride or trisodium phosphate to wash water eroded and etched into the egg cuticle and the internal surface of the shell, and lead to granulation of the external surface of the egg shell.

The effectiveness of iodine based disinfectants has been demonstrated by Knape *et al*. (2001) and McKee *et al.* (1998). The iodine containing chemical 'Enzodine', a peroxidase-catalysed compound, reduced egg shell surface contamination with SE and was found to be a viable alternative to chlorine based sanitisers (Mckee *et al*., 1998). However the efficacy of these

chemicals is largely dependent upon the amount of dissolved solids present in the wash water, with larger concentrations decreasing their bactericidal effectiveness (Knape *et al*., 2001).

The practice of using of electrolysed oxidizing water in the washing process has also been suggested as a further or alternate egg surface decontaminating process (Bialka *et al*., 2004; EFSA, 2005c). This is a novel process which utilises electrolysis and membrane separation technology to obtain two solutions from a saltwater solution. A study by Bialka *et al*., (2004) tested the efficacy of using this technology on artificially contaminated shell eggs (SE and *E. coli*) with reported $\log_{10}$ reductions of >2.1 and >2.3 for SE and *E. coli* respectively. Control eggs processed through a typical commercial wash and sanitising process showed lower reductions, with approximately 1.7 $\log_{10}$ and 2.0 $\log_{10}$ reduction for SE and *E. coli* respectively.

Other factors

The egg cuticle may also be damaged through physical processes such as from the pressure of jet sprays (Sparks and Burgess, 1993) or through the use of brushes in the washing process (Hutchison *et al*., 2003). The propensity for egg shell damage is also dependent on the original condition of the eggs being washed.

It has been shown that the presence of excess moisture on the surface of the egg is not essential for, but may enhance the ability of *S.* Typhimurium to pass through the cuticle and shell (Padron, 1990). This is not just during egg washing but may be due to water being introduced to the egg surface through other deliberate actions such as during the use of humidifiers, or may be a result of condensation formed when eggs are moved from a cool environment into a warmer one (Hutchison *et al*., 2003).

Washed eggs may also undergo a process of oiling, using a fine mist of food grade oil. This reduces the rate of moisture and carbon dioxide loss and the subsequent loss in egg quality by blocking the pores of the egg shell and is seen as a way of replacing the protective cuticle layer which may be damaged during the washing process (EFSA, 2005c; Hutchison *et al*., 2003) . Oiling of eggs has been found to reduce levels of spoilage, however the affect on the potential horizontal transmission of *Salmonella* into the egg contents is uncertain (Davis and Stephenson, 1991; Hill and Hall, 1980).

Under experimental conditions, exposing visibly clean eggs to ultraviolet (UV) radiation has been found to reduce the bacterial load on the egg surface (de Reu K. *et al*., 2006; Rodriguez-Romo and Yousef, 2005). For of eggs artificially contaminated with *Salmonella*, treatment with UV radiation at 1,500 – 2,500 $\mu W/cm^2$ for 1 – 5 minutes resulted in a reduction of salmonella of 3.4 – 4.3 log cfu/g on the shell surface (Rodriguez-Romo and Yousef, 2005). Treatment of visibly dirty eggs by UV radiation has, however, been found to have limited effect on reducing levels on the shell surface (Berrang *et al*., 1995; de Reu K. *et al*., 2006). UV radiation does not readily penetrate organic material (de Reu K. *et al*., 2006).

Summary
From the scientific data it can be concluded that, if performed correctly, the egg washing process reduces the overall surface microbial concentration, thereby reducing the likelihood of horizontal transmission and cross contamination. However, if egg washing is performed incorrectly it can pose an increased risk of pathogen contamination of the egg contents. The temperatures of wash, sanitising and rinse waters, as well as the effective use of detergents and sanitisers (*e.g.* maintaining correct pH levels) are key factors in determining the efficacy of egg washing.

2.3.3.2 Candling

Candling is a process used to identify and divert dirty, cracked or broken eggs, as well as eggs with imperfections such as blood spots, away from clean, intact shell eggs. Traditionally this involves passing eggs over a light source which allows for very fine hair-line cracks to be identified with the naked eye (Lake *et al*., 2004). Candling is a non destructive and rapid technique, however it is labour and time intensive (De Ketelaere *et al*., 2004).

More recently, automated machines utilising ultrasonic waves (acoustic detection) have been used to detect cracks in egg shells, however visual candling is still performed to identify internal imperfections. Other modern sensor technologies have been developed utilising mechanical, spectroscopic and computerised techniques to detect cracks, eggshell thickness and internal egg defects. The sensitivity of these machines is variable, with a balance between the detection of cracks and false rejection of eggs. For example, reported rates of crack detection range from 90% detection with 1% false rejection through to 99% detection with over 10% false rejection (De Ketelaere *et al*., 2004). Research is ongoing into development of faster, non-destructive and reliable commercial screening techniques for the detection of cracks and other indicators of egg quality (De Ketelaere *et al*., 2004).

Contamination of eggs with spoilage organisms such as *Pseudomonas* as well as pathogenic bacteria such as *Salmonella* will not be identified by candling or the detection techniques mentioned above (Cox, 2001).

2.3.3.3 Grading

Egg are generally sorted into various sizes based on a weight (Cox, 2001). This is normally performed by automated grading equipment. If not maintained in a clean manner, grading equipment may present a cross contamination hazard. A study undertaken in the UK by Davies and Breslin (2003b) demonstrated that *Salmonella* spp. could be readily isolated from various surfaces in egg packing plants. *Salmonella* spp. was isolated from grading tables (30.8% positive), conveyor belts or rollers (23.1%), candlers (23.8%) and floor surfaces (23.1%). In the same study, sterile eggs were passed through contaminated grading/packing plants to measure the rate of cross contamination. No *Salmonella* spp. was isolated from sterile eggs passaged once through contaminated packing plants. However, when sterile eggs were passaged three times through packing plants, the overall rate of contamination was 0.3%.

Packaging

Packaging is not considered to be a major risk factor for the introduction of *Salmonella* spp. into egg contents, however the reuse of egg cartons that are soiled with faecal material (or other material potentially contaminated with *Salmonella*) may provide an opportunity for cross contamination. Packaging materials can also add an insulative barrier around the eggs which will reduce the rate of cooling during storage (Curtis *et al*., 1995).

2.3.4 Collection of liquid egg (pulp and fractions)

The contents of an egg can be collected whole, or separated into its components of albumen and yolk. Whole liquid egg can be collected by crushing the egg and removing the shell by centrifugation, filtration or both. This process results in the egg contents being in contact with the external surface of the egg, therefore increasing the potential for microorganisms to be transmitted to the liquid egg product (called pulp or liquid whole egg). This is of particular concern if the surface of the egg is externally contaminated with faecal material which may have a high bacterial load.

It is not uncommon for egg producers to produce liquid whole egg on-farm from cracked, leaking and/or misformed eggs. In a survey of raw egg pulp collected from individual farms in Queensland during 1994 – 1995, Cox *et al*. (2002) isolated *Salmonella* spp from 23% of 856 samples. The same authors sampled whole egg, egg pulp and liquid yolk from an egg processing plant which received product from a number of farms. Of 1301 samples analysed, 326 (32%) were *Salmonella* positive. Of particular interest, 95.5% (105/110) samples of raw whole egg were contaminated with *Salmonella*. This is presumably a result from the pooling eggs from multiple batches or farms, whereby *Salmonella*-positive pulp from one farm may contaminate the rest of the pulp. Contamination of raw liquid yolk and farm pulp at the processing plant was 31% and 32% respectively There is, however, a lack of data on the levels of *Salmonella* spp. in contaminated raw pulp.

2.3.5 Quantitative exposure assessment – shell eggs

This section of the exposure assessment is based on the AECL quantitative risk model for non-SE *Salmonella* spp. in egg and egg products developed by Thomas *et al*. (2006). The scope of the model included chicken eggs from point of lay through to consumption as illustrated in Figures 2.4, 2.5 and 2.6.

The mathematical model was set up to describe the changes in the numbers of *Salmonella* in the contents of shell eggs based on the following assumptions (summarised in Figure 2.7):

1. *Salmonella* that contaminate the contents[3] 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 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 results in inactivation of *Salmonella*. The resultant reduction in numbers of *Salmonella* is dependent on the time and temperature of the cooking process.

2.3.5.1 Yolk Mean Time

Previous risk assessments undertaken for *Salmonella* in eggs use a parameter called Yolk Mean Time (YMT) to describe the time required for *Salmonella* present in the egg to begin exponential growth (FAO/WHO 2002; FSIS, 2005; Thomas *et al*., 2006; Whiting *et al*., 2000). An equation was developed based on data from experiments using eggs artificially contaminated with *S*. Enteritidis (Humphrey, 1994; Whiting *et al*., 2000). Significant variation has been observed in the ability of different *Salmonella* serovars to penetrate the vitelline membrane and begin growing in the yolk (Cogan *et al*., 2004). Therefore, the use of YMT to estimate the time required before growth of *Salmonella* may result in a conservative estimate of risk (*i.e.* over-estimate) (Thomas *et al*., 2006). The model also assumes no growth of *Salmonella* will occur until after the YMT has expired. The effect of temperature on YMT is illustrated in Figure 2.8.

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

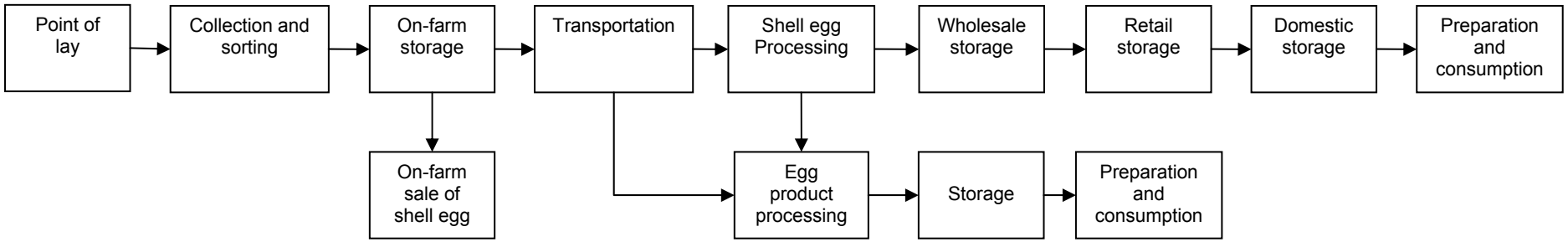


Figure 2.4 Generalised flowchart for the off-line production of shell eggs (Note: transportation steps after processing removed for clarity). (Thomas *et al*., 2006)**.**

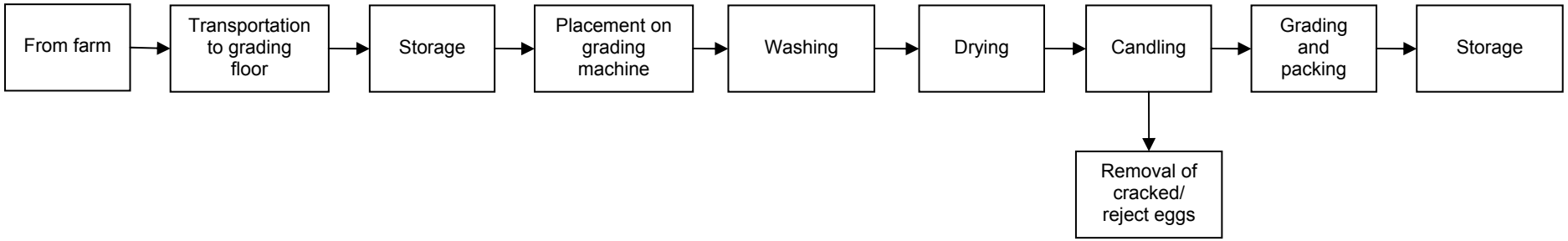


Figure 2.5 Generalised flowchart for shell egg processing (Thomas *et al*., 2006).

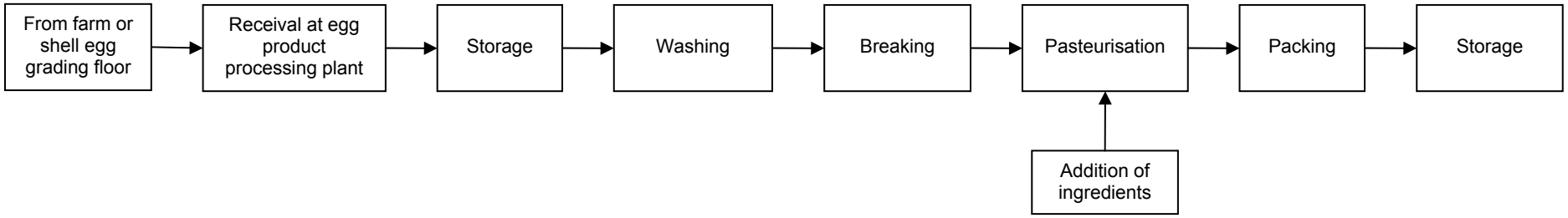


Figure 2.6 Generalised flowchart for egg product processing (Thomas *et al*., 2006).

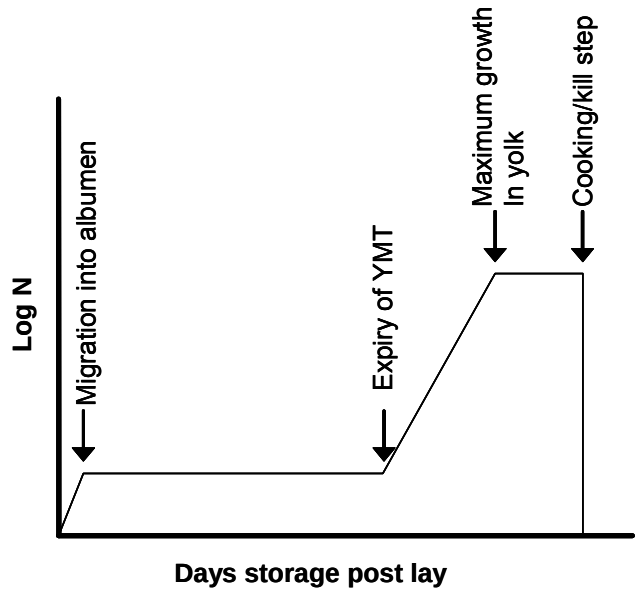


Figure 2.7 Graphical representation of invasion and growth of *Salmonella* in shell eggs from point of lay through preparation/cooking. N = number of *Salmonella* cells (Thomas *et al*., 2006).

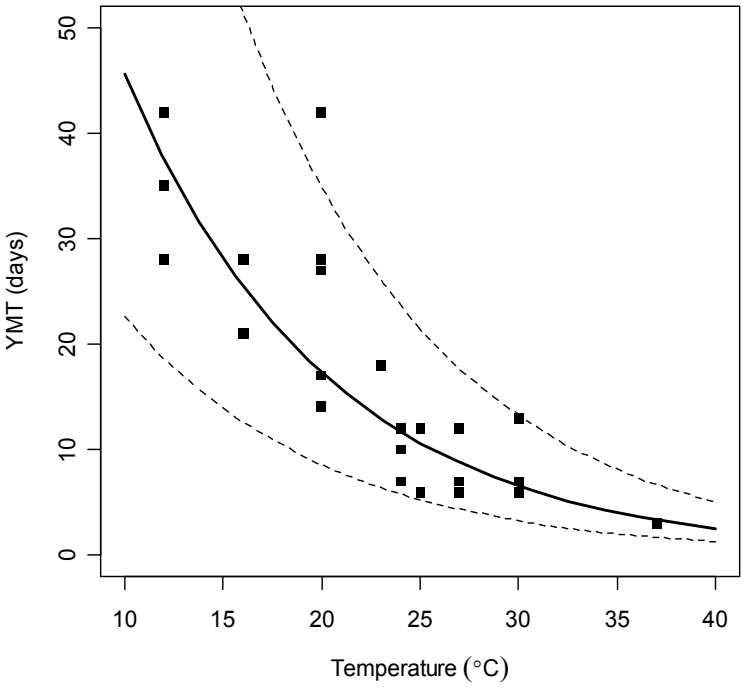


Figure 2.8 Plot of YMT vs storage temperature. The solid line shows the fitted model with confidence intervals (dashed lines). (Thomas *et al*., 2006).

2.3.5.2 Prevalence of Salmonella in eggs

To provide a meaningful estimate of exposure, it is essential to have data on the prevalence of *Salmonella* contaminated eggs (internal and external contamination).

Directly comparing results from published surveys is difficult due to differences in sample sizes and methodologies. For example testing for surface contamination of egg shells can be undertaken by swabbing a section of the shell or by rinsing the entire shell surface. Egg contents can be sampled aseptically by separating the contents without contact with the shell surface. Alternatively *Salmonella* can be isolated by crushing the egg, allowing contact of the egg contents with shell, and isolating from the mixture.

The reported prevalence of *Salmonella*-contaminated intact shell eggs internationally ranges from 0% to 9.43% (Appendix 4). In Australia a low prevalence of *Salmonella* contaminated shell eggs has been reported. In 2002, SARDI undertook a pilot microbiological survey of commercial eggs to determine the prevalence of *Salmonella* contamination (Daughtry *et al*., 2005). *Salmonella* spp. was not isolated from the external surface of 10,000 eggs sampled (Table 2.5). An additional 20,000 eggs were tested for internal *Salmonella* spp. contamination, with all samples being negative. Given the expected low prevalence of *Salmonella* contaminated eggs, these results indicate that more samples are required to determine the true prevalence of contaminated eggs in Australia.

Table 2.5 Pilot prevalence survey of *Salmonella* spp in commercial shell eggs (Daughtry *et al*., 2005)

Egg type	Pilot prevalence 95% CI[A]				Overseas average (95% CI)
	Total tested	No. Positive	Sensitivity 0.7[B]	Sensitivity 1.0	
Shell eggs upgraded - external					
- Caged	2,160	0	0 – 0.2%	0 – 0.2%	0.21% (0.04- 0.62%)
- Free range [C]	1,200	0	0 – 0.4%	0 – 0.3%	
- Barn laid [C]	1,200	0	0 – 0.4%	0 – 0.3%	
Shell eggs - graded					
- Caged external	6,476	0	0 – 0.08%	0 – 0.06%	0.03% (0.01-0.07%)
- Caged internal contents	20,000	0	0 – 0.03%	0 – 0.02%	0.004% (0.001-0.008%)

[A] All cultures negative

[B] Assumes a 0.7 sensitivity due to culturing pools of 20 eggs

[C] Sample size for free range and barn laid too small to confidently estimate prevalence

As previously discussed, internationally *S*. Enteritidis has been the predominant *Salmonella* serovar isolated from eggs and often studies report the presence of this serovar in isolation. From these published surveys, in countries where SE is endemic, SE was isolated up to 6 – 8 times more frequently than other *Salmonella* spp. (Thomas *et al*., 2006).

For the AECL quantitative model, the prevalence of eggs with internal *Salmonella* contamination was described by a distribution using data from the pilot survey (beta distribution, $\alpha=1$, $\beta=20001$) as well as data from large international studies on the prevalence of non-SE contaminated eggs (mean prevalence 0.004%) (Thomas *et al*., 2006).

There has been very little scientific publication of the prevalence of *Salmonella* contamination in/on eggs produced by poultry species other than chickens. Of the few studies published, the prevalence of *Salmonella*-contaminated duck eggs and quail eggs range from 0 – 12.4% and 0.6 – 5.7% respectively (Appendix 4).

2.3.5.3 Numbers of Salmonella in contaminated eggs at point of lay

Very few published studies are available whereby the number of *Salmonella* present in the contents of contaminated eggs at or near the point of lay has been determined. The model input for the number of *Salmonella* in contaminated eggs at the point of lay was described by a Poisson distribution with a median number of 7 cells and was based on data reported in the literature (Thomas *et al*., 2006). No assumption was made as to the method of transmission of internal *Salmonella* contamination.

2.3.5.4 Exposure assessment model

The quantitative model for shell eggs developed by Thomas *et al*. (2006) was based on eggs that are collected on-farm and graded at a central grading facility. Data from the pilot survey of egg production and processing practices was used to describe distributions for model inputs (times and temperatures at each stage of production) and is summarised in Table 2.6.

To enable evaluation of production practices on the estimated time before growth of *Salmonella* in eggs and subsequent risk of illness, inputs were separated into three levels of performance: the best 10% (shortest storage time and lowest temperature), median 10% and worst 10% (longest time storage and highest temperature) of responses. Input distributions in Table 2.6 were developed using data from each of the categories and expert opinion. In the case of the on-farm storage time, the Log Normal distribution parameter estimates were based on the best fit to the averaged cumulative percentile distribution (Vose, 2000) for each group of responses using @Risk version 4.5 (Palisade Corporation) (Thomas *et al*., 2006).

Table 2.6 Summary of on–farm collection, distribution and handling conditions for shell eggs. These factors were used as inputs for development of the Exposure Assessment model.

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.

The predictive growth model for *Salmonella* in liquid whole egg and yolk used by Thomas *et al*. (2006) was the cardinal temperature equation developed by Rosso *et al*. (1993). The model was validated by comparing it with published *Salmonella* growth rates in egg, most of which fitted within the 95% confidence intervals for the equation.

Scenarios of various storage times (up to 36 days) and temperatures (4, 16, 22 and 30°C) at retail were simulated to determine their impact on the growth of *Salmonella* in contaminated eggs. The exposure model then considered inactivation of organisms for different degrees of cooking: uncooked (no reduction), lightly cooked (2-$\log_{10}$ reduction) and well cooked (12-$\log_{10}$ reduction). These values were inputted as statistical distributions.

The final exposure to *Salmonella* (dose) was calculated by combining the probability of consuming a contaminated egg with the likely concentration of *Salmonella*, and the amount of egg consumed. This output was then used in the dose-response model to estimate the risk of illness per million servings. This is discussed further in Section 2.4.

The quantitative model was developed in Microsoft Excel and probabilistic simulations were undertaken using the @Risk add-in (version 4.5.2, Palisade Corporation). 100,000 iterations were run using Latin Hypercube sampling of distributions.

The main results presented by Thomas *et al*. (2006) included estimations of the median egg age, median YMT expired, percentage of eggs with YMT >1, and the median time before growth occurs in eggs stored at 16°C and 22°C at the end of wholesale storage (or the start of retail storage). Results were expressed for nine different scenarios (3 on farm × 3 processing factors) which describe the effects of best, median and worst industry practices for these stages of production.

2.3.5.5 Effect of industry practices on egg safety at end of wholesale storage

To understand the importance of on-farm and processing practices in the production of shell eggs, an output of the model developed by Thomas *et al*. (2006) was the predicted microbial status of the egg at the end of wholesale storage. In particular, an estimation of the percentage of YMT expired at this stage of the supply chain under various production practices is important.

The median estimated age of eggs at the end of wholesale ranged from 3 days for best practice on-farm handling and processing times to approximately 14 days for the worst case scenario (approximately 5 days for median on-farm and processing practices). The resulting median range for the proportion of the YMT expired by the end of wholesale under best and worst practices was between 20 –80%. Results from the model suggest 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. In contrast, those eggs produced and processed under "median/worst", "worst/best", "worst/best", "worst/median" and "worst/worst" on-farm and processing practices had a significant chance of having a YMT >1 and hence capable of supporting growth of *Salmonella*, should those eggs be contaminated.

2.3.5.6 Effect of retail storage on potential for growth of Salmonella

The potential for growth of *Salmonella* spp. during retail storage is dependent on the remaining YMT at the end of wholesale storage, and times and temperature of storage at retail. Table 2.7 provides a summary of the median predicted time before growth of *Salmonella* in eggs produced and processed under varying industry practices stored at different retail temperatures. The difference between predicted time before growth for eggs at retail stored at 16, 22 and 30°C was approximately 2-fold respectively. Retail storage at 4°C prevented the growth of *Salmonella* spp. and therefore the time before growth remained stable.

The median predicted time before growth of *Salmonella* for eggs stored at 22°C during retail ranged from 2.4 – 11.5 days depending on production and processing conditions (Table 2.7). The current industry Code of Practice (CoP) recommends a best-before date for packaged eggs of up to five weeks from the time of lay and storage at retail at <20°C (AECL, 2005). If stored at retail at >20°C the CoP recommends the eggs should be stored no longer than 4 days prior to sale.

Table 2.7 Predicted median time (days) 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 (Thomas *et al*., 2006).

		Retail Storage Temperature			
On-farm Conditions	**Processing Conditions**	**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

2.3.6 Quantitative exposure assessment – egg products

The quantitative model developed by Thomas *et al*. (2006) also simulated the fate of *Salmonella* spp. in pasteurised liquid whole egg, yolk and albumen. Egg products in Australia must be processed in accordance with requirements in Standard 1.6.2 of the Code and are listed in Table 2.8. The Code also prescribes a microbiological limit (Standard 1.6.1) for processed egg products of the absence of *Salmonella* in 5 × 25g samples.

Table 2.8 Australian standard for the pasteurisation of liquid whole egg, yolk and albumen.

Product	Temperature (°C)	Time (minutes)
Whole egg	64	2.5
Yolk	60	3.5
Albumen	55	9.5

Approximately 9% of eggs produced in Australia are used in the manufacture of liquid egg products (Thomas *et al*., 2006). In addition to liquid whole egg, yolk and albumen pre-prepared liquid egg mixes containing added salt and sugar (egg for scrambled egg) are also produced.

Data on current pasteurisation practices was gathered as part of the pilot industry survey (a total of 8 processors). Based on reported pasteurisation times and temperatures, some were operating below the requirements of the Code (Figure 2.9).

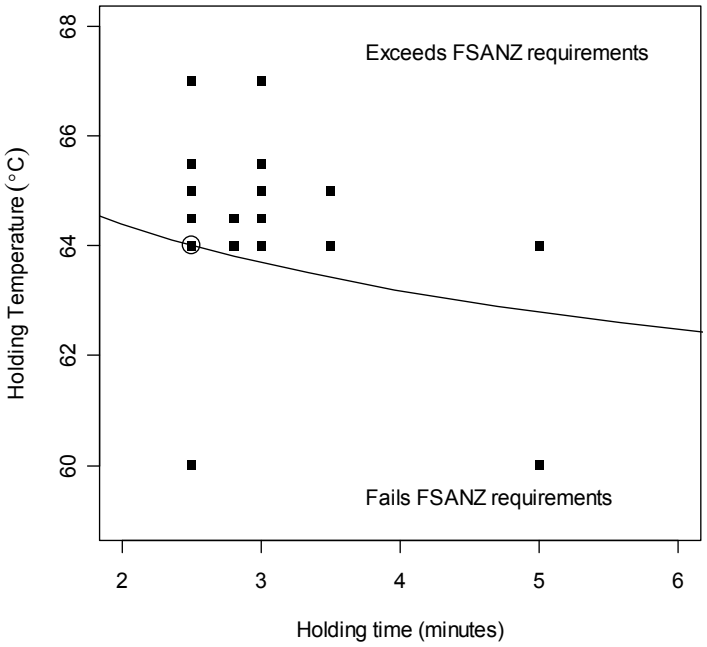


Figure 2.9 Comparison of reported industry holding conditions (squares) for the pasteurisation of whole egg pulp. The circle represents FSANZ processing requirements. The solid line shows temperature and holding times of equivalent severity (z = 4°C) to the FSANZ standard. Combinations of holding times and temperatures to the bottom of the solid line represent under-processing (Thomas *et al*., 2006).

The USDA-FSIS exposure assessment used inactivation equations for *S*. Enteritidis in whole egg, yolk and albumen (FSIS, 2005). As this serovar has not been found in Australian egg pulp, Thomas *et al.* (2006) developed a new inactivation equation based on published decimal reduction time data for non-SE *Salmonella* serovars in whole egg, yolk and albumen.

Based on the processing requirements in the Code (Table 2.8) and logarithmic inactivation rates (Figure 2.10), the median predicted degree of thermal inactivation during pasteurisation of whole egg, egg yolk and albumen are provided in Table 2.9.

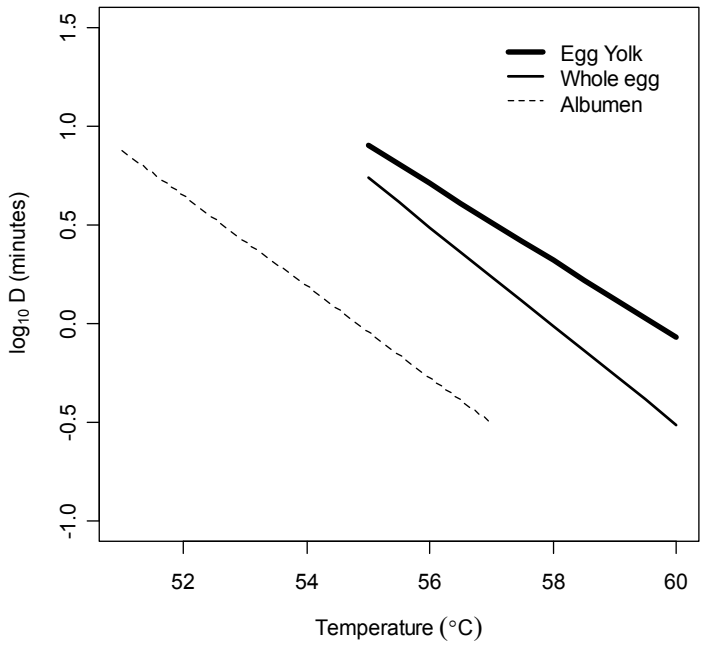


Figure 2.10 Effect of temperature on the logarithm of the decimal reduction time (minutes) for *Salmonella* in whole egg, egg yolk and albumen (Thomas *et al*., 2006).

Table 2.9 Median predicted degree of thermal inactivation from pasteurisation (Thomas *et al*., 2006).

Product	Log_{10} reduction	5^{th} percentile	95^{th} percentile
Whole egg	80.7	58.6	109.8
Yolk	4.12	3.37	5.03
Albumen	10.46	7.42	14.6

The model predictions for inactivation of *Salmonella* spp. were based on the "worst case" scenario using the largest decimal reduction times reported in the literature. The large variation in thermal inactivation rates for *Salmonella* in liquid egg products may be a result of different methodologies used. For example, studies used capillary tubes, quarts tubes, glass test tubes and glass flasks to simulate the pasteurisation process (Thomas *et al*., 2006). If smaller reported decimal reduction times were used in the model, estimates of inactivation would be orders of magnitude higher.

There is a large difference in the degree of inactivation for the three liquid egg products processed under current requirements. Pasteurisation of liquid whole egg is more than sufficient to inactivate any *Salmonella* likely to be present (predicted >80 log_{10} inactivation). In contrast, pasteurisation requirements for liquid yolk and albumen was predicted to provide approximately a 4-log_{10} and 10-log_{10} inactivation respectively (Thomas *et al*., 2006).

The probability of pasteurised product not meeting the microbiological criteria in the Code of absence of *Salmonella* in 5 × 25g samples for eggs, based on varying on-farm and processing practices is presented in Table 2.10. Due to the high inactivation rates achieved from pasteurisation of liquid whole egg, it is extremely unlikely to detect *Salmonella* from 5 × 25g samples. For egg yolk and albumen, *Salmonella* may be detected in up to 13% and 0.05% of

batches respectively, depending on the time and temperature of egg storage on-farm and during processing (Table 2.10).

Table 2.10 Predicted probability of pasteurisation failure for whole egg, egg yolk and albumen using minimum Australian processing conditions (Thomas *et al*., 2006).

		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

2.3.7 Specialty Eggs

In addition to the sale of fresh eggs from other avian species, there are small industries producing processed "specialty eggs". They are a particular delicacy in many Asian cuisines, and include salted, balut (fertilised) and century eggs produced from duck eggs.

Salted eggs are produced by soaking duck eggs in a saturated salt solution at room temperature for approximately 4 weeks. Salted eggs are processed as follows (Biosecurity Australia, 2007):

- Eggs are washed
- Eggs are soaked in the shell for 30 days in saturated saline (20% to 25% salt solution)

Salted eggs are either pre-cooked during manufacture or steamed/boiled prior to consumption. This heat treatment, whereby the core temperature may reach at least 85°C would be sufficient to inactivate any *Salmonella* present.

Alkalised duck eggs, also known as "century eggs", are produced from commercial flocks of ducks. They are processed using the following steps (Biosecurity Australia, 2007):

- Eggs are washed
- The eggs are soaked within the shell for 35 days in alkali solution with a pH of >13, in order to achieve an internal pH of 9.5 or higher
- Eggs are then washed, dried and packed.

With the internal pH of alkalised eggs reaching 9.5, it is unlikely *Salmonella* would grow in these products however the pH is not high enough to result in substantial inactivation of *Salmonella* that may be present (Biosecurity Australia, 2007; You-Min and Ting, 1997). These are also known as preserved eggs, hundred-day eggs or thousand-year eggs. In some cases the curing process used in the preparation of century eggs has been enhanced with the use of sodium hydroxide and/or the addition of catalysts, such as lead or zinc oxide.

Variations in century eggs are made by preserving duck, chicken or quail eggs. They are produced using different methods and there are several forms, including:

- "Hulidan" eggs are individually coated with a mixture of salt and wet clay or ashes for a month. This process darkens and partially solidifies the yolk.
- "Dsaudan" eggs are packed in cooked rice and salt for at least 6 months. During this time, the shell softens, the membranes thicken, and the egg contents coagulate.
- "Pidan" eggs are made by covering eggs with lime, salt, wood ashes, and a tea infusion for 5 months or more. The egg yolks become greenish grey and the albumen turns into a coffee-brown coloured jelly.

As is the case for salted and alkalised preserved eggs, the salting processes of these eggs will reduce the potential for *Salmonella* growth, but will have little impact on survival.

Balut eggs are prepared by incubating fertilised eggs at 40°C for approximately 2½ weeks. This process allows the embryo to continue to grow. Growth is stopped when the egg is removed from the incubator. Balut eggs are generally cooked (boiled) prior to consumption.

In a targeted survey in 2006, the NSW Food Authority tested 78 specialty egg products (39 salted eggs, 39 century eggs) (McCreadie *et al*., 2007). *Salmonella* spp. was not detected in any of the samples.

2.3.8 Consumption of eggs and egg products

The consumption of eggs in Australia has dropped considerably since the late 1940s, and in the mid 1990s, annual per capita egg consumption decreased to 132 (Figure 2.11). In recent years, egg consumption has increased to an estimated 165 eggs per person per year (AECL, 2006a). In comparison to other countries, Australia's egg consumption per capita is still low, for example, per capita egg consumption in Hungary and the US was 296 and 255 respectively in 2005 (IEC, 2007).

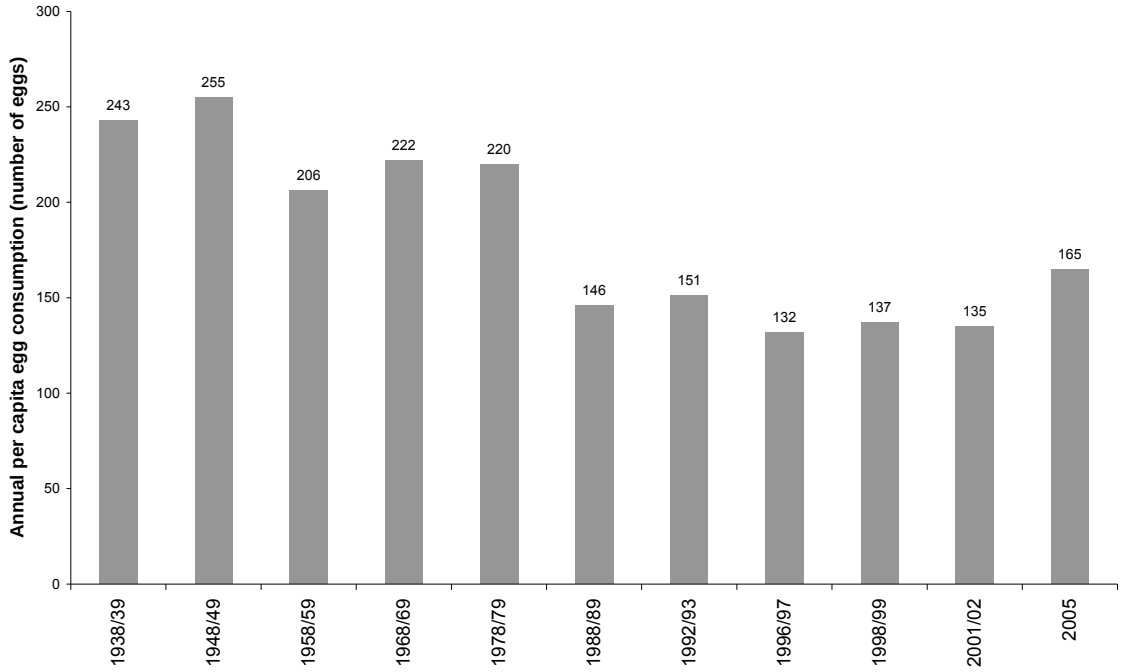


Figure 2.11 Apparent per capita egg consumption in Australia (AECL, 2003; AECL, 2006a).

Results of the Australian National Nutrition Survey (1995) (Table 2.11) showed that 1,314 (9.5%) of 13,858 respondents reported consumption of eggs and egg products (range 4.6 – 11.7%). Of those that consumed egg, the average amount consumed was 56.7g/day (range 31.1 to 81.2 g/day)

Table 2.11 Average daily consumption of eggs and egg products by sex and age (Australian Government Department of Health and Family Services, 1997).

Sex	Age	No. consumers surveyed	No. consuming eggs and egg products (% of no. surveyed)	Average amount of eggs and egg products eaten per day (g)	% of total food consumed
Male	2 - 3	170	11 (6.47)	31.31	0.10
Male	4 - 7	416	30 (7.21)	54.78	0.18
Male	8 - 11	385	35 (9.09)	58.03	0.20
Male	12 - 15	349	26 (7.45)	76.67	0.18
Male	16 - 18	215	24 (11.16)	69.39	0.20
Male	19 - 24	485	38 (7.84)	81.22	0.15
Male	25 - 44	2140	236 (11.03)	68.60	0.18
Male	45 - 64	1554	174 (11.2)	63.98	0.18
Male	65+	902	101 (11.20)	65.63	0.22
Female	2 - 3	213	25 (11.74)	40.96	0.26
Female	4 - 7	383	30 (7.83)	47.72	0.19
Female	8 - 11	354	26 (7.34)	51.92	0.17
Female	12 - 15	304	19 (6.25)	51.71	0.12
Female	16 - 18	218	10 (4.59)	52.59	0.09
Female	19 - 24	575	41 (7.13)	51.82	0.12
Female	25 - 44	2385	207 (8.68)	52.95	0.14
Female	45 - 64	1752	184 (10.50)	51.14	0.16
Female	65+	1058	97 (9.17)	49.78	0.16

2.4 Risk Characterisation

2.4.1 Shell eggs

The quantitative model developed by Thomas *et al*. (2006) estimated the number of cases of salmonellosis per million serves of shell eggs from the end of wholesale storage until 36 days of retail storage. Of particular interest is the estimated number of cases of salmonellosis per million serves for shell eggs stored at various temperatures at retail and consumed uncooked, lightly cooked or well cooked.

The degree to which eggs are cooked is a continuum from uncooked ***e.g.*** in a food such as mayonnaise, through to well cooked ***e.g.*** scrambled egg. The model predicted consumption of well-cooked egg presented little risk of illness as the cooking temperature is high enough to inactivate *Salmonella* (> 12-$\log_{10}$ reduction). As illustrated by epidemiological data, consumption of uncooked foods containing eggs has repeatedly been identified as a risk factor in outbreaks of salmonellosis. These food vehicles include egg/milk drinks, mayonnaise and desserts.

The impact of on-farm and processing practices, as well as storage temperature at retail, on the estimated risk of illness from consumption of foods containing uncooked eggs is presented in Figure 2.12. It can be seen that when eggs are stored at 4°C during retail there is no increased risk of illness when eggs are produced under "best" and "median" industry practices. Although there was slight increase in risk from eggs produced under "worst" industry after storage at 4°C for 14 days, risk was limited under these conditions.

Growth of *Salmonella* was predicted in contaminated eggs stored at retail at 16, 22 and 30°C which reflects the expiry of the YMT. The higher the temperature of storage, the time required to exceed YMT decreases (Figure 2.12).

The effect of cooking on the predicted risk of illness is summarised in Figure 2.13. Light cooking was described as an egg boiled for 4 minutes, fried (***e.g.*** sunny side up) or microwaved. Well cooked included eggs hard boiled, scrambled or used in cakes etc that are cooked. For lightly cooked and uncooked eggs, it was predicted that storage at 16°C and above for enough time (between 10 – 20 days post lay) would result in growth of *Salmonella* in contaminated eggs, leading to a high likelihood of illness in those people who consume these eggs.

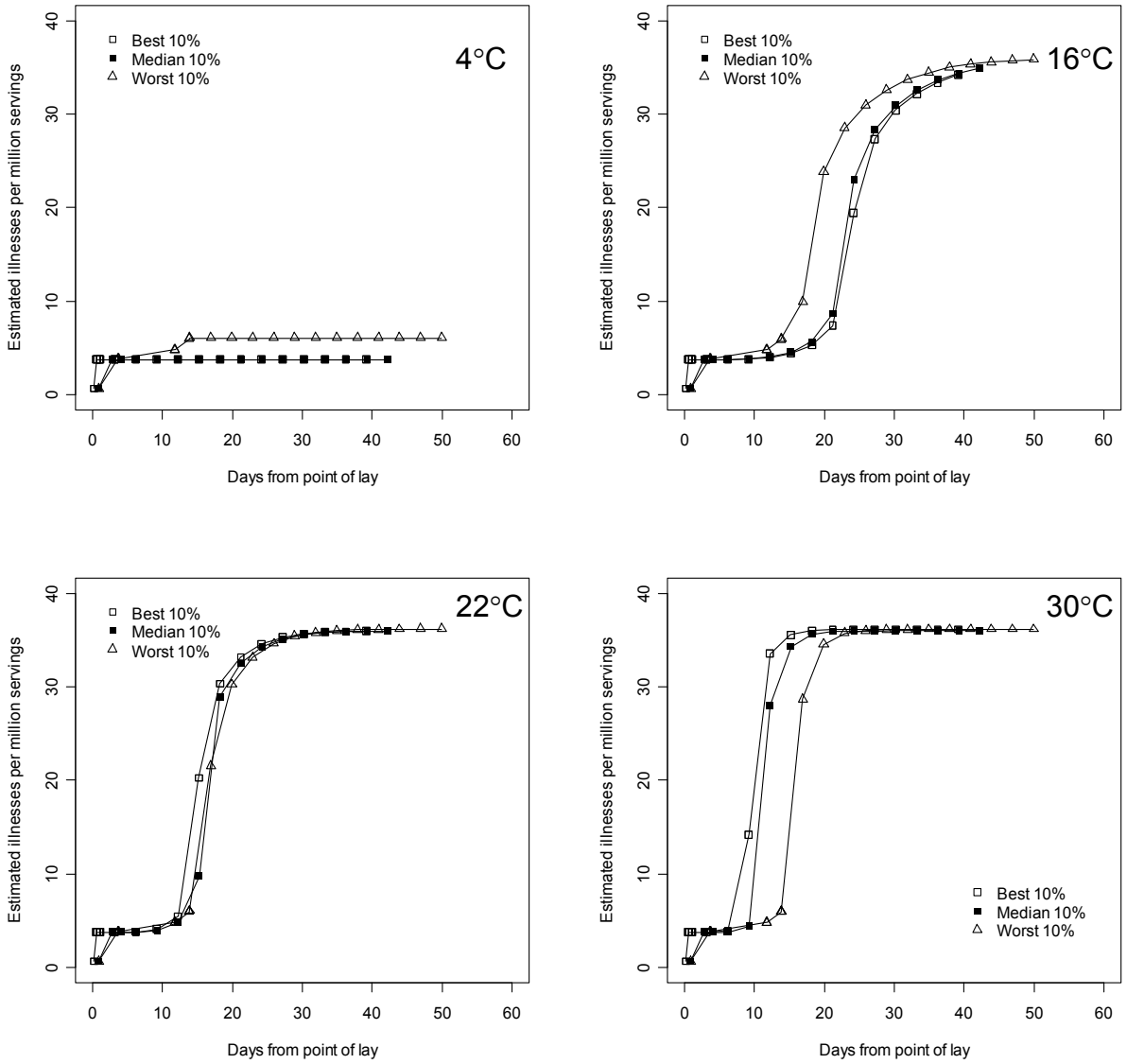


Figure 2.12 Comparison of production, processing (worst 10%, median 10% and best 10%) and retail storage temperature on estimated illnesses per million servings for eggs stored at different temperatures. Data shown is for uncooked foods (Thomas *et al*., 2006).

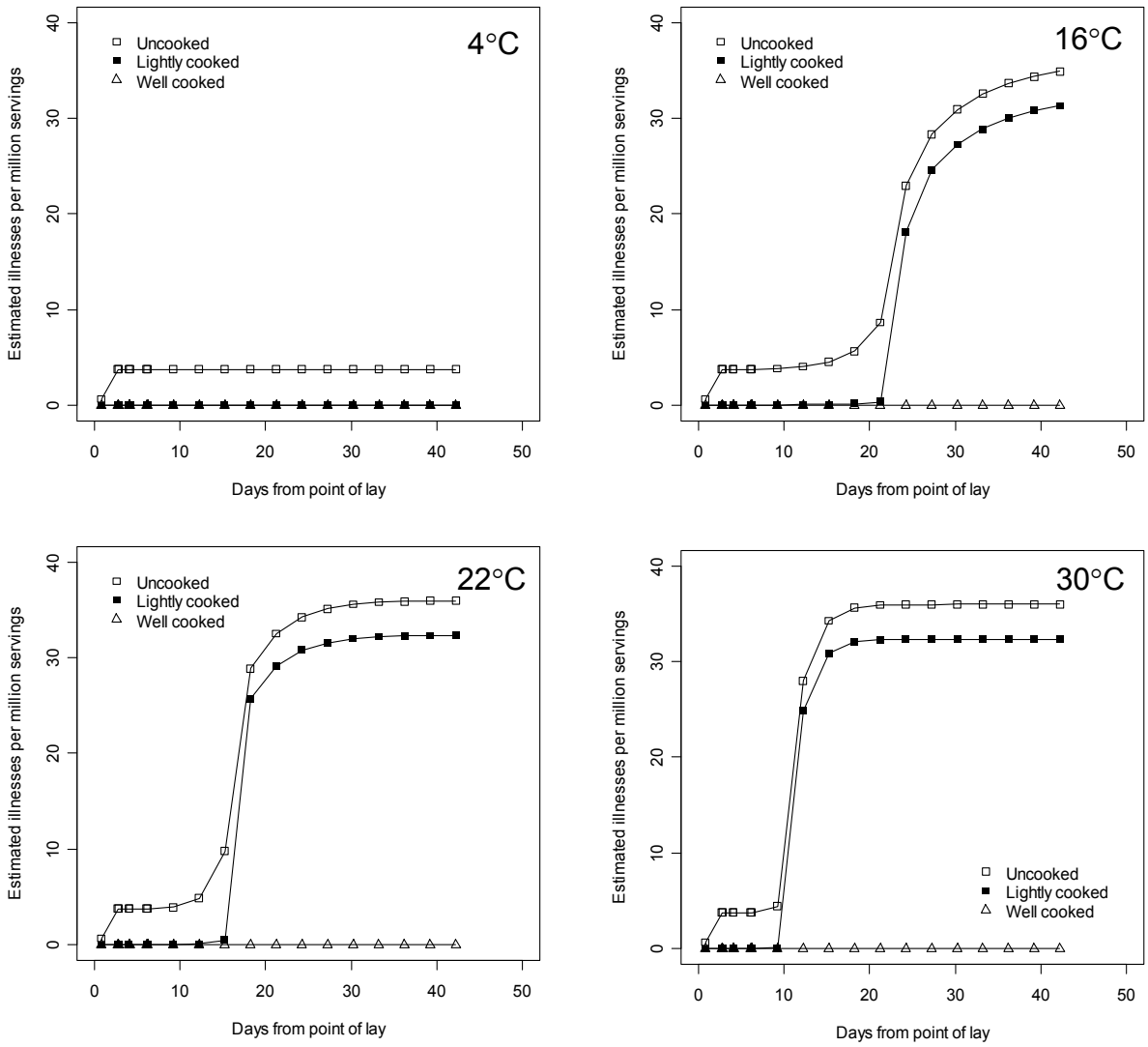


Figure 2.13 The effect of storage temperature of retail eggs on estimated illnesses per million servings for uncooked, lightly cooked and well cooked foods. Each panel represents risk associated with. Median on-farm and processing practices are assumed (Thomas *et al.*, 2006).

The risk of illness from the consumption of uncooked eggs is dependent on the prevalence of *Salmonella* contaminated eggs. Regardless of retail storage, a one-fold increase (or decrease) in prevalence with result in an approximately one-fold increase (or decrease) in risk of illness if consumed after YMT has expired. For example, if prevalence of contaminated eggs is reduced by 50%, the predicted number of illnesses if eggs are consumed after YMT has expired will decrease from approximately 36 to about 19 cases per million serves (data not shown).

The number of *Salmonella* in contaminated eggs at the point of lay had a variable impact on the risk of illness following consumption of uncooked food containing eggs. For example, once the YMT had expired (*e.g.* storage at 16°C for 20 days), the initial number of organisms

had minimal impact on risk. However prior to the potential for exponential growth, the risk of illness was dependent on the initial number of organisms in the contaminated egg.

2.4.1.1 Variability and uncertainty

Figure 2.14 demonstrates the variation in the risk estimates for consumption of uncooked foods containing eggs that were stored at temperatures that may permit the growth of *Salmonella*. This variation reflects the uncertainty and variability associated with model inputs such as the prevalence and initial concentration of *Salmonella* in contaminated eggs, and variation in the calculation of YMT. Factors such as regional differences and differences in behaviour of individual strains of *Salmonella* have not been included in the model.

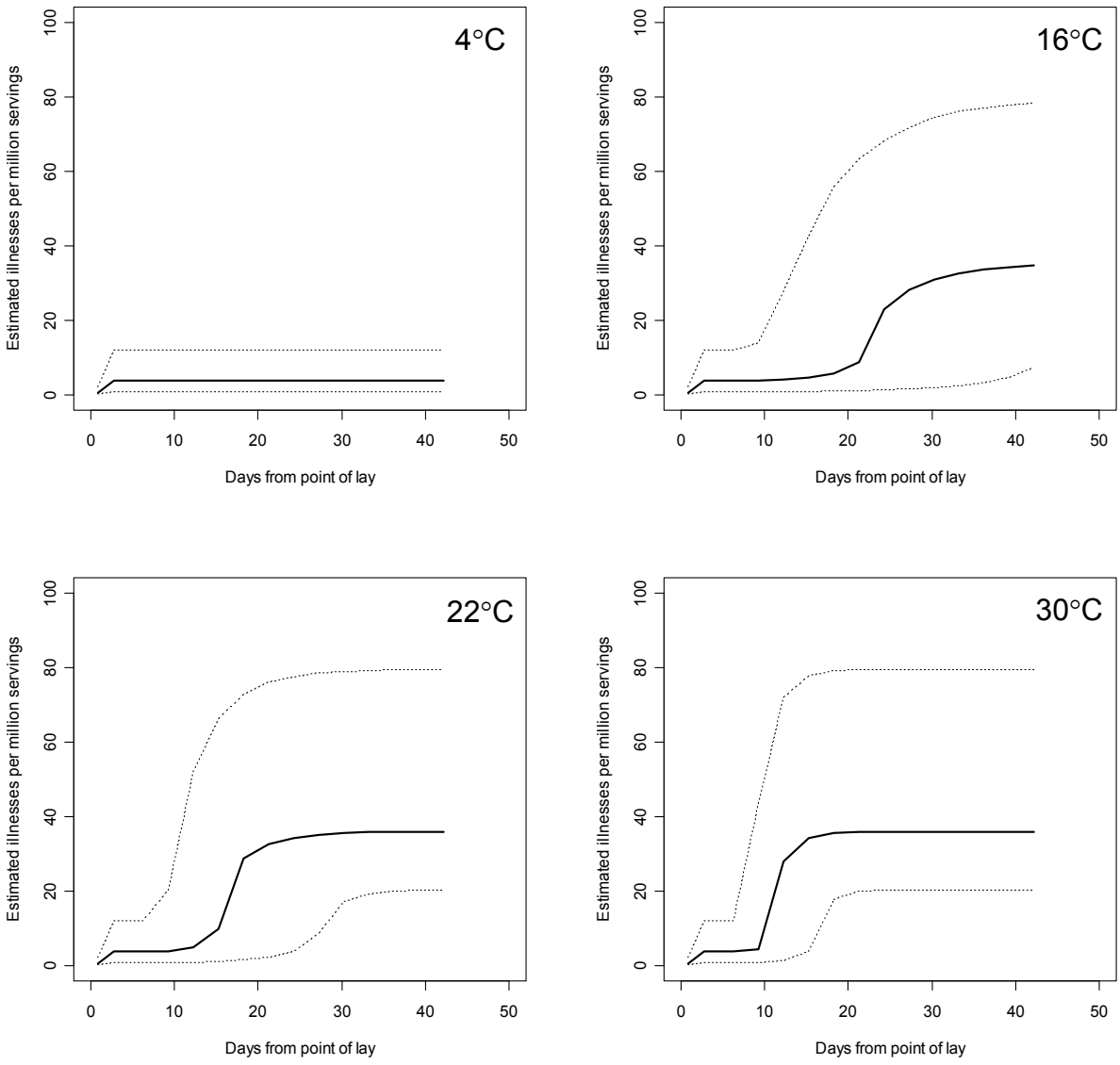


Figure 2.14 Comparison of different retail storage temperatures on estimated illnesses per million servings of uncooked foods. Median on-farm and processing practices are assumed. Median risk of illness represented by solid line, 5th and 95th percentile represented by dashed line (Thomas *et al*., 2006).

2.4.2 Egg products

As a worst case scenario, the model developed by Thomas *et al*. (2006) estimated the probability of illness from the consumption of uncooked foods containing pasteurised egg products (as opposed to cooked products) by combining the output from the exposure assessment and the dose-response model for non-SE *Salmonella* (Table 2.12). As part of the exposure assessment it was assumed that the serving size for liquid whole egg was 100 g (approximately 2 eggs) and 50 g both for liquid yolk and albumen (Thomas *et al*., 2006).

Table 2.12 Predicted median probability of illness per serving from the consumption of uncooked foods containing pasteurised whole egg, egg yolk and albumen (Thomas *et al*., 2006).

		Probability of illness per serving		
On-farm Conditions	**Processing Conditions**	**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	$<10^{-25}$	1.4×10^{-17}*	$<10^{-25}$

* 95th percentile = 0.026

The median estimated probabilities of illness per serve for the three egg products produced and processed under different conditions were very low ($<10^{-25}$, 10^{-17} and 10^{-25} for whole egg, yolk and albumen respectively). Reflecting the inherent uncertainty and variability associated with the model, the distribution of risk estimates was large. For example, the estimated probability of illness at the 95th percentile for pasteurised egg yolk produced and processed under "worst" conditions was estimated to be 0.026. In other words, it was estimated that 5% of uncooked foods containing pasteurised yolk produced and processed under "worst" conditions would have a probability of illness of >2.6% per serve. Even with this very conservative estimate, when you take into account the probability of being exposed to a product produced and processed under "worst" industry practices, the risk of illness from consumption of uncooked products containing pasteurised egg products is low.

There is little data available to estimate the consumption of uncooked foods containing pasteurised liquid egg (frequency and amount of egg product consumed). Consumption of these products is likely to be infrequent.

2.4.3 Specialty Eggs

The physical properties of specialty eggs (salted eggs and alkalised eggs) would prevent the growth of *Salmonella* spp. if present. In addition, for salted eggs and balut eggs, the product is predominantly cooked/steamed prior to consumption at temperatures high enough to inactivate *Salmonella* spp.

2.5 Discussion

The literature and epidemiological data confirm that the principal pathogenic microorganism associated with consumption of eggs and egg products is *Salmonella* spp. This has been highlighted internationally with the emergence of *S*. Enteritidis (SE) which has the ability to directly contaminate eggs as they are formed (vertical transmission). Fortunately, SE is not endemic in Australian laying flocks. Therefore horizontal transmission of non-SE *Salmonella* spp. through the shell is considered the main route of internal egg contamination in Australia.

There are many physical and chemical properties of the egg that protect its contents from bacterial invasion and/or growth. The shell is porous to allow exchange of respiratory gases and water vapour. Protecting the ingress of micro-organisms and other material into the egg via these pores is the cuticle covering the surface of the egg, as well as various internal shell membranes. In addition to these physical barriers, the albumen contains a number of bacteriostatic compounds that limit the growth and/or survival of microorganisms.

A number of factors impact on the efficacy of these defence mechanisms and the subsequent likelihood of bacterial penetration and/or growth. This includes the presence and load of external contamination, temperature differential between the egg and the environment, humidity, porosity of the egg, and condition of the shell, cuticle and membranes. Practices during the production and processing of eggs and egg products that impact on these factors will affect the likelihood of horizontal transmission of *Salmonella* into the egg contents.

In addition to increasing the likelihood of transmission of *Salmonella* into the egg contents, faecal contamination of the shell provides a source of cross-contamination throughout the egg supply chain, including during food preparation.

There is little epidemiological data in Australia implicating clean, intact eggs as the source of egg-associated outbreaks. However, it is important to recognise that there are considerable numbers of sporadic cases of salmonellosis where it is difficult to identify the food vehicle. A review of epidemiological data on outbreaks associated with the consumption of eggs and egg products highlights the multi-factorial nature of foodborne illness. A major risk factor identified in outbreaks associated with consumption of uncooked/undercooked foods containing raw egg was the use of eggs with visible surface faecal contamination. Contributing factors in these outbreaks included cross-contamination during food preparation and/or temperature abuse of the food containing raw egg.

Numerous factors during primary production have a potential to introduce *Salmonella* into a laying flock, including:

- Feed
- Water
- Pests
- Laying environment
- Personnel
- New stock (*i.e*. day-old chicks or replacement pullets)
- Equipment

Due to the multi-factorial nature of transmission of *Salmonella* spp. into laying flocks, and a lack of quantitative data, it was not possible to determine which factors have greater impacts on flock contamination than others. Despite this, limiting the opportunity for flocks to become infected with *Salmonella* spp. from these sources will have an impact on the potential for egg contamination. Many of these factors can be managed in biosecurity programs which aim to reduce transmission of avian diseases into layer flocks. Control of biosecurity in Australian laying farms is, however, voluntary and the true adoption of these measures is unknown.

Once infected, birds may excrete large numbers of *Salmonella*, providing many opportunities to contaminate the egg either during lay (contamination of the surface of the egg as it the leaves the vent) or via contact of the egg with faecal material in the layer environment. Studies have demonstrated that faecal contamination of the egg surface impacts on the extent of horizontal transmission (trans-shell). Practices on-farm that minimise the opportunity for contact of eggs with faecal material are likely to reduce the potential for horizontal transmission of *Salmonella* into the contents of the egg. Factors that impact on the likelihood of external contamination of eggs include:

- Animal health (infection status, degree of faecal shedding)
- Design and maintenance of facilities (***e.g.*** nest boxes, conveyer belts, collection and removal of faeces)

Following egg collection, the sorting, washing and grading of eggs has the potential to impact on exposure to *Salmonella* contaminated eggs. Generally, eggs grossly contaminated with faecal and/or other material such as litter are removed from the grading process and diverted for disposal or further processing (***i.e.*** pasteurisation). It has been demonstrated that washing eggs, when conducted under appropriate conditions, results in a reduction in the microbial load on the egg surface. Critical to the efficacy of egg washing is the type and concentration of detergent and sanitising agents used and the temperature of wash water. The presence of organic matter (***e.g.*** faecal material and litter) has the potential to reduce the efficacy of sanitising agents. Indeed, if performed incorrectly, washing of eggs may actually increase potential for egg contamination (via cross contamination) as well as the likelihood of horizontal transmission of *Salmonella* through the shell.

As discrete from identifying quality imperfections such as internal blood spots, the candling process is also performed to detect cracks in the egg shell. As previously mentioned, the shell and cuticle form the primary barrier to penetration of *Salmonella* from the surface of the egg to the egg contents. The presence of cracks may increase the likelihood of transmission of *Salmonella* through the shell to the shell membranes.

Surveys on *Salmonella* contamination in graded and un-graded eggs in Australia, have reported a low prevalence of contamination (not detected in 20,000 eggs sampled). Environmental sampling undertaken during egg-associated outbreak investigations have, however, isolated *Salmonella* spp. from the surface of implicated eggs. The true prevalence of *Salmonella* contamination in intact, clean, graded eggs is difficult to determine due to the extremely large sample size required to be confident to detect contamination at such a low prevalence. Although the reported prevalence of *Salmonella* contaminated eggs is low, this must be considered with regard to the large number of eggs produced in Australia (approximately 203 million dozen annually).

Results from the quantitative risk assessment model developed by Thomas *et al*. (2006) estimated the risk of illness following consumption of raw eggs that have been stored under conditions that permit growth (i.e. YMT>1) was 36 cases per million serves. Once the YMT has expired (permitting growth of *Salmonella*) the estimated cases of illness largely reflected the prevalence of *Salmonella* contaminated eggs. Therefore, it is predicted that a 50% reduction in the prevalence of *Salmonella* contaminated eggs would result in an approximately 50% reduction in the risk of illness from eggs consumed after the YMT has expired.

For eggs stored at 16°C at retail it was predicted that growth of *Salmonella* in contaminated eggs could occur approximately 18 days from the end of wholesale storage when produced under median industry practices. If eggs were stored at 20°C at retail, the estimated time before growth of *Salmonella* in contaminated eggs would be 10 days. Current industry guidelines recommend a shelf life of eggs of 35 days or less from the point of lay. Retail storage at 4°C prevented the growth of *Salmonella* and therefore resulted in no increased risk of illness over time. Although not explicitly considered in the quantitative model due to a lack of data, consumer storage of eggs will also affect the potential for growth of *Salmonella* spp. in contaminated eggs. For eggs that have been stored under conditions that do not permit the growth of *Salmonella* spp. and consumed raw, the risk of illness was estimated to be approximately four cases per one million serves of uncooked egg.

The quantitative model did not consider the potential for cross-contamination during food preparation or multiple serves of uncooked food containing raw egg such as raw egg-containing sauces, desserts etc. If these foods are prepared from contaminated eggs that have been stored under conditions that permit growth of *Salmonella*, there is a potential for a number of consumers of this food to be exposed to levels of *Salmonella* that may result in illness.

The distribution of the risk estimate was affected by variability in model parameters such as in the estimation of YMT, as well as sources of uncertainty such as the prevalence of *Salmonella* contaminated eggs and initial levels of *Salmonella* in these contaminated eggs.

Raw egg pulp has been identified as often being contaminated with *Salmonella* spp. (23% and 95% contamination of farm pulp and bulk pulp respectively). At temperatures above 7°C, *Salmonella* has the ability to grow in liquid egg products. Sufficient heat treatment, such as pasteurisation, is required to inactivate *Salmonella* spp.

For liquid egg products the predicted inactivation of *Salmonella* during pasteurisation was estimated using a mathematical model based on reported thermal inactivation rates and times and temperatures of pasteurisation. There was a large difference in the degree of inactivation predicted for pasteurisation of liquid whole egg, liquid albumen and liquid yolk. In summary, pasteurisation requirements for liquid whole egg resulted in a large predicted inactivation of *Salmonella* (>80-$\log_{10}$ reduction), with much less for liquid albumen and yolk (10.5-$\log_{10}$ and 4.1-$\log_{10}$ respectively).

Consumption of liquid egg products was assumed to be 100g for whole egg and 50g for liquid yolk and albumen. The median estimated probability of illness per serve for the three liquid egg products was extremely low (10^{-25} – 10^{-17} probability of illness per serve). Due to the uncertainty and variability with a number of the model inputs, the distribution of risk estimates was large. As a worst case scenario, the estimated probability of illness at the 95th percentile for consumption of pasteurised liquid yolk produced and processed under "worst" industry practices was 0.026 (2.6%). The quantitative model was conservative in nature. For example, the predictions for inactivation of *Salmonella* spp. during pasteurisation were based on the largest decimal reduction times reported in the literature.

2.6 Data gaps and opportunities for further research

A number of data gaps were identified throughout the egg and egg product supply chain that contributes towards uncertainty in the outputs of the risk assessment. Research into these data gaps may assist in better describing factors along the farm-to-fork continuum that impact on the likelihood of illness from consumption of eggs and egg products in Australia, as well as help reduce uncertainty in the risk assessment.

- The relative contribution of on-farm factors towards contamination of eggs with *Salmonella* spp.
    - Numerous on-farm factors have been identified as potential sources of *Salmonella* infection in laying flocks; however their relative impact on the likelihood of egg contamination could not been determined. Examples of these factors include housing design, feed contamination, water contamination, new litter contamination, pest and vector contamination etc. This data may assist in determining those on-farm factors that have the greatest impact on the likelihood and extent of egg contamination.

- On-farm control measures
    - There is limited quantitative and/or qualitative data on the effect of on-farm control measures, either individually or collectively, on the *Salmonella* status of layer flocks. Examples of these control measures include manure management, biosecurity measures, feeding practices, pest management, flock size.

- Information for non-chicken egg producing species
    - There is little data both domestically and internationally on the prevalence of *Salmonella* contaminated eggs and the impact of production methods for non-chicken egg producing species such as ducks, turkeys and quails. This data could be used to validate assumptions used in the risk assessment in relation to the extent and mechanisms of egg contamination for non-chicken egg producing species.

- Prevalence of *Salmonella* contaminated eggs (external and internal contamination)
    - With the low prevalence of *Salmonella* contaminated eggs in Australia, previous surveys have had insufficient sample sizes to detect the organism either on the egg surface or in egg contents. Commissioning a survey of the size necessary to confidently determine the prevalence of contaminated eggs would be costly. However, using estimates of prevalence in the quantitative model, the relative risk from changes in the rate of *Salmonella*-contaminated eggs could be determined.

- Levels of *Salmonella* in contaminated eggs
    - Very few studies have determined the initial level of *Salmonella* in contaminated eggs at, or near, the point at lay. Results from the quantitative model estimate that the risk of illness from consumption of raw eggs that have been stored and consumed prior to the opportunity for growth of *Salmonella* is dependent on the initial number of organisms in the egg contents. Available data on the level of *Salmonella* in contaminated eggs has been generated from experimentally infected laying hens which may not be representative of eggs

from naturally infected hens. A survey on the levels of *Salmonella* in contaminated eggs would need to consider variabilities such as the breed, age and health status of hen, and the serotype and strain of *Salmonella*.

- Levels of *Salmonella* in raw egg pulp
    - Limited data is available on the level of *Salmonella* contamination in raw egg pulp. Levels of *Salmonella* in egg pulp will be affected by factors such as the source and quality of eggs, method of pulping and the temperature and time of storage (potential for growth). The initial level of *Salmonella* in raw pulp is, however, not considered important for pasteurised whole egg as the predicted inactivation is so great, that no *Salmonella* would be likely to survive (predicted >80-$\log_{10}$ reduction).

- Levels of *Salmonella* in separated raw liquid albumen and yolk
    - Similar as for raw egg pulp, very little information is available either nationally or internationally on the level of *Salmonella* in separated raw liquid albumen and yolk. Levels of *Salmonella* in these products will be influence by many factors such as the initial prevalence of *Salmonella*-contaminated eggs, storage time and temperature of eggs prior to separation, as well as the equipment and practices used for separation. The predicted inactivation of *Salmonella* during pasteurisation of liquid yolk is relatively low (approximately 4-log) compared with that for albumen and whole egg. The potential for survival of *Salmonella* in pasteurised liquid yolk is therefore highly dependent on the initial level of *Salmonella* in the raw liquid yolk.

- Vertical transmission of non-SE *Salmonella* serovars
    - For non-SE Salmonella serovars, horizontal transmission is considered the main route of internal egg contamination. Studies have shown, however, that some non-SE *Salmonella* serovars have the ability to colonise the reproductive tissue of hens under experimental conditions. Equivalent studies to determine the possibility/extent of vertical transmission using *Salmonella* serovars isolated from Australian laying flocks may validate assumptions made in the risk assessment in relation to the transmission of non-SE Salmonella serovars into the egg content.

- Mechanisms and extent of horizontal (trans-shell) transmission of *Salmonella* into egg contents
    - Many factors are associated with the potential transmission of *Salmonella* through egg shell (and membranes) into the egg contents. Ideally, studies should be conducted on contaminated eggs from naturally infected hens and penetration of *Salmonella* through the shell determined under conditions observed during production and processing of eggs in Australia.

- Virulence of different *Salmonella* serovars in humans
    - The outcome of human exposure to *Salmonella* spp. is dependent on the virulence of the particular organism (*i.e.* serovar and/or strain) as well as the health status of the individual. Further data is required on the virulence of *Salmonella* serovars that have been associated with eggs in Australia.

- Integrated *Salmonella* surveillance data
    - Integration of *Salmonella* surveillance data from animal, environment/inputs and human sources would assist in gaining a better understanding of the changes in the distribution of *Salmonella* serovars between sources, location and time. This would require a development of a systematic surveillance system to monitor the presence of *Salmonella* in various sources, including standardised methods for detection and classification.

- Extent and cause of sporadic human cases of egg-associated salmonellosis
    - Outbreak data is not necessarily indicative of the incidence and causes of sporadic egg-associated cases of salmonellosis. Attribution of sporadic cases of salmonellosis to consumption of eggs is difficult due to factors such as the retrospective nature of foodborne illness investigation, the often non-point source nature of exposure, association of exposure with mixed foods, and low prevalence of *Salmonella* contaminated eggs. More robust epidemiological data on sporadic human cases of egg-associated salmonellosis would assist in determining the extent of illness attributed to consumption of eggs in Australia.

- Proportion of eggs consumed after expiration of YMT
    - The potential for growth of *Salmonella* in contaminated eggs greatly influences the risk of illness if the egg is consumed raw or lightly cooked. The quantitative model provided scenarios of risk illness from consumption of eggs following expiration of the YMT. This will be affected by the time and temperature of storage along the entire supply chain, including consumer storage. Limited quantitative data is available on the time and temperature of egg storage particularly during the retail and consumer stage.

- Frequency and amount of consumption of raw eggs (and uncooked foods containing raw egg)
    - Limited data is available on the frequency and amount of consumption of raw egg or lightly cooked egg in Australia. This data is difficult to gather as exposure to raw egg when used as an ingredient in uncooked food is often unknown, particularly when the consumer has not prepared the food (*i.e.* food service). For example, it may not be known if a product such as mayonnaise was made on the premises using raw (non-pasteurised) egg or if a commercial product containing pasteurised liquid egg was used.

Availability of the following data may assist in estimating the total number of cases of salmonellosis per year from consumption of eggs and/or egg products in Australia:

- Prevalence of *Salmonella* contaminated eggs (internal and external contamination).
- Levels of *Salmonella* in contaminated eggs.
- Time and temperature conditions which eggs are subject to throughout the supply chain.
- The frequency and amount of consumption of raw and lightly cooked egg.

3 RISK ASSESSMENT – CHEMICALS IN EGGS AND EGG PRODUCTS

3.1 Potential sources of chemical contamination in eggs

There is a range of potential sources of chemical contaminants in eggs and egg products. Exposure to chemicals may occur at the primary production stage through the ingestion, dermal contact or inhalation by layers of feed and water, veterinary treatment, air, soil, or from housing materials. Further along the processing chain additional chemical inputs may occur, including food additives and processing aids.

A paddock-to-plate flowchart identifying potential chemical inputs into eggs and egg products is presented in Figure 3.1.

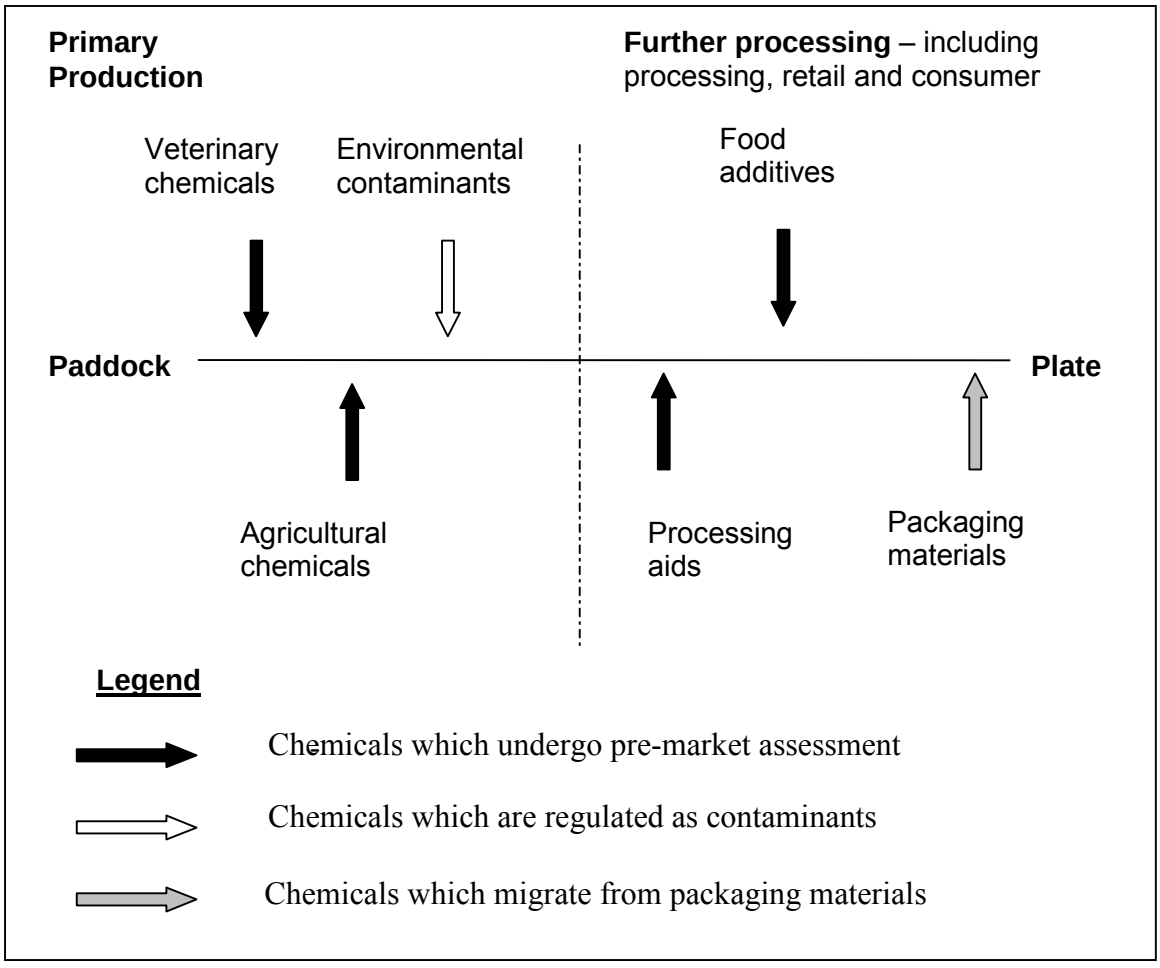


Figure 3.1 Potential chemical inputs in egg production

For the purposes of the Code, chemical substances present in eggs and egg products are either *intentionally added* to food or *unintentionally present* in food. FSANZ uses an evidence-based scientific process to identify and characterise hazards and to evaluate the level of chemical exposure. This information is used to characterise the potential risk associated with chemical hazards.

Substances in food that arise from *intentionally used* chemicals include agricultural and veterinary chemicals, food additives and processing aids. The use of these substances is generally supported by extensive safety data suitable to identify and characterise any risks and in most cases to establish a safe level of human exposure. These substances have undergone a pre-market safety evaluation and approval process and generally have maximum levels of use identified in the Code.

Substances that are *unintentionally present* in food serve no technological function and are generally considered to be contaminants. For contaminants, FSANZ assesses the risk on the basis of the best available data and, where possible, establishes a maximum level in food where there is a potential risk to public health and safety from excessive exposure.

This risk assessment considers the range of potential sources of chemicals in eggs and egg products, including biological sources, agricultural practices and food processing. Contaminating chemicals, such as heavy metals, endogenous plant toxicants, mycotoxins, or anthropogenic chemicals (produced by humans), such as polybrominated flame retardants, may be ingested by laying birds as a result of their presence in the soil or feed. Agricultural chemicals such as herbicides and pesticides are used in association with egg production, and therefore residues may be found in eggs. Veterinary medication to maintain a healthy flock may include the administration of antimicrobials (including coccidiostats) and anthelmintics, which are sometimes found at low concentrations in eggs.

Agricultural and veterinary chemicals, which are used extensively in primary production, are assessed as part of a pre-market evaluation and approval process. The registration and use of these chemicals is regulated by the Australian Pesticide and Veterinary Medicines Authority (APVMA). Maximum residue limits for food are set by the APVMA and are included in the Code. On-farm Quality Assurance (QA) and legislated food safety programs require farmers to use all agricultural and veterinary chemicals according to label instructions, accurately identify treated layers, keep records of all chemical use, and separate eggs from treated layers for the duration of the prescribed withholding periods. Compliance is monitored by the APVMA and State and Territory jurisdictions. Therefore, this risk assessment does not consider agricultural and veterinary chemicals in any detail. The use of agricultural and veterinary chemicals in a way not exactly specified on the label is known as 'off-label' use. This is not illegal within certain limits, however control of use legislation relating to off-label use of veterinary medicines varies between states. Under certain circumstances, registered veterinarians may give instruction for products to be used in a way that is not specifically stated on the label, however they cannot prescribe a veterinary drug in a way that is contrary to the label instructions (e.g. 'not to be used on poultry producing eggs for human consumption'). Where chemical residues occur in foods for which there are no maximum residue limits, these foods are not compliant with the Code and enforcement action may be taken.

Food additives and processing aids, including sanitisers, undergo pre-market evaluation and approval and have maximum use levels identified in the Code.

There are a wide variety of regulatory controls for contaminants at both the primary production and food manufacturing levels. Within food regulations, maximum levels (MLs) are established for many heavy metals and also for a variety of organic chemicals found in the environment that may contaminate food. For some metals, there are also 'generally

expected levels' (GELs) established, which are non-regulatory measures designed to identify contamination outside the normal range. The general principle used for all contaminants is that the levels found in food should be as low as reasonably achievable (the ALARA principle).

Packaging of eggs and egg products may also lead to the unintentional migration of chemicals from the packaging material into the food. FSANZ regulates food contact uses of primary packaging materials in general terms through Standard 1.4.3 – Articles and Materials in Contact with Food. The Standard does not specify individual packaging materials for food contact or how they should be produced or used. With respect to plastic packing products, the standard refers to the Australian Standard for Plastic Materials for Food Contact Use, AS 2070-1999. This reference provides a guide to industry about the production of plastic materials for food contact use. AS 2070, in turn, refers to regulations of the United States (US) and European Economic Community (EEC) directives relevant to the manufacture and use of plastics.

The issue of chemical migration of chemicals from packaging material to eggs and egg products has not been considered as part of this risk assessment as packaging contamination can potentially occur in a wide range of foods and so will be considered by FSANZ as a broader issue at a later time. This process may impact upon the packaging of eggs and egg products in the future.

Regulations that control the use of chemicals in food are outlined in the general standards applicable to all food in Chapter 1 of the Code. There are six Standards in Chapter 1 of the Code that regulate chemical inputs that are relevant to eggs and egg products. The Standards are the following;

Primary production

Standard 1.4.1 – Contaminants and Natural Toxicants

Standard 1.4.2 – Maximum Residue Limits (Agricultural and Veterinary Chemicals)

Further processing

Standard 1.3.1 – Food Additives

Standard 1.3.3 – Processing Aids

Standard 1.4.3 – Articles and Materials in Contact with Food

Related Standards

Standard 1.3.4 – Identity and Purity

3.1.1 Stockfeed and water

A significant potential source of exposure of laying birds (and hence potentially the eggs produced) to chemicals is from feed. Table 3.1 lists some commodities used in hen feed. In addition to the plant materials listed in the table, fish or meat meal may also be used in some poultry diets. Vitamin and mineral premixes may also be added.

Table 3.1 Potential feed ingredients for laying hens (APVMA, 2002)

Feed type	Examples
Grains	Wheat, oats, barley, triticale, rice, maize/corn, millet, sorghum, rye
Pulses/legumes	Succulent or mature dried seed and immature pods of leguminous plants, peas (e.g. field pea, chick pea, cow pea, pigeon pea), beans (e.g. adzuki, fava, kudzu, mung, navy, winged), lentils, soya beans, lupins
Processed grain fractions	Pollard, bran, millrun, wheat germ, brewers grain, malt combings, biscuits, bread, hominy, semolina
Oilseeds	Cotton seed, sunflower seed, safflower seed, rape/canola seed, linseed, sesame seed
Plant protein meals	Oilseed meals, peanut meal, soya bean meal, copra meal, palm kernel meal
Fruit by-products	Citrus pulp, pineapple pulp, pome fruit pomace, grape marc, grape pomace
Other	Vegetables, vegetable by-products (e.g. potato peels), cannery waste and by-products, oils/fats (e.g. vegetable oils, tallow), fodder, forage, fodder vegetable (e.g. field turnips, kale) and animal by-products *e.g.* meat meal.

Stockfeed is regulated by the states and territories and is required to be suitable for its purpose. Some States have set limits on particular contaminants (e.g. NSW Stock Food Regulation 2005 and Queensland Agricultural Standards 1998). The relevant pieces of legislation are listed in Table 3.2.

Table 3.2 State Stock Feed Legislation

State	Legislation	Details
South Australia	Livestock Regulations 1998. Regulations 42, 43, 44, 45[4]	Regulatory limits for prohibited substances, antioxidants, organochlorine pesticides, and registered veterinary medicines.
Queensland	Qld Agricultural Standards 1998[5]	Regulatory limits for cadmium, copper, fluorine, lead, mercury, added selenium, a range of seeds of various toxic plants e.g. heliotrope, aflatoxin b1 (for ducks and laying chickens), ergot, PCBs, urea.
New South Wales	NSW Stock Food Regulation 2005[6]	Schedule one has limits for a range of contaminants including ergot, some pesticides and some metals.
Western Australia	Veterinary Preparations and Animal Feeding Stuffs Regulations 1998[7]	Contains limits for a range of contaminants including aflatoxin B1, ergot, heavy metals, some agricultural chemicals and lists permitted additives.

[4] http://www.austlii.edu.au/au/legis/sa/consol_reg/lr1998237/sch2.html
[5] http://www.legislation.qld.gov.au/LEGISLTN/CURRENT/A/AgrStandR97.pdf
[6] http://www.austlii.edu.au/au/legis/nsw/consol_reg/sfr2005229/
[7] http://www.agric.wa.gov.au/servlet/page?_pageid=449&_dad=portal30&_schema=PORTAL30&p_start_url=/pls/portal30/docs/FOLDER/IKMP/PW/CHEM/STOCKFEEDREGS.HTM
http://www.austlii.edu.au/au/legis/wa/consol_reg/vpaafsr1998585/

Nationally, maximum residue limits have been set for pesticides in animal feed by the Australian Pesticide and Veterinary Medicines Authority, however this covers only fodders and forage, which may be high in residues, but are generally used in cattle and sheep farming rather than egg production (APVMA, 2007b).

Within Australia, the stock feed industry operates under a number of industry codes of good manufacturing practice. Some egg farms produce their own feed or source feed only from accredited suppliers (*e.g.* Egg Corp Assured certified) and/or will require vendor declarations that the product is free from chemical residues at unsafe levels. The National Agricultural Commodities Marketing Authority (NACMA) also has standards for stockfeed with which some feed suppliers will comply (Azadeh Laghai, personal communication). In addition, the Stock Feed Manufacturers' Council of Australia (SFMCA) initiated a quality assurance program for the Australian stock feed industry in March 2003 (SFMCA, 2007). This program is known as FeedSafe® and is based on the principles of Good Manufacturing Practice (GMP). It was developed in conjunction with the Chief Veterinary Officers from the states and endorsed by the Primary Industries Ministerial Council. This program requires feed manufactures to meet minimum standards in relation to: premises and mill buildings; personnel training and qualifications; plants and equipment; raw material sourcing, quality and storage; feed formulation and manufacture; product labelling; loading, transport, and delivery to clients; product inspection, sampling and testing; and customer complaint investigation.

Despite these systems, there is still potential for feed to be contaminated with undesirable chemicals, for example through occasional contamination events or where producers do not have systems in place to comply with the guidelines. Potential feed contaminants have been discussed in Section 3.2 below. The regulation of additives used in feed, e.g. veterinary chemicals, enzymes, pigments and vitamins and minerals are discussed in Section 3.3 – Agricultural and veterinary chemicals. Although these chemicals are extensively regulated, non-compliance could result in undesirable chemical residues in eggs.

Drinking water and the water used in fogging or in ponds to which some free-range hens and ducks may have access is also a potential source of exposure of laying birds to chemical contaminants. Environmental contaminants and heavy metals may be present in some water sources. In addition to the potential for exposure to contaminants through contaminated water, drinking water is the administration route commonly used for veterinary medicines.

Although there are guidelines for human drinking water, these are not applicable to water used for egg producing hens. However, poultry drinking water quality guidelines exist (Peter Scott, personal communication). Potential water contaminants are discussion below in Section 3.2.

3.2 Contaminants

Food standards, when used to establish maximum levels (MLs) for contaminants in various foods, operate within a broader risk management structure to reduce public health risks. Other regulations that encourage practices that in turn reduce contamination of food operate at all levels of government in Australia. These include waste management/disposal programs, water quality programs, industrial zoning regulation and environmental safeguards.

In many cases, the potential for contamination of food is self-limiting because of these other regulations and specific regulation may be unnecessary. When a food standard is considered necessary for a particular contaminant as a risk management option, this is achieved by establishing an ML in particular food commodities. MLs are the legal limits enforced through the State and Territory Food Acts and are, in general, used only when other mechanisms of control are considered insufficient or inadequate to safeguard the health of consumers.

FSANZ regulates the presence of contaminants in food through Standard 1.4.2 – Contaminants and Natural Toxicants. This Standard sets out the MLs of specified metal and non-metal contaminants and natural toxicants in nominated foods. As a general principle, regardless of whether or not a ML exists, the level of contaminants and natural toxicants in all foods should be kept as low as reasonably achievable (the ALARA principle).

Contaminants to which layers may be exposed are listed in Table 3.3.

Table 3.3 Potential chemical contaminants in eggs

Contaminant	Source	Potential adverse effects
Arsenic	Environmental contaminant. Use of arsenic-based anticoccidial agents.	Human carcinogen (induces primary skin cancers).
Cadmium	Environmental contaminant.	Nephrotoxic agent.
Lead	Environmental contaminant. Contamination of specialty duck eggs such as century eggs.	Human neurodevelopmental toxin with children being particularly sensitive.
Mercury	Contamination of poultry fishmeal starter rations.	Human neurotoxin (developing foetus particularly sensitive).
Selenium	Contamination of poultry fishmeal starter rations. Essential trace mineral (may be added intentionally to feed).	Adverse effects on nervous system.
Dioxins	Environmental contaminant.	Potential human carcinogen. Very low tolerable monthly intake.
Polychlorinated biphenyls	Environmental contaminant.	Potential human carcinogen. Very low tolerable monthly intake.
Polybrominated diphenylethers	Flame retardant in manufactured goods. Environmental contaminant.	Adverse effects on liver in rats.
Aflatoxin B_1, B_2, G_1, G_2	Feed contaminant. *Aspergillus flavus, and A. parasiticus* contamination of corn, peanuts and other feed ingredients.	Aflatoxin B_1 is a potential human carcinogen.
Trichothecenes T-2 and HT-2 toxin Deoxynivalenol (Vomitoxin)	Feed contaminant. *Fusarium graminearum, F. crookwellense* and *F. culmorum* contamination of wheat, barley and corn.	Acute food poisoning.
Zearalenone	Feed contaminant. *Fusarium graminearum, F. crookwellense* and *F. culmorum* contamination of wheat and corn.	Possible carcinogen (affects the reproductive system of laboratory animals and pigs).
Ochratoxin A	Feed contaminant. *Aspergillus ochraceus* and *Penicillium verrucosum* contamination of barley, wheat and many other commodities.	Nephrotoxin, possible human carcinogen.
Fumonisin B_1	Feed contaminant. *Fusarium moniliforme* plus several less common species contamination of corn.	Nephrotoxin, possible human carcinogen.
Pyrrolizidine alkaloids	Endogenous plant toxin in some forage plants and weeds (e.g. comfrey, Patterson's curse, heliotrope).	Hepatotoxin: hepatocellular injury, cirrhosis and veno-occlusive disease.
Lupin alkaloids	Lupins.	Acute toxicity.
Ergot alkaloids	*Claviceps purpurea* contamination of rye, wheat, barley, triticale, oats, millet, sorghum and maize.	Ergotism, mainly affects livestock.

Contaminant	Source	Potential adverse effects
Phomopsins	Produced by *Diaporthe toxica* fungus. Lupins are the main host for this fungus.	Cytotoxicity (targets the liver) liver carcinogen in rat.
Cyclopropinoic acid	*Penicillium* species *(e.g. P. commune* and *P. camembertii*) and *Aspergillus flavus and A. versicolor*.	Neurotoxicity, Kodua' poisoning in humans.

These contaminants were selected for the following reasons and are discussed individually in Sections 3.2.1 – 3.2.4.

- Arsenic - widespread use of arsenic based anticoccidials in the poultry industry without residue permissions in Standard 1.4.2 – Maximum Residue Limits;
- Cadmium, lead, dioxins and PBDEs - environmental contamination;
- Mercury and selenium - use of fishmeal starter rations;
- Polychlorinated biphenyls - current MLs in the Code;
- Pyrrolizidine alkaloids and lupin alkaloids - potential exposure of free range layers to plant toxins and/or contamination of poultry feed with weed seeds; and
- Mycotoxins - potential contamination of poultry feeds.

3.2.1 Dioxins and dioxin-like polychlorinated biphenyls

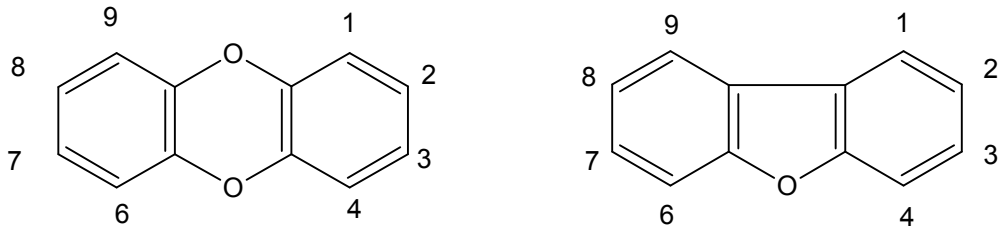
Dioxins enter the food chain when animals eat contaminated plants or inhale smoke from burning organic matter. The dioxins can accumulate in the animal fat, increasing in concentration as they migrate up the food chain. The consumption of animal products with high fat content can therefore theoretically increase human exposure to dioxins. Dioxins and dioxin like polychlorinated biphenyls are carried over from contaminated feed into eggs: the carry-over rate varies for the different congeners, and ranges from 4 – 76% of that consumed (Hoogenboom *et al*., 2006).

The Code does not contain a ML for dioxins. However, FSANZ carried out a dietary exposure assessment and risk characterisation of dioxins and dioxin-like polychlorinated biphenyls (PCBs) in food as part of the National Dioxins Program (NDP) in 2004 (FSANZ, 2004; Office of Chemical Safety, 2004).

The term 'dioxins' is used to describe a group of environmentally persistent halogenated aromatic hydrocarbon chemicals that include polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polybrominated dibenzodioxins (PBDDs), polybrominated dibenzofurans (PBDFs). The chlorinated compounds predominate and are the focus of this review.

PCDDs, PBDDs, PBDFs and PCDFs are not manufactured intentionally but are by-products of combustion. They are formed naturally by volcanoes and forest fires, as well as by industrial processes such as waste incineration and the synthesis of certain chemicals.

The PCDDs and PCDFs are chlorinated tricyclic aromatic hydrocarbons, made up of two benzene rings joined by either two oxygen atoms at adjacent carbons on each of the benzene rings (PCDDs) or by one oxygen atom and one-carbon-carbon bond (PCDFs); their basic structure is given in Figure 3.2 (Office of Chemical Safety, 2004).



Dibenzo-*p*-dioxin

Dibenzofuran

Figure 3.2 Structures of dibenzo-p-dioxin and dibenzofuran

Both groups of chemicals may have up to eight chlorine atoms attached at carbon atoms 1 to 4 and 6 to 9. Each individual compound resulting from this is referred to as a congener. The number and position of chlorine atoms around the aromatic nuclei distinguish each specific congener. In total, there are 75 possible PCDD congeners and 135 possible PCDF congeners. The most widely studied of the PCDDs and PCDFs is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). TCCD is often generically referred to as 'dioxin', and represents the reference compound for this class of chemicals (Office of Chemical Safety, 2004).

Certain polychlorinated biphenyls (PCBs), the non-*ortho* and mono-*ortho* congeners, can adopt a coplanar conformation that is structurally similar to the PCDD/PCDFs and appear to elicit dioxin-like responses through similar modes of action.

PCDDs, PCDFs and dioxin-like PCBs are commonly referred to as 'dioxin-like compounds'.

In general, dioxin-like compounds have very low water solubility, high octanol-water partition coefficients, low vapour pressure and absorb strongly to particles and surfaces and are resistant to chemical degradation under normal environmental conditions. Thus, they are persistent in the environment and their high fat solubility results in their bioconcentration into biota and biomagnification up the food chain (Office of Chemical Safety, 2004).

Toxic equivalency factors

When found in the environment, biological tissue and industrial sources, dioxins are usually present as complex mixtures; this complicates hazard and risk assessment because different congeners vary significantly in their toxicity. However, the potency of different dioxins can be ranked relative to TCDD, the most toxic member of the dioxin class. These rankings are known as toxic equivalency factors (TEFs). To be included in the TEF scheme, a compound must be structurally related to PCDDs and PCDFs, bind to cellular aryl hydrocarbon (*Ah*) receptor, elicit *Ah* receptor-mediated biochemical and toxic responses, must be persistent, and accumulate in the food chain.

Several schemes for assigning TEFs to PCDDs, PCDFs and PCBs have been used previously. However, the most recent review of TEFs was that of the World Health Organization (WHO) in 2005 (Van den Berg *et al*., 2006). However, the FSANZ dietary exposure assessment and risk characterisation of dioxins and dioxin-like polychlorinated biphenyls (PCBs) in food conducted as part of the National Dioxins Program (NDP) in 2004 used the TEFs recommended by the WHO in 1998 (Van den Berg *et al*., 1998). Under the 1998 WHO TEF scheme, TCDD is assigned a TEF of 1.0, and other PCDDs, PCDFs and PCBs have TEF

values ranging from 1.0 down to 0.00001. To estimate the toxic potency of a given dioxin mixture, the mass concentration of each individual component is multiplied by the respective TEF, and the products are summed to represent the TCDD toxic equivalence (TEQ) of the mixture.

Intake of dioxins for the purpose of this report will be expressed in units of TEQs applying the 1998 WHO TEFs (Office of Chemical Safety, 2004).

Hazard Identification and Characterisation

The most widely studied of all the dioxin-like compounds is TCDD. It has been shown to affect a wide range of organ systems in many animal species and can induce a wide range of adverse biological responses. The binding of TCDD to the aryl hydrocarbon (*Ah*) receptor in cells appears to be the first step in a series of events that manifest themselves in biological responses, including changes at the biochemical, cellular and tissue level.

In humans, the most widely recognised and consistently observed effect following high dose exposure to TCDD is chloracne. The condition can disappear after termination of exposure or can persist for many years. Other effects on the skin include hyperpigmentation and hirsutism. TCDD can cause long-term alteration in glucose metabolism and there is some evidence of a weak correlation between incidence of diabetes and occupational or accidental exposure to dioxins; however, background exposure to dioxins is not a significant risk factor for diabetes. TCDD exposure has been suggested to cause slight changes in thyroid function, but clinical illness associated with immune system disorders does not appear to have been associated with TCDD in any cohort studied. There is suggestive evidence of toxicity to the cardiovascular system. Overall, epidemiology studies on populations exposed occupationally or environmentally to TCDD have not demonstrated any significantly increased all-cause or non-cancer mortality (Office of Chemical Safety, 2004).

Experimental studies demonstrate that TCDD is carcinogenic in all species and strains of laboratory animals tested. It has been characterised as a multi-site carcinogen. Epidemiological evidence from the most highly-exposed occupational cohorts studied produces the strongest evidence in humans of an increased cancer risk from exposure to dioxins, when the data is considered for all cancers combined. There is weaker evidence of an increased cancer risk when cancers from particular sites is considered (Office of Chemical Safety, 2004). The International Agency for Research on Cancer concluded that TCDD is carcinogenic to humans (IARC, 1997b).

Australia established a Tolerable Monthly Intake (TMI) for dioxins of 70 pg TEQ/kg body weight (bw)/month from all sources combined. This tolerable intake is equal to the PTMI set by JECFA (JECFA, 2002) and includes polychlorinated dioxins, polychlorinated furans and dioxin-like PCBs, as specified under the WHO 1998 TEF scheme.

Exposure

Eggs were analysed for dioxin levels as part of the National Dioxin Program. This program involved the analysis of a range of food samples for the 29 PCDD, PCDF and PCB congeners for which the WHO derived TEFs for human risk assessment. In this study, 13 composite egg samples were analysed for PCDD/F and PCBs. The analytical results are shown in Table 3.4.

Individual composite sample PCDD/F and PCB results are summarised in a FSANZ Technical Report (FSANZ, 2004).

Table 3.4 Mean levels of PCDD/F and PCBs in eggs (FSANZ, 2004)

	PCDD/F		PCB	
	Lower bound pg/g FW	**Upper bound pg/g FW**	**Lower bound pg/g FW**	**Upper bound pg/g FW**
Eggs (13 samples)	0.0026	0.045	0.0062	0.012

All samples are composites of three or four purchases.
All results are reported in picograms TEQ per gram of food on a fresh weight basis.
Lower Bound – assumes results reported as below the LOR are zero for each congener. The levels of the individual congeners are then summed.
Upper Bound – assumes results reported as below the LOR are at the LOR for each congener. The levels of the individual congeners are then summed.

Comparison of dioxin concentrations in food across different monitoring programs is difficult since there are differences in food sampled, analytical methodologies and calculation and reporting of TEQs. Generally Australian foods have levels of PCDD/Fs and PCBs that are similar to those reported in New Zealand and lower than those reported from other areas of the world.

Table 3.5 Comparison of mean PCDD/F and PCB concentrations in eggs from different areas of the world

Australia	**New Zealand**[1,2]	**UK**	**Netherlands**[3]	**Europe**[1]	**North America**[1]
(Office of Chemical Safety, 2004)	(MFE, 1998)	(Food Standards Agency, 2003)	(Freijer *et al*., 2001)	(Codex, 2003)	(Codex, 2003)
Mean PCDD/F (pg TEQ/g lipid)					
0.013-0.42	0.017-0.12	0.24-0.24	1.52	0.5-2.7	0.044-0.3[4]
Mean PCBs (pg TEQ/g lipid)					
0.04-0.11	0.05-0.11	0.11-0.20	0.87	0.2-0.6	0.029[4]

[1] Results reported in I-TEQs, that are 10-20% lower than WHO-TEQs.
[2] Results reported in the range of lower to middle bound.
[3] Results reported as lower bound only.
[4] Reported on a fresh weight basis.

Dietary exposure assessment indicated that for Australian consumers, eggs contributed around 0-2% to mean dietary PCDD/F exposures for each population group. The contribution of eggs to mean dietary PCB exposure was 1% for all population groups (Office of Chemical Safety, 2004).

Risk Characterisation

For the general population, over 95% of exposure to dioxin-like compounds is through the diet, with foods of animal origin such as meat, dairy products and fish being the main sources (Office of Chemical Safety, 2004).

Eggs and egg products are relatively low contributors to the total dietary exposure of the Australian population to dioxins and dioxin-like compounds. Overall, however the levels of exposure from all foods are well within the JECFA PTMI.

Australian eggs have relatively low PCDD/F and PCB concentrations compared to other areas of the world (Table 3.6); bearing in mind that there are differences in analytical methodologies and calculation of the reporting of TEQs. The overall dietary exposure to dioxins and dioxin-like PCBs in Australia and New Zealand is well below that of values recorded in the U.K., The Netherlands and Europe (FSANZ, 2004).

From this study, on the basis of the available data, taking into account all the inherent uncertainties and limitations, it was concluded that the public health risk for all Australians from exposure to dioxins from foods, including eggs and egg products is very low.

Table 3.6 An international comparison of mean or range of estimated dietary intakes of dioxins

Country/region	Reference	PCDD/Fs (pg WHO-TEQ/kg bw/month)	PCBs (pg WHO-TEQ/kg bw/month)	Total Dioxins (pg WHO-TEQ/kg bw/month)
Australia[1]	(FSANZ, 2004)	0.9-10.2	2.8-5.4	3.7-15.6
New Zealand[2]	(MFE, 1998; MFE, 2001)	6.6	4.5	11.1
UK[3,4]	(Food Standards Agency, 2003)	9	9-12	15-21
The Netherlands[4,5]	(Freijer *et al*., 2001)	20.7	18.6	39
Europe[6,7]	(European Commission, 2000)	12-45	24-45	36-90

[1] Range is lower bound to upper bound for all persons 2+years of age
[2] Medium bound estimate for adult males
[3] Range is lower bound to upper bound for the population average
[4] Sum of PCDD/F and PCB (total dioxins) may not equal sum of separate intakes due to rounding
[5] Lower bound estimate, mean lifelong-averaged (1-70 years) exposure.
[6] I-TEQs. WHO-TEQs are 10-20% higher than I-TEQs.
[7] Average dietary exposure for an adult.

Conclusion
There are no public health and safety concerns with the current levels of dioxin in eggs and egg products.

3.2.2 Polybrominated diphenyl ethers

Polybrominated diphenyl ethers (PBDEs) are anthropogenic chemicals that are added to a wide variety of consumer/commercial products in order to improve their fire resistance. PBDEs have come under increased scrutiny because of their potential to impact upon human health and the environment. FSANZ recently commissioned an analytical survey of PBDEs in a limited range of Australian foods, which taken together with data on the concentration of PBDEs in breast milk, were used in a population exposure assessment and health risk appraisal (FSANZ, 2007).

Hazard Identification and Characterisation

The nature of the adverse effects associated with PBDE exposure has been described in several national and international reviews. In Australia, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) has reviewed the available toxicological data and identified an unacceptably low margin of exposure (MOE) in women of child bearing age when considering all sources of exposure, which includes food and house dust. As a consequence, NICNAS has taken action against the more toxic PBDEs by implementing an interim suspension on the import and manufacture of penta-BDE and octa-BDE flame retardants (NICNAS, 2007).

In 2005 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that due to the paucity of reliable toxicity data it was not possible to establish a common mechanism of action that would permit a single congener to be used as a surrogate for the purpose of establishing toxic equivalence factors for all PBDEs (JECFA, 2005a). Moreover, the database lacked appropriate mechanistic studies which would allow the toxicological relevance of some studies to be determined. While it would be normal practice to establish a tolerable weekly or monthly intake (TWI or TMI) for bio-accumulative contaminants such as the PBDEs, the incompleteness of the available database for each congener does not enable a provisional TMI or TWI to be established. Following an independent review of the available toxicological data FSANZ concurs with JECFA's conclusion. The limited toxicological database suggests that adverse effects for all congeners in the rat (liver weight increase with corresponding histopathology) are unlikely to occur at doses much less than 0.1 mg/kg bw/day. This threshold dose has been used as the basis for determining the magnitude of the MOE for all the PBDEs.

Exposure

In the FSANZ survey, PBDEs were detected in boiled eggs with an upper bound mean value of 954.6 pg/g fresh weight, however the source and mode of transmission of PBDEs into eggs is not known. Of all foods, the highest levels of PBDEs were detected in boiled eggs, grilled pork chops, bacon and cream. The concentrations and types of PBDEs detected in Australian food appear to be similar to those reported in other areas of the world. Australian breast milk has previously been found to contain PBDEs at levels below those reported for North America but higher those in Europe and Japan. With the exception of fully breast-fed infants, the main contributors to dietary exposure across the majority of population groups were bread, eggs, vegetables and meat, with eggs contributing to between 11 and 16% of exposure from food.

Risk characterisation

In characterising the risk associated with PBDE exposure from food, the estimated dietary exposures for various age groups were compared to the threshold dose of 0.1 mg/kg bw/day. The margin of exposure (MOE) is calculated by dividing the dose at which adverse effects were observed in laboratory animal studies by the estimated intake of PBDE from food. The lower the MOE, the greater is the public health risk.

Dietary exposure of the general population to PBDEs in food is low with the MOEs for the majority of population groups at or above 10,000. Other population groups with comparatively high exposures included 3-month old fully breast fed infants (MOE = 1960)

and 9-month old breast fed infants (MOE ~3000). However, as these exposures are still over 1,000-fold below any adverse effect dose observed in laboratory animals they are unlikely to constitute a risk to infant health.

On the basis of the available data and taking into account all the inherent uncertainties and limitations it can be concluded that the Australian public health risk arising from exposure to PBDE in food including eggs is low.

3.2.3 Metals

Metals can potentially contaminate eggs through their presence in the soil, uptake by crops used in stockfeed or metal contamination during processing. Metals may enter the soil through agricultural practices, for example, as components of fertilisers and /or industrial contamination. In addition to the consumption of crops grown in soils with high metal contents, hens with access to the external environment may directly consume soil. The bioavailability of trace elements in soil is about 1.5 times lower than in feed (Van Hooft, 1995).

There are no MLs in the Code for heavy metals in eggs and egg product. The available data indicate eggs are an insignificant dietary source of these heavy metals and therefore do not require a control such as an ML.

Arsenic, cadmium, lead, mercury and selenium have been considered below.

3.2.3.1 Arsenic

Arsenic occurs naturally in both organic and inorganic forms; drinking water contains largely the inorganic form of arsenic, whereas food contains more than 90% of its arsenic in the organic form. It is widely distributed in the environment and has been used in agriculture; therefore arsenic is present in most human foods. The use of phosphate fertilisers on agricultural land may be a significant source of arsenic and, in some circumstances this could lead to elevated levels in crops. The level of arsenic varies in plants and therefore levels in eggs may be increased when layers consume plants with high levels of arsenic. Some veterinary medicines, such as arsenic based anticoccidials, may be a source of exposure for layers.

Hazard Identification and Characterisation

A risk assessment on arsenic was last performed by FSANZ[8] in Proposal 157 – Review of the maximum permitted concentrations of metal contaminants in food (ANZFA, 1999b). Arsenic in its inorganic form is toxic to humans. The most relevant toxicological data, other than industrial exposure, are derived from studies of human populations exposed to arsenic in drinking water, with chronic toxicity and cancer the most sensitive indicators of toxicity.

Chronic ingestion of low doses of inorganic arsenic initially produces cutaneous vasodilatation, then hyperpigmentation and hyperkeratosis with subsequent atrophy and degeneration of the skin leading over a period of time to the development of skin cancers.

[8] As the Australia New Zealand Food Authority (ANZFA)

FSANZ established a lowest observed effect level (LOEL) for inorganic arsenic, based on population studies in Taiwan, where drinking water exposures for periods of 12 years to whole-of-life were associated with cancers (skin, liver, bladder, and lung). Only skin cancer was detected at the lowest LOEL. There is growing evidence for a threshold in a dose-response relationship between inorganic arsenic and various cancers. The lowest LOEL for human skin cancer was approximately 0.0029 mg/kg bw/day, based on a review of epidemiological data. On the basis of the available data, this level is considered to be close to a 'threshold' value, below which increased incidence of skin cancer was not associated with arsenic exposure.

The provisional tolerable daily intake (PTDI) for inorganic arsenic is 0.003 mg/kg bw/day. While based on exposure to drinking water rather than food, it is considered appropriate for use in assessing the risk from inorganic arsenic in food. It should be noted however, that this PTDI for arsenic does not incorporate any safety factors (ANZFA, 1999b). While it is usual to apply a 10-fold safety factor for the variability in response among humans, one was not used in this case because the PTDI is based on epidemiological data involving a large number of individuals.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has assigned a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg bw per week for inorganic arsenic (WHO, 1989), noting that the margin between the PTWI and intakes reported to have toxic effects in epidemiological studies, was narrow. The provisional status of the maximum weekly intake was continued due to the desire to lower the arsenic intake of those individuals exposed to high levels of inorganic arsenic in drinking water.

The International Agency for Research on Cancer (IARC) has classified inorganic arsenic into group 1 (carcinogenic for humans), for the ability to induce primary skin cancers (IARC, 1987).

Dietary exposure

A dietary exposure assessment was conducted as part of the assessment of arsenic for Proposal P157 – Review of the maximum permitted concentrations of metal contaminants in food. Inorganic arsenic as derived in the 1995 Australian National Nutrition Survey (ANZFA, 1995) was an estimate based on a proportion of total arsenic. For the general community the main foods contributing to inorganic arsenic dietary exposure, excluding water, were prawns (51.9%), marine fish (14.3%), milk (9.8%) and rice (5.5%).

The 20th Australian Total Diet Survey estimated exposure to total arsenic between 9 -48% of the PTDI set for inorganic arsenic. In the 19th and 20th ATDS, eggs were analysed for arsenic (limit of reporting of 0.01 mg/kg). Samples tested ranged from ‘not detected’ to 0.04 mg/kg (ATDS, 2001; ATDS, 2003). These results demonstrate consistent non-detectable or very low levels of arsenic in eggs.

3.2.3.2 Cadmium

Cadmium is a widespread contaminant in many agricultural products worldwide. The use of phosphate fertilisers on agricultural land may be a significant source of cadmium and, in some circumstances this could lead to elevated levels in crops. Since cadmium is retained in

the topsoil, concentrations can increase if the application of these materials to soils continues over long periods. Exposure of animals to cadmium results from feed intake and uptake of soil during feeding (e.g. free-range birds or cage birds consuming soil contaminated feed) and is a function of the concentration of cadmium in the feed and the amount of feed consumed.

A risk assessment on cadmium was last performed by FSANZ[9] in Proposal 144 – Review of the maximum permitted concentration of cadmium in food (ANZFA, 1997). Cadmium has been most recently assessed by JECFA in 2005 (JECFA, 2005b) (JECFA, 2003a).

Hazard Identification and Characterisation

Cadmium has an extremely long biological half-life in humans and accumulates in the kidneys over time. The kidney has been identified as the critical organ in relation to chronic exposure to relatively low levels of cadmium and in particular the renal cortex.

An early feature of the adverse renal effects in humans is the impairment of the reabsorption functions of the tubules with an increase in urinary excretion of low-molecular weight proteins. Renal injury may progress and, in severe cases, involve glomerular damage with proteinuria, aminoaciduria, glucosuria and phosphaturia. It has generally been found that tubular proteinuria, once manifest, persists even when exposure ceases. Intakes of cadmium in the range of 140-255 μg/day have been associated with increased low-molecular weight proteinuria in the elderly.

Low-molecular weight proteinuria is not accompanied by any specific histological changes and the pathological significance of this finding is unclear. However, it can be used to as an indicator of the threshold of a possible toxic effect and it is appropriate to set the provisional tolerable weekly intake on the basis of the dose-response data for this endpoint (JECFA, 1989).

The critical health outcome with regard to cadmium toxicity is renal tubular dysfunction. JECFA established a provisional tolerable weekly intake (PTDI) for cadmium of 7 μg/kg bw per week (JECFA, 2003a).

This level was to ensure that cadmium concentration does not exceed 50 μg/g in the renal cortex assuming an absorption rate of 5% and a daily excretion rate of 0.005% of body burden, over a period of 50 years.

The IARC has classified cadmium and cadmium compounds into group 1 (carcinogenic for humans) (IARC, 1997a).

Cadmium is carcinogenic in experimental animals when given by injection or inhalation, and exposure of workers by inhalation has been shown to result in pulmonary cancer. There was no evidence that cadmium is carcinogenic to humans exposed by the oral route (JECFA, 2001c).

[9] As the Australia New Zealand Food Authority (ANZFA)

Dietary exposure

A dietary exposure assessment was conducted as part of the assessment of cadmium for Proposal P144 – Review of the maximum permitted concentrations of cadmium in food.

A revised dietary exposure assessment for cadmium was conducted on the basis of additional survey information (ANZFA, 2000). Cadmium concentration data used in this assessment were sourced both within FSANZ as well as submissions from external sources. Cadmium was not detected in eggs in either the 19th ATDS or the 20th ATDS (limit of reporting of 0.005mg/kg) (ATDS, 2001; ATDS, 2003). The primary foods that contribute to dietary cadmium exposure in the Australian population were cereals (9.3%), meat and offal (9.5%), cocoa (5%), fruit (14.7%), potatoes (28%), other roots and tubers (6%) and other vegetables (13.6%).

Estimated dietary exposure to cadmium, based on the 1995 Australian National Nutrition Survey (ANZFA, 1995) (whole population aged 2 years and over) resulted in a mean dietary exposure of 13-16% of the PTDI and dietary exposure at the 95th percentile (consumers only) of 34-41% of the PTDI. Cadmium dietary exposure from the consumption of eggs and egg products presents a negligible risk to the consumer.

3.2.3.3 Lead

A risk assessment on lead was last performed by FSANZ[10] in Proposal 157 – Review of the maximum permitted concentrations of metal contaminants in food (ANZFA, 1999c).

Lead (Pb) is a soft, silvery grey metal which is highly resistant to corrosion. Solubilities in water vary, lead sulphide and lead oxides being poorly soluble and the nitrate, chlorate and chloride salts reasonably soluble. Lead also forms salts with such organic acids as lactic and acetic acid, and stable organic compounds such as tetraethyllead and tetramethyllead, the latter two important as fuel additives and as such are sources of environmental lead. In recognition of its toxic effects, more than 40 years ago lead was removed from paint produced in Australia and petroleum products are now lead-free reducing the potential for environmental exposure to lead.

No organic forms of lead have been reported to occur in food. Thus lead in foodstuffs exists exclusively as salts, oxides or sulphydryl complexes. The elimination of lead solder from food cans has reduced the hazard of exposure to lead from canned food, particularly from canned milk and infant formula.

Lead can potentially contaminate eggs and egg products through environmental contamination or through contamination of water supplies. The illegal use of lead as a processing aid in specialty eggs will also cause contamination (see Section 3.6.2).

Hazard Identification and Characterisation

In humans, blood levels exceeding 300 μg/l as a consequence of occupational exposure have been related to a number of toxic effects such as anaemia, renal toxicity and subsequent carcinogenicity, cardiovascular and neurological/behavioural effects, and impairment of the

[10] As ANZFA

reproductive system (Gardella, 2001; Gonick and Behari, 2002; Silbergeld, 2003). The most important and best-documented effect of lead at the concentrations most commonly encountered outside occupational settings is retardation in the neurobehavioral development observed in children of mothers having been exposed to lead (Lidsky and Silbergeld, 2003). The most recent research on developmental toxicity in children suggests that detectable deficits may occur even at exposure levels previously considered safe (Canfield *et al*., 2003; Lanphear *et al*., 2000; Selevan *et al*., 2003).

The IARC has classified lead into group 2A (probably carcinogenic for humans) (IARC, 2004).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has established a provisional tolerable weekly intake (PTWI) for lead in 1986. It is set to 25 μg/kg bwt/week for infants and children (equivalent to 3.6 μg/kg bw/day) on the basis that lead is accumulating in the body and an increase of the body burden should be avoided (JECFA, 1986). In 1993 and 2000, the Committee reconfirmed this PTWI and extended it to all age groups (EFSA, 2004c).

Exposure Assessment

A dietary exposure assessment was conducted as part of the assessment of lead for Proposal P157 – Review of the maximum permitted concentrations of metal contaminants in food.

Estimated dietary exposure to lead, based on the 1995 Australian National Nutrition Survey (whole population aged 2 years and over) (ANZFA, 1995) resulted in a mean dietary exposure of 2.2-5.6% (6.5-9.9% including water) of the PTWI and dietary exposure at the 95th percentile (consumers only) of 6.2-13.2% (16.1-22.6% including water) of the PTWI.

The primary foods that contribute to dietary lead exposure in the Australian population (aged 2 years and older), excluding water, were cattle meat (29.9%), pig meat (11.7%), wine (9.8%), peach (8.7%), pineapple (5.4%) and sugar (5.0%). Egg was not a major contributor to lead dietary intake.

Lead was detected in two of 28 egg samples at very low levels in the 20th ATDS. The overall intake of lead from all food sources was determined to be with the acceptable health standard (ATDS, 2003).

Lead was detected in the yolks and shells of eggs from hens that had been exposed to chips of lead-based paint in the environment. The levels of lead in the yolk strongly correlated with blood lead levels and ranged from <20 – 400 ppb (Trampel *et al*., 2003). Routine consumption of highly contaminated eggs such as these, ***e.g.*** from a back-yard farm, represents a potential public health concern, particularly for children[11].

3.2.3.4 Mercury

Mercury occurs naturally in the environment with levels in the topsoil varying between 0.02 and 0.15 mg/kg. Therefore, despite barriers to bioavailability, there is potential for ingestion

[11] Consumption of a 60 g egg with 400 ppb lead (assuming lead level in white is equal to that in the yolk) by a 10kg child would represent approximately 67% of the PTWI expressed on a daily basis.

of low levels of mercury by free-range birds. Mercury may be a contaminant in fish meal used in some layer diets.

A risk assessment on mercury was last performed by ANZFA in Proposal 157 – Review of the maximum permitted concentrations of metal contaminants in food (ANZFA, 1999d).

The primary route of exposure to mercury, primarily in the form of methylmercury, is through the food supply. Occupational exposure to mercury is generally from mercury vapour.

The different chemical forms of mercury can exhibit quite distinct pharmokinetic and toxicological properties. From the perspective of exposure via food, inorganic mercury appears to represent a lesser hazard than organic forms of mercury. There are essentially two reasons for this. Firstly, the levels of inorganic mercury in food are low and secondly, absorption of inorganic mercury from the gastrointestinal tract is also low, therefore it appears unlikely that many people would be subject to the levels of oral intake that might be expected to have an adverse effect.

The predominant source of environmental methylmercury is the methylation of inorganic mercury. This reaction is typically carried out by microorganisms in aquatic sediments, soils and faecal material. Although intake of the methylated form is of primary interest, surveys of contaminants in food typically only measure total mercury (ANZFA, 1999d).

Hazard Identification and Characterisation

In humans, methylmercury can induce toxic effects in several organs such as the nervous system, kidney liver and reproductive systems. Neurotoxicity is considered the most sensitive endpoint. The majority of toxicological data, on which tolerable limits were previously set, have come from large scale poisonings of human population with methylmercury in Japan and Iraq. Data from these incidences confirmed an association between the consumption of fish contaminated with methylmercury and the development of neurological symptoms in adults and infants exposed *in utero*. The data indicated that the most sensitive section of the population to methylmercury poisoning is the unborn foetus (JECFA, 2003b).

The IARC has classified methylmercury into group 2B (probably carcinogenic for humans – sufficient evidence in animals and inadequate data in humans) and metallic mercury and inorganic mercury compounds into group 3 (not classifiable as carcinogenic to humans) (IARC, 1997a).

In June 2003, JECFA evaluated new information that became available on methylmercury. This information included results of studies performed on laboratory animals and humans, and epidemiological studies investigating possible effects of prenatal methylmercury exposure on child neurodevelopment. A new PTWI of 1.6 μg/kg bw was recommended. This PTWI is considered sufficient to protect the developing foetus, the most sensitive subgroup of the population (JECFA, 2003b).

Exposure Assessment

A dietary exposure assessment was conducted as part of the assessment of mercury for Proposal P157 – Review of the maximum permitted concentrations of metal contaminants in

food. Fish is by far the greatest contributor to dietary mercury exposure. Eggs were not a major contributor to mercury dietary intake.

Mercury was not detected in egg samples in the 19th or 20th Australian Total Diet Surveys. The limit of reporting was 0.01mg/kg (ATDS, 2001; ATDS, 2003).

3.2.3.5 Selenium

A risk assessment on selenium was conducted by ANZFA as part of Proposal 157 – Review of the maximum permitted concentration of metal contaminants in food (ANZFA, 1999e).

Selenium is a metallic group VI element that is abundant and which can exist in four oxidation states (-2, +1, +2 and +6). Selenium in food is predominantly in the form of organoselenium compounds; selenocysteine is usually the primary form obtained from animal based foods. The selenium content of food varies depending on the selenium content of the soil. Organ meats, such as kidneys or livers, contain the highest levels of selenium, but some seafood products contain almost as much (IPCS, 1986). Selenium in poultry meat was investigated by FSANZ as part of Proposal P282 - Primary Production and Processing Standard for Poultry Meat, due to a concern that some poultry meat might contain high levels of selenium from the consumption by poultry of fishmeal rations. However, selenium is an essential trace element which is sometimes added to poultry feed in order to avoid deficiency in birds fed grain-based diets low in selenium. Selenium enriched eggs are also being produced as a niche product (Peter Scott, personal communication).

Hazard Identification and Characterization

Selenium is an essential element necessary for good health, and the contribution of selenium deficiency to specific diseases are well described (ANZFA, 1999e). In the diet only organoselenium compounds are present. Exposure to inorganic selenium only occurs through supplementation or contamination of foods. In excessive quantities in the diet, selenium compounds can cause systematic toxicity in people, stock animals and laboratory species.

Absorption of selenium is efficient and is not regulated. More than 90% of selenomethionine, the major dietary form of the element, is absorbed by the same mechanism as methionine itself. Two pools of reserve selenium are present in humans and animals. One of them, the selenium present as selenomethionine, depends on dietary intake of selenium as selenomethionine. The amount of selenium made available to the organism from this pool is a function of turnover of the methionine pool. The second reserve pool of selenium in the selenium present in liver glutathione peroxidase.

The mechanism that regulates production of mammalian excretory metabolites has not been elucidated, but excretion has been shown to be responsible for maintaining selenium homeostasis. The excretion occurs largely in the urine.

Prolonged exposure to high levels of selenium induces pathological changes to the hair and nails as well as adverse effects on the nervous system. Common clinical features are hair loss and structural changes in the keratin of hair and of nails, the development of icteroid skin, and gastrointestinal disturbances. Nervous system effects include peripheral anaesthesia, pain in the extremities, and paresthesis. A positive association between dental caries and urinary selenium have been reported. Changes in biochemical parameters have also been reported.

The human data from the Enshi district in China, albeit limited, is considered the best, available for the estimation of a LOEL. A chronic dietary intake of 0.75 mg Se/day was noted as the minimum level at which increasing amounts of dietary selenium was associated with a decrease in the plasma/erythrocyte selenium ratio in human blood. The biological significance of the decrease in this ratio is not clear, but may indicate changes in the selenium compartmentation and may be interpreted as the most sensitive biochemical indication of chronic selenosis. Nail changes considered the most sensitive clinical marker of chronic selenosis were observed at 0.85-0.95 mg Se/day. However, the effect on the plasma selenium to erythrocyte selenium ratio could be considered a more acceptable conservative biochemical marker of sub clinical selenium toxicity (ANZFA, 1999e).

Based on the sub clinical observation of the plasma/erythrocyte plasma selenium ratio in human blood, ANZFA proposed a PTDI of 0.75 mg/day (equivalent to 12.5 μg/kg bw/day) for selenium. Furthermore, there are homeostatic mechanisms present in adults, which act to compensate for an excessive intake of selenium and hence clinical signs of toxicity, are reversible (ANZFA, 1999e). A subsequent report utilizing an upper tolerable nutrient intake level (UL) as a reference, provisionally set an intake of 400 μg/day for selenium (FAO/WHO, 2001).

Exposure Assessment

A dietary exposure assessment was conducted as part of the assessment of selenium for Proposal P157 – Review of the maximum permitted concentrations of metal contaminants in food.

The primary foods that contribute to dietary selenium exposure in the Australian population aged 2 years and older, were chicken meat (19%), marine fish (11%), pork (10%), eggs (10%), wheat flour (5%) and milk and dairy (5%). Australian Total Diet Survey data indicate selenium levels in eggs range from 0.18 – 0.47 mg/kg (ATDS, 2001; ATDS, 2003).

Estimated dietary exposure to selenium, based on the 1995 Australian National Nutrition Survey (whole population aged 2 years and over) resulted in a mean dietary exposure of 7.3-12.6% of the PTDI (8.6-13.8% of the PTDI when water was included) and dietary exposure at the 95th percentile (consumers only) of 18.9% and 20.8% including water (ANZFA, 1995).

3.2.3.6 Risk characterisation for metals in eggs

An evaluation on arsenic, cadmium, lead, mercury and selenium was performed to establish whether there are potential public health and safety risks with the consumption of eggs and egg products.

As discussed in the individual sections above, these five metals can result in serious adverse effects when consumed at high concentrations. However, data from the Australian Total Diet Surveys on the concentrations of these metals in eggs indicates that levels are low. This data is from chicken eggs, however on the basis of physiology and good agricultural practices, it could be expected that the levels of metal contaminants in eggs from other species e.g. duck or quail, will be similar. An exception to this may be some specialty egg products, such as alkalised eggs, which are sometimes produced with lead oxide as an additive/processing aid. The use of lead in food is not permitted. Specialty eggs are discussed further in Section 3.6.2

below. In addition, routine consumption of eggs from highly contaminated sites represents a potential public health concern, particularly for children in regard to lead.

Overall, based on work previously conducted by FSANZ, intakes of these metals from the total diet are within safe levels. In conclusion, dietary exposure to arsenic, cadmium, lead, mercury and selenium from eggs and egg products does not raise any public health and safety concerns.

3.2.4 Plant, fungal and bacterial toxins

Mycotoxins and bacterial toxins are secondary metabolites derived from fungi or pathogenic bacteria and may be natural contaminants of food and stockfeed. There are approximately 6000 known mycotoxins, but few of these have complete toxicological profiles. Some toxins can be carried over from the layers' feed into the eggs where they have been found at low concentrations. Whether a contaminant is transferred from feed to the eggs is determined by various kinetic processes, and will be dependent on the nature of the individual contaminants, the levels in the feed and the period over which exposure occurs. However, it is important to note that feed contaminated with these toxins will potentially have significant detrimental effects on animal health and cause subsequent production losses. Concentrations which do not manifest in systemic toxicity in birds are unlikely to result in concentrations of concern in eggs.

The susceptibility of stockfeed to contamination by plant, fungal and bacterial toxins will vary according to the type of feed used and the geographic location. Measures such as vendor declarations from feed suppliers, feed producers implementing on-farm controls for crop management (GAP), and appropriate storage of feed grains, may assist in reducing the risk of the use of contaminated feed.

Egg grading and sorting machinery may also be a source of fungal contamination of the external surface of the egg (Davis *et al*., 1999). However, there is no evidence that fungal contamination of machinery leads to internal contamination of the egg.

Some of the more common naturally occurring toxins, their sources and an indication of whether the toxin is carried over into eggs and egg products, are listed in Table 3.7.

Table 3.7 Naturally occurring toxins of plant and fungal origin

Type	Toxin	Pathogen	Source/Host	Known Residues in Eggs?	Reference
Endogenous plant toxins	Pyrrolizidine alkaloids	N/A[a]	Forage plants and weeds (e.g. comfrey, Patterson's curse, heliotrope)	Yes	(Edgar and Smith, 2000)
	Lupin alkaloids	N/A[a]	Lupins	Unknown	
Mycotoxins	Aflatoxin	*Aspergillus sp.*	Stored grains e.g. corn, sorghum, peanuts, cottonseed and cottonseed meal	Yes	(Bintvihok *et al*., 2002; Oliveira *et al*., 2000)
	Phomopsins	*Diaporta toxica*	Mainly lupins	Unknown	
	Ergot alkaloids	*Claviceps purpurea*	Rye, wheat, barley, triticale, oats, millet, sorghum, maize	Unlikely	(Dingle and Blaney, 2003)
	Ochratoxin	*Aspergillus sp. and Penicillium sp.*	Forage and stored grains, e.g. barley, wheat	Yes to some extent	(IPCS, 1979; JECFA, 2001b; Krogh *et al*., 1976)
	Trichothecene toxins (T-2, nivalenol, deoxynivalenol)	*Fusarium sp*	Forage and stored grains, particularly wheat and corn	Very limited if any	(El-Banna *et al*., 1983; IPCS, 1990; Kubena *et al*., 1987; Prelusky *et al*., 1989; Sypecka *et al*., 2004; Valenta and Dänicke, 2005)
	Zearalenone	*Fusarium sp.*	Forage and stored grains	Unknown	
	Fumonisins	*Fusarium sp.*	Forage and stored grains, particularly corn and sorghum.	Unknown	
	Cyclopiazonic acid	*Penicillium spp., Aspergillus spp.*	Cereal grains	Yes, low levels	(Dorner *et al*., 1994)

[a] not applicable

3.2.4.1 Pyrrolizidine alkaloids

Pyrrolizidine alkaloids (PAs) are plant toxins that may find their way into human and animal food in Australia. They are derived mainly from the plants *Heliotropium europaeum* (common heliotrope or potato weed), *Echium plantagineu* (Pattersons' curse), *Senecio* spp. (ragwort), *Symphytum* spp. (comfrey), and *Crotalaria retusa* (rattleweed). The *Sympthytum* spp. may be deliberately ingested (e.g. in traditional medicine preparations) while the remaining species are weeds in various grain crops. There is a long history of toxicity in livestock caused by grazing on PA-containing plants and through the consumption of grains contaminated with weed seeds.

There are more than 50 types of PAs, some of which have been shown to be toxic to animals at very low doses. There have also been a number of outbreaks of human poisoning as a result of ingestion of contaminated grain as well as case reports of poisoning caused by intentional ingestion of herbal medicines containing PAs (FSANZ, 2001c).

No Maximum Levels for PAs in food have been established. Queensland and New South Wales have regulations limiting the number of seeds of heliotrope, yellow burr weed (*Amsinkia* spp) and Pattersons' curse which may be contaminants in stock feed (NSW, 2005; Qld, 1997).

Hazard Identification and Characterisation

The PAs of relevance to human health are the hepatotoxic PAs which are esters of 1-hydroxymethyl dehydropyrrolizidine. Such compounds are metabolised in the liver to electrophilic derivatives referred to as pyrroles. These pyrroles cause damage in the hepatocytes in which they are generated, but depending on their persistence in aqueous media, can pass from the hepatocytes into the adjacent sinusoids and damage endothelial lining cells of the sinusoids and smallest hepatic veins. These effects give rise in humans to hepatocellular injury, cirrhosis and veno-occlusive disease.

The available data on cases of veno-occlusive disease in humans indicates a tentative no-observed-effect level (NOEL) of 10 µg/kg bw/day can be established. If an uncertainty factor of 10 to account for human variability is applied to this NOEL, the provisional tolerable daily intake (PTDI) for PAs in humans is 1 µg/kg bw/day (FSANZ, 2001c).

Dietary exposure

Apart from the deliberate use of herbal remedies and nutritional supplements containing PAs, humans can become inadvertently exposed through consumption of contaminated food. The foods which have been found to contain PAs include grains, honey, milk, offal and eggs. FSANZ (2001) reports that pyrrolizidine alkaloids have been detected in eggs, with levels ranging from 5 to 168 µg/kg.

No free pyrrolizidines were detected in eggs of hens experimentally fed plant alkaloids from *Senecio vernalis* (Eroksuz *et al*., 2003). However another study found pyrrolizidine alkaloids in eggs at levels ranging from 19 – 168 µg/kg, from hens which had been exposed to feed containing the seeds of *Heliotropium europaeum* (0.6% by weight), and lesser quantities of yellow burr weed and sheepweed (*Buglossoides arvensis*) seeds (Edgar and Smith, 2000).

Substantial contamination of grain commodities has been recorded in various countries due to both contaminations by seeds of PA-containing weeds growing in the crop as well as plant dust fragments from the same plants. The levels of PAs found in various grain commodities in Australia have ranged from <50 to >6000 µg/kg, but there has been no systematic analysis of the levels in grains entering the food supply (FSANZ, 2001c).

On the basis of the very limited data available, the major source of dietary exposure to PAs is grains; eggs, offal, honey and milk are minor dietary contributors.

Risk characterisation

While PAs can cause liver cancer in rats, there is no evidence from the significant human epidemics that have occurred that PAs cause liver cancer in humans.

While there is survey data to suggest that significant levels of PAs can be found in some foods, and particularly in grains, there is virtually no data on the levels of PAs in foods as consumed. While it appears that PAs are carried over into eggs from hens' diets, eggs are only minor dietary contributors to overall exposure. However further data would assist in further characterising the public health and safety risk.

3.2.4.2 Lupin alkaloids

The quinolozidine alkaloid, found in the *Lupinus* genus, is of major concern to human and animal health. The levels of alkaloids in seeds or meal can be reduced to approximately 500 mg/kg through a de-bittering process involving soaking or washing with water.

In Australia, lupin varieties with low alkaloid content ("sweet lupins") have been developed through plant-breeding programmes, and levels of alkaloids have been reduced to 130 – 150 mg/kg. Humans consume lupins in the form of seed flour and meal that can be used to prepare pastas, pastries and dairy product substitutes. Lupins are also used in traditional fermented foods such as tempe, miso and soy sauces in Indonesia and Japan (FSANZ, 2001a).

Several species of lupin are poisonous to livestock, producing death in sheep and "crooked calf disease" in cattle (Lopez-Ortiz *et al*., 2004).

An ML for lupin alkaloids in lupin flour, lupin kernel flour, lupin kernel meal and lupin hulls was included in Table to clause 5 in Standard 1.4.1 of the Code. The ML for lupin alkaloids in mixed foods was set at 200 mg/kg.

Hazard Identification and Characterisation

Humans appear to be the most sensitive species for alkaloid toxicity. Human poisonings due to lupin alkaloids indicate that the acute lethal dose is approximately 30 mg/kg bw, where the major alkaloid is sparteine. Traditional consumption of de-bittered lupins in Europe suggests a dose of 0.35 mg/kg bw per day is without chronic effect for adults. If a safety factor of 10 is applied to account for the uncertainties in the data and particularly to take into account likely human variation, the provisional tolerable daily intake (PTDI) for humans is 0.035 mg/kg bw per day (FSANZ, 2001a).

Exposure Assessment

Lupins have been shown to dramatically impair egg production and quality parameters when included in the feed at 25%, particularly in the absence of methionine supplementation (Hammershoj and Steenfeldt, 2005). Therefore it is undesirable to include significant amounts of lupins in hen feed, thus also limiting any potential for carry-over.

Human exposure to lupin alkaloids is considered to be largely from direct consumption of lupin meal and not from carry-over of the alkaloids in eggs; however there is currently no data available on the levels of lupin alkaloid in eggs.

Risk Characterisation

There is no data available on potential presence of lupin alkaloids in eggs and therefore the potential public health and safety risk cannot be characterised.

3.2.4.3 Aflatoxins

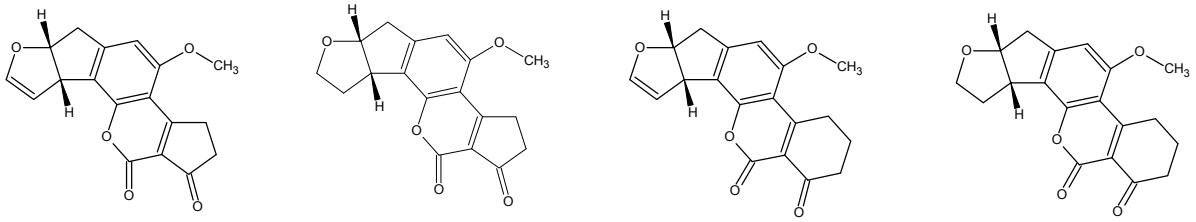
The safety of aflatoxins was last assessed by FSANZ[12] in Proposal 158 – Review of the maximum permitted concentration of non-metals in food (ANZFA, 1999a).

Aflatoxins are a group of naturally occurring toxic secondary metabolites produced primarily by two species of ubiquitous Aspergillus fungi: *A. parasiticus* and *A. flavus*. These fungi are present in soil and decaying plant material, cause heating and the decay of stored grain, and may invade corn in the field.

Crops and feed ingredients most susceptible to fungi and aflatoxins development include corn, peanuts, peanut meal, cottonseed and cottonseed meal. Conditions favouring aflatoxin development include drought stressed, insect-damaged feed stored at high temperatures (25°C – 32°C) and high relative humidity.

Among the naturally occurring aflatoxins (B_1, B_2, G_1 and G_2), aflatoxin B_1 is the most important compound with respect to both, prevalence and toxicity for humans and animals (EFSA, 2004a). Aflatoxin dietary intake in humans mainly arises from contamination of maize and groundnuts and their products (JECFA, 1998). The chemical structures of aflatoxins B_1, B_2, G_1 and G_2 are given in Figure 3.3.

[12] As ANZFA



Aflatoxin B_1 Aflatoxin B_2 Aflatoxin G_1 Aflatoxin G_2

Figure 3.3 Chemical structures of aflatoxins B_1, B_2, G_1, and G_2

Hazard identification and characterisation

Aflatoxins are amongst the most toxic of the known mycotoxins and have been implicated in the deaths of humans and animals that have consumed mouldy food. While the liver is the target organ for aflatoxicosis, aflatoxins are also found in other animal tissues and products, such as meat, milk and eggs.

The aflatoxins are among the most potent mutagenic and carcinogenic substances known. Extensive experimental evidence in test species shows that aflatoxins are capable of inducing liver cancer in most species studied (JECFA, 1998). However, assessment of the risk of liver cancer in humans has proved to be difficult because of confounding factors influencing tumour formation. Sensitivity to aflatoxins varies from one species to another, and, within the same species, the severity of toxicity depends on dose, duration of intake, age and breed of the animals and their dietary protein content.

The liver is the primary target organ in most species, but tumours of other organs also have been observed in animals treated with aflatoxins.

Aflatoxins are metabolised in humans and test species to an epoxide, which usually is considered to be the ultimate reactive intermediate. The effective dose of aflatoxins B_1 for induction of liver tumours varies widely over a wide range of species when the carcinogen was administered by continuous feeding, generally for the lifetime of the animal. Epidemiological studies indicate that individuals who are carriers of persistent viral infection with hepatitis B virus and who are exposed to aflatoxin in their diets are at increased risk for progression to liver cancer (JECFA, 1998). Some epidemiological evidence indicates the possibility that humans are at substantially lower risk from aflatoxins than other species. While some studies suggest that intake of aflatoxins poses a detectable risk in the absence of other factors, other studies suggest that it poses risks only in the presence of confounding factors such as hepatitis B infection (JECFA, 1998).

IARC has concluded that aflatoxins are carcinogenic to humans (Group 1) (IARC, 2002a).

JECFA has concluded that aflatoxins should be treated as carcinogenic food contaminants, the intake of which should be reduced to levels as low as reasonably achievable. However, JECFA did not believe that there was a firm foundation for setting absolute limits for aflatoxins intake by humans at this time.

Dietary exposure

Analysis of Australian and New Zealand commodities have indicated that problems associated with aflatoxins are almost entirely confined to peanuts and nut products (ANZFA, 1999a).

The 20th ATDS reports that there were no detections of aflatoxins (B1, B2, G1 and G2) in foods which may potentially contain these substances (i.e. breads, biscuits, rice, oats, processed wheat bran, breakfast cereals, instant coffee, peanut butter, almonds and milk chocolate) (FSANZ, 2002).

Although there is no data on aflatoxins residue levels in Australian eggs, internationally aflatoxin B1 and aflatoxicol have been detected in eggs. Under experimental conditions aflatoxins have been shown to be carried over from feed into the eggs, with the ratio of the feed level to the residue level varying but at a ratio of approximately 4000-5000:1(Bintvihok *et al*., 2002; Oliveira *et al*., 2000).

Queensland and New South Wales limit amount of aflatoxin B1 permitted in stockfeed for laying hens with a maximum level of 0.02 mg/kg (NSW, 2005; Qld, 1997).

Risk characterisation

Aflatoxins are regarded as human carcinogens, the intake of which should be reduced to levels as low as reasonably achievable.

It is possible that secondary exposure to aflatoxins may occur through consumption of egg products produced by layers fed aflatoxins-containing feed. Sourcing aflatoxin-free layer feed is therefore important. However, despite the lack of data on Australian eggs, aflatoxin is rarely a problem in Australian grains unless they have been stored improperly.

In conclusion, the potential for aflatoxins in eggs and egg products is low and so long as appropriate feed is sourced, there are no public health and safety concerns.

3.2.4.4 Phomopsins

The phomopsins are a family of mycotoxins produced by the fungus *Diaporthe toxica* (the teleomorph form of *Phomopsis leptostromiformis*). Lupins are the main host for the fungus, which is capable of infecting most parts of the plant.

Infection of the vegetative parts of the plant can result in high levels of phomopsin being present on the stubbles, which is the major source of animal exposure to phomopsin. Under certain storage conditions, infected lupin seed can also exhibit significant levels of phomopsin contamination. While the majority of lupin seed is used in animal feed, lupin products are also increasingly being introduced into food for human consumption.

Therefore, whole lupin seed and flour may be a source of human exposure to phomopsins, which have been shown to be stable to processing, including cooking (FSANZ, 2001b).

An ML for phomopsins in lupin seeds and the products of lupin seeds is included in Table to clause 3 of Standard 1.4.1. The ML for phomopsins was set at 0.005 mg/kg.

Hazard Identification and Characterisation

Overall phomopsins are potent cytotoxic agents which predominantly target the liver and which are clearly liver carcinogens in the rat. Phomopsins may be less toxic by the oral route than other routes, although they are still capable of causing severe disease, e.g., lupinosis in sheep. Also, some animal species appear more vulnerable than others to the toxic effects of phomopsins. The cytotoxic nature of phomopsins suggests that humans would also be vulnerable to its toxic effects; however, the available animal studies do not allow a determination of a safe level of dietary exposure to phomopsins.

Given these concerns, particularly with regard to the potential carcinogenicity of phomopsins, it would be prudent to ensure that human exposure be kept as low as is reasonably achievable. The paucity of toxicity data available does not make it possible at this time to identify a NOEL in animal studies or assign a tolerable level for human exposure (FSANZ, 2001b).

Dietary exposure

Levels of phomopsins in lupin seed (from Australia) vary from <6 to 360 µg/kg and levels as high as 4522 µg/kg in seed have also been detected. Laying hens may be given lupin seed as part of their diet; however it would be undesirable to give feed containing high levels of phomopsins as this may be detrimental to the birds' health.

There is no data available on the levels of phomopsins carried over to lupin flour. Therefore, it is not clear to what extent the milling process may remove phomopsin contamination. In addition, no data is available for other potential sources of exposure such as other lupin products, offal, milk or eggs. Therefore, there is insufficient survey information to enable a dietary exposure assessment to be carried out. However, sub-population groups most likely to have high exposure to phomopsins would be those consuming large amounts of lupin products (FSANZ, 2001b).

Risk Characterisation

Phomopsins have been shown in animal studies to be potent liver toxins and carcinogens in rats. Although no direct evidence of toxicity in humans is available, their mechanism of action is such that humans are likely to be susceptible to their toxic effects. Phomopsins appear to be less toxic by the oral route than by other routes but still capable of causing severe liver disease in sheep following ingestion. If affected, animals show signs of liver disease and may die within a few days. Although there is no data available on whether phomopsins are carried over into eggs, they are unlikely to be a risk to public health and safety as hens fed diets high in phomopsin-contaminated lupins would show signs of systemic toxicity, including reduced egg production.

3.2.4.5 Ergot

Ergot alkaloids (ergolines) are produced by the fungus *Claviceps purpurea* that infects the florets of grasses and cereals, forming sclerotia. All the common cereals can be infected with ergot, including rye, wheat, barley, triticale, oats, millet, sorghum and maize. The ergolines,

contained within the sclerotia, are derivatives of lysergic acid and fall into three groups, ergotamine, ergotaminine and clavines.

The ML for ergot is set at 500 mg/kg in cereal grains. Queensland and NSW regulate the maximum amount of ergot (*Claviceps* spp.) other than sorghum ergot (*Claviceps africana*) in stock feed at 200 mg per kg, and sorghum ergot in stock feed at 3000 mg per kg (NSW, 2005; Qld, 1997).

Hazard Identification and Characterisation

Ergotism is relatively uncommon in humans but it can affect livestock, producing the following effects: behavioural effects, convulsions, lack of coordination, lameness, and difficulty in breathing, excessive salivation, diarrhoea and dry gangrene of the extremities. Reproductive effects including abortion, high neonatal mortality, reduced lactation, reduced feed intake and weight gain. These are species-specific effects, which depend upon the ergot source, amount consumed, period of exposure and age and stage of production of the animal (EMAN, 2005).

Laying hens appear to be more tolerant to the effects of ergot than other stock animals such as ruminants, horses and swine, but egg production is affected at higher levels of ergot consumption (0.4 - 9%) (Dingle and Blaney, 2003).

Exposure assessment

All the common cereals including rye, wheat, barley, triticale, oats, millet, sorghum and maize can be infected with ergot, although rye is the most susceptible (EMAN 2005).

Sorghum, naturally contaminated with 4-6% ergot was fed to laying hens to determine if residues could be detected in eggs. Diets were estimated to contain 0, 5, 9, or 19 mg dihydroergosine/kg. No residues were detected in the eggs, however a slight difference in optical density between control and treated eggs, may indicate very low levels of ergot alkaloid in the treated eggs (<0.002 mg/kg) (Dingle and Blaney, 2003). Egg production is significantly affected at levels of ergot in the diet above 12 mg dihydroergosine/kg.

There are no literature reports of rye ergot residues in eggs. The limited evidence available does not indicate that ergot alkaloids accumulate in animal tissues, including milk and eggs, and therefore animal products are not expected to be a significant source of exposure (EFSA, 2005a).

Risk characterisation

Although ergot alkaloids have toxic effects in animals there is no evidence that there is carry-over of ergot into eggs and therefore there are no public health and safety concerns associated with ergot residues in eggs.

3.2.4.6 Ochratoxin A

Ochratoxins, of which ochratoxin A is the most prevalent, are secondary fungal metabolites of some toxigenic species of *Aspergillus* or *Penicillium*. Ochratoxin A consists of a

chlorinated dihydroisocoumarin moiety linked through a 7-carboxyl group by an amide bond to one molecule of L-β-phenylalanine (Bakker and Pieters, 2002).

Ochratoxin has been found in barley, wheat and many other commodities contaminated with *A. ochraceus* and *P. verrucosum* (FSANZ, 2005b). It is nephrotoxic and a possible human carcinogen (IARC, 1993; JECFA, 2001b).

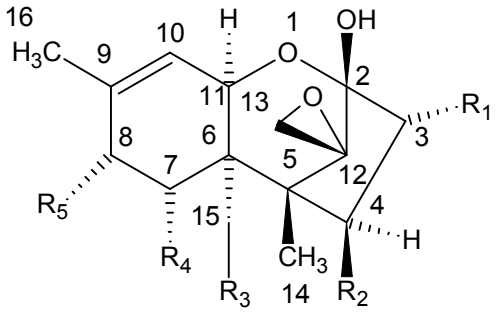
There are limited studies on the presence of ochratoxin A residues in non-ruminant food animals. Under experimental conditions, groups of hens were exposed to ochratoxin A in the diet for 1-2 years at levels of 0.3 and 1 mg/kg, although ochratoxin residues were found in the kidney, liver and muscle, no residues were detected in the eggs (IPCS, 1979; Krogh *et al*., 1976). In another study, ochratoxin was found in eggs when the birds were fed 10 mg/kg bw (JECFA, 2001b; Juszkiewicz *et al*., 1982). In laying Japanese quail, ochratoxin was found in the eggs of birds given 5 and 20 mg/kg bw, but not those given 1 mg/kg bw (JECFA, 2001b).

There is little evidence that Australian consumers are exposed to ochratoxin A in significant amounts, however monitoring and exposure data would be required to enable a definitive risk characterisation to be made. In conclusion, no public health and safety concerns have been identified in relation to exposure to ochratoxin A from eggs and egg products.

3.2.4.7 Trichothecene toxins

Trichothecene mycotoxins are produced by several field fungi, including *Fusarium graminearum* and *Fusarium culmorum*, and are common in cereals and grains, particularly in wheat, barley and maize. Co-occurrence with other *Fusarium* toxins, including zearalenone as well as well as the group of fumonisins, is regularly observed. Trichothecene mycotoxins include type A (T-2 and HT-2 toxin) and type B toxins (deoxynivalenol and nivalenol). The chemical structures of the trichothecene mycotoxins T-2, HT-2, deoxynivalenol (DON) and nivalenol (NIV) are given in Figure 3.4.

Among the naturally occurring trichothecenes in foods, T-2 toxin is the most potent, followed by NIV. DON, also known as vomitoxin, was the least toxic in acute toxicity studies. In experimental animals, T-2 toxin produces acute systematic effects, with necrosis of epithelial tissues and suppression of haematopoiesis. In contemporary outbreaks of disease, only gastrointestinal symptoms have been reported (IPCS, 1990). Many outbreaks of acute human disease involving nausea, vomiting, gastro-intestinal upset, dizziness, diarrhoea and headache have been attributed to DON in Asia (IPCS, 2001).



Trichothecene	R1	R2	R3	R4	R5
T-2 Toxin	-OH	$-OCOCH_3$	$-OCOCH_3$	-H	$-OCOCH_2CH(CH_3)_2$
HT-2 Toxin	-OH	-OH	$-OCOCH_3$	-H	$-OCOCH_2CH(CH_3)_2$
Nivalenol (NIV)	-OH	-OH	-OH	-OH	=O
Deoxynivalenol (DON)	-OH	-H	-OH	-OH	=O

Figure 3.4 Trichothecene toxins, T-2, HT-2, DON and NIV

Hazard identification and characterisation

Reported cases of human disease associated with trichothecene exposure are limited in number and information. Symptoms of digestive disorders and throat irritation develop rapidly after ingestion of food contaminated with trichothecenes. The symptoms described include abdominal pain or a feeling of fullness in the abdomen, dizziness, headache, throat irritation, nausea, vomiting, diarrhoea, and blood in the stool (SCF, 1999). At present, there is no evidence of human cancer cause by trichothecenes (IPCS, 1990).

T-2 toxin, HT-2 toxin, DON and NIV cause similar toxic effects, and appear to cause similar effects at the biochemical and cellular level. However, substantial differences in the spectrum of toxic effects *in vitro* have been observed. Large, non-systematic potency differences between these toxins were seen when different endpoints are considered. There are very few studies addressing the combined effects of these toxins. Moreover, in most of these case studies naturally contaminated feed was used which makes the attribution of a potential effect to a single toxin very difficult (SCF, 1999).

The EU Scientific Committee on Food (SCF) has assigned temporary daily intakes (TDIs) to DON, NIV, T-2 toxin and HT-2 toxin pending among other things, a group evaluation. The TDIs for NIV and T-2 toxin were also made temporary because of gaps in the database. Therefore the Committee established a full TDI for DON (TDI = 1 μg/kg bw per day) only and confirmed the t-TDI for nivalenol (t-TDI = 0.7 μg/kg bw per day) and the combined t-TDI for T-2 toxin and HT-2 toxin (t-TDI = 0.06 μg/kg bw per day) (SCF, 2002).

Exposure assessment

There is no data available regarding trichothecene residues in Australian eggs and egg products. However, evidence indicates that the transmission of DON to eggs is limited (Prelusky *et al*., 1989). Residues of DON (detection limit 10 ng/g), were not found in the

eggs from laying hens fed 5 ppm for 190 days, 18 ppm for 28 days, and 83 ppm for 27 days (El-Banna *et al*., 1983; Kubena *et al*., 1987; Lun *et al*., 1986). Sypecka *et al* (2004) reported transmission of DON from naturally contaminated grain incorporated into chicken feed into eggs occurred only at levels corresponding to 0.002-0.004% (Sypecka *et al*., 2004). Valenta and Dänicke (2005) found no, or only trace amounts of DON and its metabolite de-epoxy-DON in eggs from hens fed DON at a concentration of 11.9 mg/kg in a maize-based diet for 16 weeks (Valenta and Dänicke, 2005). NIV was not detected in the yolk or albumin of eggs from hens fed a diet containing 5mg NIV /kg. Less than 1% of the administered dose of T2 is transferred to the eggs of laying hens (IPCS, 1990).

Risk characterisation

There is no data on the exposure of the Australian population to trichothecene toxins. However, it is thought that human exposure to DON is predominately through cereals and grains, and that animal products, including eggs, do not contribute significantly to this exposure (EFSA, 2004b). In conclusion, secondary exposure to trichothecene toxins through consumption of eggs and egg products derived from layers fed trichothecene-containing feed, presents a negligible risk to the consumer.

3.2.4.8 Zearalenone

Zearalenone is a non-steroidal estrogenic plant growth regulator produced by some plants and also produced as a mycotoxin by several field fungi including *Fusarium graminearum* (*Gibberella zeae*), *F. culmorum*, *F. cerealis*, *F. equiseti* and *F. semitectum* (SCF, 2000). The main metabolites of zearalenone are α- and β-zearalenol and the glucuronide conjugates of both the parent compound and its metabolites (JECFA, 2000). α-zearalenol (zearanol) is also used as a hormonal growth promotant in livestock and has been previously assessed by JECFA as a veterinary medicine (JECFA, 1988).

Zearanol residues can be differentiated by the presence or absence of zearalenone metabolites. If zearanol occurs with other zearalenone metabolites it is more than likely due to the ingestion of zearalenone from pasture, grain, or plant material infected by *Fusarium* spp. containing zearalenone by the cattle. In the absence of other zearalenone metabolites, zearanol is suggestive of the administration of a hormonal growth promotant containing zearanol.

The chemical structures of the zearalenone and α- and β- zearalenol are given in Figure 3.5.

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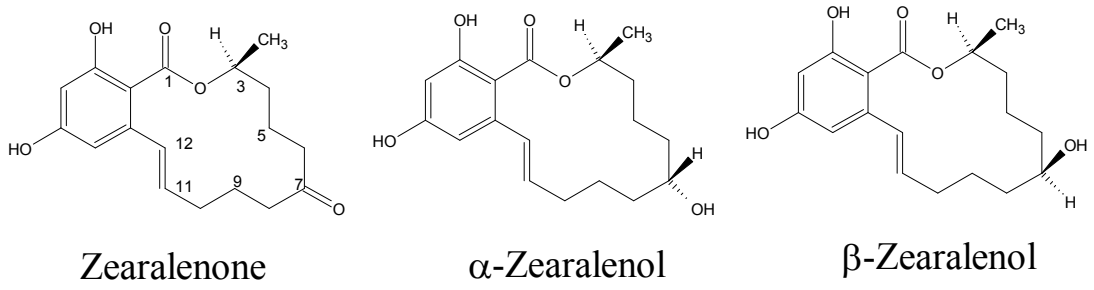


Zearalenone α-Zearalenol β-Zearalenol

Figure 3.5 Chemical structures of zearalenone and primary metabolites

Hazard identification and characterisation

Zearalenone causes alterations in the reproductive tract of laboratory animals and domestic animals. Various estrogenic effects, including decreased fertility, increased embryo-lethal resorptions, reduced litter size, changed weight of adrenal, thyroid and pituitary glands and change in serum levels of progesterone and estradiol, have been observed but no teratogenic effects were found in mice, rats, guinea pigs and rabbits (JECFA, 2000; Kuiper-Goodman *et al*., 1987).

JECFA concluded that the safety of zearalenone could be evaluated on the basis of the dose that had no hormonal effects in pigs, the most sensitive species. JECFA established a provisional maximum tolerable daily intake (PMTDI) for zearalenone of 0.5 μg/kg bw. This decision was based on the NOEL of 40 μg/kg bw/day obtained in a 15-day study in pigs (JECFA, 2000). The Committee also took into account the lowest observed effect level of 200 μg/kg bw/day in this pig study and the previously established ADI of 0-0.5 μg/kg bw for the metabolite α-zearalenol, evaluate as a veterinary drug (JECFA, 1988). The Committee recommended that the total intake of zearalenone and its metabolite (including α-zearalenol) should not exceed this value (JECFA, 2000).

Dietary exposure

There are no reports of residues of zearalenone in eggs and egg products in Australia, although it has been speculated that due to its lipophilic nature, prolonged exposure of layers to this toxin could lead to an accumulation of the toxin in the egg yolk (Sypecka *et al*., 2004). No zearalenone or its metabolites could be detected in the eggs of hens fed 0.5 mg zearalenone/kg feed (Sypecka *et al*., 2004), nor in eggs from hens fed 1.1 mg zearalenone/ kg feed (Dänicke *et al*., 2002).

Estimated average dietary intakes of zearalenone based on individual diet records have been presented by FAO, indicating an exposure of 0.03 to 0.06 μg/kg bw/day, thus remaining below the PMTDI of 0.5 μg/kg bw/day set by JECFA.

Data from the EU Scientific Cooperation (EU SCOOP) taskforce showed that the mean intake of zearalenone, estimated from various European countries, might range from 1 to 420 ng/kg bw/day. Bread and other cereal products were the most prominent sources of exposure (EFSA, 2004d).

Thus although only few analyses have been performed on residues of zearalenone in animal derived products, the available information indicates that due to rapid metabolism and excretion of zearalenone, the contribution of products from animal origin, including eggs, to dietary exposure of zearalenone is very limited (EFSA, 2004d).

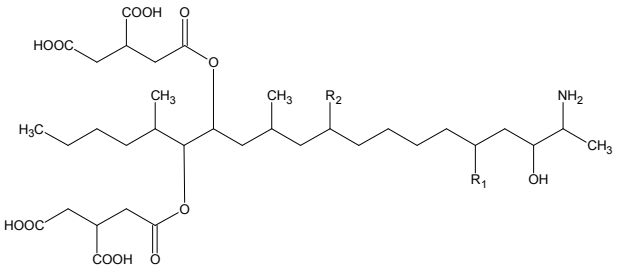
Risk characterisation

Estimated average dietary exposure internationally is below the PMTDI of 0.5 μg/kg bw/day. A small number of studies suggest that zearalenone is not transferred into eggs from layers to any significant degree. Secondary exposure to zearalenone through consumption of eggs and egg products derived from layers fed zearalenone-containing feed is very low compared to direct exposure via cereal and grain products.

In conclusion, there are no public health and safety concerns in relation to levels of dietary exposure to zearalenone from eggs and egg products.

3.2.4.9 Fumonisin

Fumonisins are mycotoxins produced by fungi of the genus *Fusarium* that commonly contaminate maize. Fumonisin B_1 contamination of maize has been reported worldwide at mg/kg levels. Fumonisin B_1 is the diester of propane-1,2,3-tricarboxylic acid and 2*S*-amino-12*S*, 16*R*-diemthyl-3*S*, 5*R*, 10*R*, 14*S*, 15*R*-pentahydroxyeicosane in which the C-14 and C-15 hydroxy groups are esterified with terminal carboxyl group of propane-1,2,3-tricarboxylic acid (JECFA, 2001a). The chemical structures of fumonisin B_1 and closely related chemical substances fumonisin B_2, fumonisin B_3, and fumonisin B_4 are given in Figure 3.6.



Fumonisin	R_1	R_2
B_1	-OH	-OH
B_2	-H	-OH
B_3	-OH	-H
B_4	-H	-H

Figure 3.6 Chemical structures of fumonisins

Hazard Identification and Characterisation

In all species studied, fumonisins are poorly absorbed from the digestive tract and are rapidly distributed and eliminated. The liver and kidney retain most of the absorbed material, and fumonisin B_1 persists longer in rat liver and kidney than in plasma.

In all animal species studied, the liver was a target for fumonisin B1; the kidney was also a target in many species. In kidney, the early effects are often increases in sphingoid bases, renal tubule-cell apoptosis, and cell regeneration. In liver, apoptotic and oncotic necrosis, oval-cell proliferation, bile-duct hyperplasia, and regeneration are early signs of toxicity (JECFA, 2001a).

A specific role for fumonisins in the development of neural tube defects has been proposed. The hypothesis includes a critical role of fumonisins in disruptions of folate membrane transport, but no specific studies have been designed to confirm this mechanism (JECFA, 2001a).

The IARC has classified fumonisin B_1 into group 2B (possibly carcinogenic to humans – sufficient evidence in animals, and inadequate data in humans) (IARC, 2002b).

Nephrotoxicity, which was observed in several strains of rats, was the most sensitive toxic effect of pure fumonisin B_1. Since the available studies clearly indicate that long-term renal toxicity is a prerequisite for renal carcinogenesis, the potential for the latter is subsumed by the dose-response relationship for renal toxicity. Therefore, the pivotal studies that could

serve as the basis for a tolerable intake of fumonisin B_1 were the short-term and long-term studies of toxicity in rodents. On the basis of these studies, the overall NOEL for renal toxicity was 0.2 mg/kg bw/day (JECFA, 2001a).

JECFA allocated a group provisional maximum tolerable daily intake (PMTDI) for fumonisins B_1, B_2, and B_3, alone or in combination, of 2 μg/kg bw/day on the basis of the NOEL of 0.2 mg/kg bw/day in rats and a safety factor of 100 (JECFA, 2001a).

Dietary exposure

Only few analyses have been performed on carry-over residues of fumonisins in animal derived products. Although, fumonisin B_1 levels in animal feedstuff can be high, and reach maximum values of 330, 70, 38, 9 and 2 mg/kg in North America (USA), Europe (Italy), Latin America (Brazil), Africa (South Africa) and Asia (Thailand), respectively (IPCS, 2000), the available information indicates that fumonisins are poorly absorbed in the laying hen with accumulation of ^{14}C-labelled compounds in tissues estimated to be less than 1% of the dose (Vudathala *et al*., 1994). No residues were found in eggs laid in the 24 hours post dosing (Vudathala *et al*., 1994).

Maize is the only commodity that contains significant amount of fumonisins (IPCS, 2000). Estimated mean dietary intakes of fumonisin B_1 based on regional diets and published distributions of concentrations of fumonisin B_1 in maize, indicating a mean intake of fumonisin B_1 ranging from 0.2 μg/kg bw/day in European-type diet to 2.4 μg/kg bw/day in the African diet (JECFA, 2001a).

Even if fumonisin B1 were found in low concentrations in eggs and egg products, these foods should not contribute significantly to human dietary exposure.

Risk characterisation

Secondary exposure to fumonisin B_1 through consumption of eggs and egg products derived from layers fed fumonisin B_1-containing feed is would be very low or negligible and represents a negligible risk to the consumer as there appears to be little carry-over of the toxin into eggs.

In conclusion, there are no public health and safety concerns in relation to levels of dietary exposure to fumonisin B_1 from eggs and egg products.

3.2.4.10 Cyclopiazonic acid

Cyclopiazonic acid (CPA) is a toxic indole tetramic acid that is produced by a number of different fungi that infect different foodstuffs, for example, *Penicillium* species (e.g. *P. commune* and *P. camembertii*) and *Aspergillus flavus* and *A. versicolor*. As it can be formed by *A. flavus*, a species that is a major producer of aflatoxins, it has the potential to co-occur with these mycotoxins in a range of food commodities, including eggs (Dorner *et al*., 1994). It is also found in dairy products such as milk, cheese and butter.

Hazard Identification and Characterisation

CPA only appears to be toxic when present in high concentrations. It has been found to be a neurotoxin when injected intraperitoneally into rats and the LD50 in male rats was 2.3 mg/kg.

Oral administration produced no convulsions and LD50 values found in rats for administration by this route were 19 - 36 mg/kg and 63 mg/kg for males and females respectively (Morrissey *et al*., 1985). In addition, lesions in the liver, kidney, spleen and other organs were observed. The effects reported include decreased weight gain, diarrhoea, dehydration, depression hyperaesthesia, hypokinesis, convulsion and death. It is reported that some of its effects in the body are due to its interference with the uptake and release of Ca^{2+} so it could pose a particular risk to humans taking drugs such as calcium antagonists designed to carefully control calcium homeostasis (EMAN 2005).

CPA is mutagenic for *Salmonella typhimurium* TA98 and TA100 in the Ames assay and its ability to co-occur with aflatoxins and may enhance the overall toxic effect when this happens. There is a dearth of human exposure data and this precludes an assessment of possible health effects. However, 'Kodua' poisoning in India resulting from ingestion of contaminated millet seeds has been linked to this toxin.

An attempt to estimate an acceptable daily intake has been reported based on a no observed effect level (NOEL) of 1 mg/kg/day, which takes into account data for several animal species and species variation. This indicates that an appropriate acceptable daily intake (ADI) would be approximately 10 µg/kg bw per day, or 700 µg/day. In the context of human exposure, if the uppermost limit of CPA found in cheese is 4 µg/g and the average individual consumes 50 g of cheese daily, this allows an intake of 200 µg, less than one third of a traditionally established ADI (EMAN 2005).

CPA is highly toxic to laying hens; 5/5 and 4/5 hens fed CPA at levels of 10mg/kg bw and 5mg/kg bw respectively died in a nine day study. In eggs from hens fed 2.5mg/kg bw or 1.25 mg/kg bw, CPA accumulated almost exclusively in the egg whites within 24 hours of dosing. The highest level detected was 430 ng/g, however the concentration was usually in the range of 60-160 ng/g (Dorner *et al*., 1994).

Dietary exposure

There is no data for CPA levels in eggs and egg products in Australia, however under experimental conditions the highest residue detected in egg white was 430 ng/g (Dorner *et al*., 1994). CPA has been detected in cheese, milk, stockfeed (maize, millet, peanuts, pulses, hay) and mixed feeds in Europe at levels up to 10 mg/kg or higher. Some cheeses are surface ripened with the species *P. camembertii* that can produce CPA, so there is intensive scrutiny of the strains used to ensure that they are non-toxin producers (EMAN 2005).

FSANZ does not have any data on dietary exposure to CPA for the Australian population. However it is likely that other foods, such as cereals and dairy products, may potentially contribute more significantly to CPA intake than eggs and egg products.

Risk characterisation

The occurrence of CPA in eggs and egg products is potentially of concern due to its high toxicity to major organs and due to its interference with the uptake and release of Ca^{2+}; toxic effects have been shown in different animals and in humans ('Kodua' poisoning). In addition, CPA can be produced by a number of species of *Aspergillus* and *Penicillium*, which increases the potential for natural CPA contamination of stockfeed.

However, the incidence of CPA in food is likely to be very low, as it occurs in the same products susceptible to aflatoxin contamination, and is therefore indirectly controlled by regulations in place for the aflatoxins. It is also likely that eggs and egg products would not be significant contributors to CPA intake.

High levels of CPA in poultry feed are detrimental to the birds' health and reduce production efficiency, so are undesirable to egg producers. More data would be required to complete a full risk characterisation of CPA.

3.3 Agricultural and veterinary chemicals

Residues of agricultural and veterinary chemicals may occur in eggs from a range of sources, including registered products (NRS, 2006). Routes of mass medication include medicated drinking water, feed additives, injection, and other methods such as eye drops, wing stabs, inhalants, fogging, dusts and sprays. Some chemicals that are no longer registered for use in Australia, e.g. some organochlorine (OC) pesticides and polychlorinated biphenyl (PCB), can remain in the soil for long periods of time, so unintended exposure of plants and animals to these compounds may still occur.

In Australia, the Australian Pesticide and Veterinary Medicines Authority (APVMA) is responsible for registering agricultural and veterinary chemical products, granting permits for use of chemical products and regulating the sale of agricultural and veterinary chemical products. Following the sale of these products, the use of the chemicals is then regulated by State and territory 'control of use' legislation. Before registering such a product, APVMA must be satisfied that the use of the product will not result in residues in food that would present an unacceptable public health and safety risk. As agricultural and veterinary chemicals are extensively regulated by the APVMA, they have not been considered in detail in this assessment.

Maximum residue limits (MRLs) for agricultural and veterinary chemicals in food are established in the Code. FSANZ evaluates the potential dietary exposure associated with the proposed MRLs and ensures that this exposure does not represent an unacceptable risk to public health and safety. MRLs are listed in Standard 1.4.2 – Maximum Residue Limits of the Code.

The inclusion of the MRLs in the Code allows produce treated according to Good Agricultural Practice (GAP) to be legally sold, provided that the residues in the treated produce do not exceed the MRL. Changes to Australian MRLs reflect the changing patterns of agricultural and veterinary chemicals available to farmers. These changes include both the development of new products and crop uses, and the withdrawal of older products following review.

Standard 1.4.2 lists the maximum permissible limits for agricultural and veterinary chemical residues present in food. Schedule 1 lists all of the agricultural and veterinary chemical limits in specific foods and Schedule 2 lists all extraneous agricultural chemical limits in specific foods. If a maximum residue limit for an agricultural or veterinary chemical in a food is not listed in the schedules there must be no detectable residues of that agricultural or veterinary chemical in that food. Also, if an agricultural or veterinary chemical is not listed in the schedules, there must be no detectable residue of that chemical or its metabolites in any food.

When an agricultural or veterinary chemical is registered for use or a permit for use granted, the APVMA includes MRLs in the APVMA MRL Standard. These MRLs are then adopted into control of use legislation in some jurisdictions and assist States and Territories in regulating the use of agricultural and veterinary chemicals.

State government agencies have responsibility for administering controls regarding the use of agricultural and veterinary chemicals, from the point of retail sale. These agencies are mostly contained in departments of agriculture, although in some jurisdictions, some responsibilities are performed by health departments (WA) or the Environmental Protection Agency (NSW). Regulation of agricultural and veterinary chemicals by States includes:

- promoting best practice and developing codes of practice for chemical use;
- licensing pest control operators and aerial spraying operators;
- establishing and administering rules and regulations in relation to chemical use, e.g. prohibited uses, allowed on- and off-label uses (includes how veterinarians can use veterinary chemicals), and control of off-target movement, e.g. spray drift; and
- audit, compliance and enforcement activities.

As of January 2007, Standard 1.4.2 had MRLs for 163 chemicals in Schedule 1 – Maximum Residue Limits and seven chemicals listed in Schedule 2 – Extraneous Residue Limits in association with eggs (Appendix 7). The list includes veterinary medicines used for prophylaxis and growth promotion, and agricultural chemicals used as crop and grain protection agents.

Clearly not all of these products are used widely in the poultry industry. Products gain and lose favour and in respect to pesticides and veterinary drugs, registrants seek to maintain registration to fill niche markets or for other commercial reasons though use may at times be limited. In some cases, registrants may choose to maintain a product registration but not offer the product for sale.

Current analytical technology can detect chemicals at very low concentrations. The detection of a residue is not a matter for concern except when the use of the relevant chemical is unauthorised or its concentration is greater than the MRL set on the basis of Good Agricultural Practice.

The two main sources of data on agricultural and veterinary chemicals in eggs are the National Residue Survey (NRS, conducted annually) and the Australian Total Diet Survey (ATDS, also conducted annually). The NRS was established under the *National Residue Survey Administration Act 1992* for the purposes of monitoring and reporting levels of contaminants in food, inputs to production and or the environment.

The ATDS is part of FSANZ system for monitoring the food supply to ensure that existing food regulatory measures provide adequate protection to consumer health and safety. The ATDS includes testing eggs for residues of agricultural chemical and veterinary medicines.

Contemporary survey results from the NRS[13] and ATDS indicate that there is a high level of industry compliance associated with agricultural and veterinary chemical MRLs in eggs. This is supported by the limited analyses conducted by individual egg producers on an *ad hoc* basis. These results indicate that dietary exposure to agricultural and veterinary chemicals through eggs and egg products presents a negligible risk to the consumer, although non-compliance with existing regulations may lead to undesirable residues in eggs.

3.4 Feed additives

Feed additives are any substance added to the basic feed mix for continuous long-term administration for specific purposes. For example, enhancing production, or maintenance of health above the levels obtained from the basis feed, improvement of storage qualities and/or palatability of the basis feed mix (APVMA, 2007a). Enzymes, e.g. 6-phytase, endo-1,3(4)-β-glucanase, endo-1,4- β-xylanase, α-amylase, and cellulose may be used in layers diets to increase feed efficiency. These are naturally occurring proteins that are derived from fungi and bacteria (e.g. *Penicillium* and *Aspergillus*). Phytases improve the digestibility of phytate phosphorus in plant sources and reduce the need for inorganic phosphorus supplementation, also reducing the likelihood of excess phosphorus excretion into the environment (King and Cutler, 2007a). Other enzyme preparations that improve the digestibility of carbohydrates in grains may also be used in laying hens.

Some producers may include pigments in the diets of laying hens in order to improve yolk colour. Diet plays an important role in determining yolk colour, as the carotenoids responsible for the yellow/red colour of the yolk are not synthesized in animals and must be obtained from plant foods or feed additives. The natural plant carotenoid pigments known as xanthophylls, and include lutein and zeaxanthin, provide the natural yellow pigments in egg yolk (EFSA, 2005b). Duck eggs may have a deeper orange colour due to the presence of beta-carotene and canthaxanthin, which in wild ducks can be obtained from water insects and small crustaceans (McGee, 2004).

Yolk colour is measured on a scale from one (pale yellow) to 15 (reddish orange) and reflects the different combinations of yellow and red carotenoids in the diet. Laying hens may obtain lutein and zeaxanthin from alfalfa and corn feeds; wheat based diets tend to produce a paler yolk than corn and alfalfa diets, and producers may supplement feed with marigold petals or other additives to produce the desired colour (McGee, 2004).

[13] In the 2004-2005 and 2005-2006 NRSs, 75 egg samples were subject to analyses for antibiotics and anticoccidials each year. In the 2004-2005 survey, two samples showed anticoccidial residue levels above the Australian Standard, one for lasalocid (as a result of a possible mix-up with feed) and one for nicarbazin (reason unknown) (NRS, 2005). In the 2005-2006 survey, nicarbazin, an anticoccidial for which there is no MRL was detected in two egg samples. This residue was traced back to a mix up with feed. Nicarbazin is a commonly used veterinary medicine in poultry (e.g. used in chicks and in broiler chickens). Accidental overuse in broiler feed in Queensland recently lead to the deaths of around 50,000 birds (The Australian, 2006). All other samples complied with Australian standards. In the 2005-2006 survey, lasalocid, at levels below the MRL, was detected in two samples (NRS 2006)

A number of natural and synthetic pigments are permitted for use as feed additives in the European Union, including capsanthin (from paprika oleoresin), β-apo-8'-carotenal, ethyl ester of beta-apo-8'-carotenoic acid, lutein, cryptoxanthin (naturally abundant in fruit, and feed ingredients such as corn and alfalfa), zeaxanthin, and citranaxanthin (naturally occurring in citrus fruit peels) (EFSA, 2006a). In Australia these pigment feed additives are not explicitly regulated, as they do not meet the definition of a veterinary medicine, however they are not considered to be of concern in term of impact on public health and safety as they are generally used to replace the naturally occurring pigments that hens might acquire from a diet high in plant products containing these compounds. Other common foods also contain these carotenoids, and a number of them are food colours permitted to be used at GMP in a range of foods in the Code (additive numbers 160e, 160f, 161b and 161c).

In November 2006, duck eggs containing the dye Sudan IV were detected in China and withdrawn from sale. It was reported that the ducks had been given the dye in their feed to produce redder yolks. Levels up to 0.137 mg/kg of eggs were detected (Patton, 2006). There is questionable evidence that Sudan I dye may be associated with cancer formation in laboratory animals, but there is no evidence that they can cause harm in humans, however sudan dyes are not permitted in food in Australia.

Other additives that may be used in stockfeed, including diets formulated for laying hens, include mould inhibitors, dietary acids, binders and metabolic modifiers (King and Cutler, 2007b). These are used in the diets only at very low levels, and there is no evidence as to whether or not residues of these might be found in eggs and egg products. Due to the low concentrations of these products in the feed, and the fact that they are tolerated by the hens, it is likely that they are of low public health and safety concern. Yeasts, plant extracts and natural earth products, may also be used as feed additives (Peter Scott, personal communication). For example, bentonite may be used as a filler to lower the nutrient density of the feed and limestone may be used as a source of calcium. Generally specifications for this material will be complied with, however it is possible that variant material could occasionally be used which may have heavy metal or other contamination.

The use of digestive enzymes, carotenoid pigments and other feed additives at low levels in layer feed is not considered by FSANZ to pose a risk to public health and safety from the consumption of eggs.

3.5 Food additives

In Australia, food additives are regulated under Standard 1.3.1 – Food Additives, of the Code. Premarket approval, including a risk assessment and evaluation of technological need, is required before food additives can be used; unless expressly permitted in this standard, food additives must not be added to food.

Food additives are used in the food supply for a number of reasons. For example, they can be used to ensure a safe product which has good keeping qualities and stability. They may also be used to enhance and improve the taste and appearance of the product. Food additives are used in relatively small quantities and may only be used in the lowest levels to achieve the desired technological function.

A food additive is approved for use by FSANZ only if it can be demonstrated that no harmful effects are expected to result from the requested use. Extensive testing of food additives is required, and FSANZ evaluates this data to determine if the food additive is safe. In addition, a dietary exposure assessment is undertaken, with the total dietary exposure compared to the ADI to determine if the levels of exposure will be without adverse effect over a lifetime.

Maximum use levels for food additives permitted in particular foods are listed in Schedule 1 of Standard 1.3.1, based on technological need and to ensure exposure remains within safe limits. There are no permissions for the use of food additives in whole eggs, however some additives are permitted in liquid egg products, frozen egg products and dried and/or heat coagulated egg products. Additives permitted in these egg products are listed in Schedule 2 – Miscellaneous additives permitted to GMP in processed foods specified in Schedule 1, of Standard 1.3.1. In addition, nisin (INS 234) is permitted at GMP in liquid egg products, and triethyl citrate (INS 1505) is permitted in liquid egg white with a maximum level of 1250 mg/kg. Colours listed in Schedules 3 and 4 of Standard 1.3.1 are not permitted in egg products.

If used, food additives are required to be listed in the ingredients list.

Given the existing regulation of food additives, FSANZ does not consider the use of currently permitted food additives in egg products poses a risk to public health and safety.

3.6 Processing aids

Like food additives, premarket approval, including a risk assessment, is required for food processing aids. Permitted processing aids and maximum use levels in egg products are detailed in Standard 1.3.3 – Processing Aids of the Code. The use of processing aids not specified in the Code is not permitted.

Processing aids listed in the Table to clause 3 of Standard 1.3.3 are generally permitted processing aids, all of which could be used as processing aids in egg products. Also, the food additives listed in Schedule 2 of Standard 1.3.1 can be generally permitted processing aids.

The use of permitted processing aids is not considered to pose a rise to public health and safety.

3.6.1 Sanitising agents

Egg washing can be conducted to reduce the risk of microbiological contamination of the contents of intact eggs. See Section 2.3.3.1 of the microbiological risk assessment, for a discussion of appropriate washing techniques.

Eggs may be washed on farm or at a central collection/processing location. Permitted washing agents and corresponding maximum permitted levels are listed in the Table to clause 12 of Standard 1.3.3 of the Code. Antifoam agents may also be used in washing water. Permitted antifoam agents (processing aids) and corresponding maximum permitted levels are specified in the Table to clause 4 of Standard 1.3.3

Chlorine- and iodine-based sanitisers may produce disinfection by-products (e.g. trihalomethanes) which are potentially carcinogenic; therefore it was considered whether the use of washing agents would affect the safety of eggs. The risk posed by the potential formation of disinfection by-products must be balanced against the benefit derived from the disinfection process. In the case of the surface sanitisation of eggs, disinfection is useful for reducing the level of pathogens on the surface of the food, therefore there is potential for substantial benefit to be obtained.

When halogens are used for disinfection, the most common by-products formed are trihalomethanes, although a number of other halogenated compounds may also be produced. In the case of water disinfection, by-products are usually formed as a result of the reaction between the disinfectant and naturally occurring organic substances present in water. These organic substances result from the decay of vegetable and animal matter and are present to varying levels in most water supplies. Removal of these organic chemicals from the water prior to its disinfection limits the potential for the formation of disinfection by-products. Most municipal water treatment plants routinely remove these organic substances from the water supply prior to disinfection, which is usually the final step in water treatment.

In relation to the use of chlorine as a disinfecting agent, it has been recognised that active chlorine can react both with the organic matter in water as well as food itself (CCFAC, 2002). The same types of by-products found following water disinfection may also be found following the surface disinfection of fruits and vegetables, with the most frequently encountered product being the trihalomethanes, although a number of other chlorinated compounds may also be produced. It is reasonable to suggest that these are also found following disinfection of eggs.

The types of by-product that will form are dependent on the characteristics of the organic constituent, the halogen species and the reaction conditions. Most of these organic chemical reactions have been investigated under conditions that involve molar concentrations of reactants that should favour product formation. This will not normally be the case in the disinfection situation where the organic reactants are expected to be at low concentrations.

Overall, FSANZ considers the risk posed by disinfection by-products to be low. Given the short period of time for which the egg is in contact with the sanitising solution, and that it is normal for a rinsing step to be included, it is unclear if disinfection by-products will be present at all on the surface of the egg shell.

Any increase in levels of iodine or chlorine within eggs following washing will be dependent on the temperature of the wash water and the duration of contact with wash water. The increase in iodine residues in raw liquid egg following a 30 mg/kg iodine wash was 0.074 mg/kg (FSANZ, 2005a). No data is available on the potential level of chlorine; however it is no more likely to enter eggs than is iodine. These very low levels of transfer do not raise any public health and safety issues.

A Joint FAO/WHO project to assess the benefits and risk of the use of ‘active chlorine’ in food production and food processing is currently underway (IPCS, 2007). FSANZ will have regard to the findings of this project.

3.6.2 Preserved eggs

3.6.2.1 Salted eggs

Salted eggs are prepared by immersing fresh duck eggs in a salt solution (generally around 35% salt), or coating eggs individually with a paste of salt, water and clay or mud, and allowing them to pickle for approximately 30 days. These eggs are boiled before consumption.

A recent survey by the New South Wales Food Authority (NSW Food Authority) tested salted eggs from New South Wales for a range of trace metals and heavy metal contaminants. No heavy metals were detected at levels causing concern.

3.6.2.2 Alkali-cured eggs

Alkali-cured duck eggs are known as Pidan, century eggs or thousand-year-old eggs. Traditionally these eggs were preserved in an alkaline clay paste/mud made of salt, wood ash, lime (calcium oxide) and coated in rice husks for several months. A similar result is produced with a modern recipe where eggs are cured in an alkaline solution for between 1-6 months. The solution is made up of salt and an alkaline material such as wood ash, lime, sodium carbonate, sodium hydroxide (lye), calcium bicarbonate or a combination of these ingredients. Tea leaves (oolong or black tea) and/or food colouring may also be added. Some recipes suggest the addition of lead oxide to speed the curing process or stabilize set egg white; however this leads to lead residues in the final food, which is a potential public health and safety concern. There is no permission in the Code for lead as a processing aid. A recent survey conducted by the NSW Food Authority found lead levels of up to 12.07 mg/kg in century eggs and mud coated century eggs (NSW Food Authority, 2007). A recent (June 2006) report from Taiwan indicated that out of twenty alkalised eggs tested for lead, four had high levels of lead and failed to meet the Council of Agriculture Certified Agricultural Standard. The exact lead levels were not reported.

FSANZ has no data on consumption patterns of alkali cured eggs by Australian consumers and so exposure to contaminants from this source cannot be estimated. However, the use of unsafe and non-permitted processing aids such as lead oxide is a potential public health and safety concern.

3.7 Discussion

In relation to chemical hazards in eggs and egg products, there are already extensive regulatory and non-regulatory measures in place to ensure that chemicals used or present in eggs and egg products present a very low public health and safety risk. The chemical risk assessment has identified and examined chemicals introduced along the egg primary production and processing chain, from the farm environment through to eggs and egg products for retail sale.

The major potential source of contamination is through exposure of the laying birds to chemicals in their feed and water, for example natural plant toxins, mycotoxins, and agricultural and veterinary chemicals. Chemical inputs may also occur from the environment in which the layers are housed. The regulations in place which control the chemicals to which laying birds are exposed include State and Territory stock feed legislation and the APVMA's

legislation around the use of agricultural chemicals and veterinary medicines in birds producing eggs for human consumption. Although the available data on chemical residues in eggs does not indicate a concern, in order to maintain this situation continuing regard should be given to the use of stock feed of appropriate quality, housing of birds in non-contaminated environments and the appropriate use of agricultural and veterinary chemicals.

Once the egg has been laid, further chemical inputs include processing aids and food additives, which are regulated through pre-market assessment and listed in the Code. Aside from the unapproved use of lead as a processing aid in alkalised eggs, compliance with the Code appears to be good.

In summary, monitoring of chemical residues in eggs over recent years has demonstrated a high level of compliance with regulation, and it appears that the regulatory measures currently in place are achieving their aims. On the whole, undesirable chemical residues in eggs and eggs products are absent or low and of little public health and safety risk.

4 CONCLUSION

The purpose of this risk assessment was to determine the microbiological and chemical risks associated with consumption of eggs and egg products in Australia and identify where in the supply chain are the hazards associated with this risk are introduced and what factors impact on their levels.

It was clear from epidemiological data and the literature that *Salmonella* spp. is the primary microbiological pathogen of concern in relation to foodborne illness associated with eggs.

Consumption of well-cooked egg (or foods containing egg) presents little risk of salmonellosis. Results of the quantitative model, as well as epidemiological evidence, demonstrates that consumption of uncooked or lightly-cooked foods containing raw egg represents a potential for foodborne illness. A common risk factor identified in outbreaks was the use of eggs with visible surface faecal contamination. Contributing factors included cross-contamination during food preparation and/or temperature abuse of the food containing raw egg.

Numerous factors during primary production have the potential to introduce *Salmonella* into a laying flock including feed, water, pests, laying environment, personnel, new laying stock and equipment. Due to the multi-factorial nature of transmission of *Salmonella* spp. into laying flocks, and a lack of quantitative data, identification of those factors have the greatest impact on flock contamination was not possible.

Factors that impact on the likelihood of horizontal transmission of *Salmonella* spp. into the egg contents includes the presence and load of external contamination *(e.g.* faecal material), temperature differential between the egg and the environment, humidity, and condition of the shell (*e.g.* cracks), cuticle and membranes. Practices during the production and processing of eggs and egg products that impact on these factors will affect the likelihood of transmission of *Salmonella*.

The output of the quantitative model included an estimation of the number of cases of illnesses per million serves for eggs stored at various temperatures at retail and consumed uncooked, lightly cooked or well cooked. The following is a summary of the key outputs from the quantitative model:

- The model confirmed that the consumption of well-cooked eggs presented little risk of illness as the cooking step is high enough to inactivate *Salmonella* (>12-$\log_{10}$ reduction).
- The length of time until there is potential for rapid growth of *Salmonella* spp. in contaminated eggs is dependent on the temperature of the egg from point of lay through to consumption. It was predicted that for eggs produced and processed under median industry practices, growth of *Salmonella* could occur in contaminated eggs after approximately 10 days retail storage at 22°C. For eggs stored at 16°C during retail, the estimated time before growth of *Salmonella* in contaminated eggs would be 18 days. No growth of *Salmonella* was predicted if eggs were stored at 4°C.
- The predicted risk of illness is dependent on the prevalence of *Salmonella* contaminated eggs. The prevalence of *Salmonella* contaminated eggs was described in the model by a distribution based on results from a pilot microbiological survey of

graded eggs in Australia (n=20,000) and data from large international surveys on the prevalence of non-SE contaminated eggs, with an overall mean prevalence of 0.004%.

- For eggs stored under conditions that would permit the growth of *Salmonella* (*i.e.* yolk mean time has expired) the estimated number of cases of salmonellosis was 36 per one million serves of uncooked egg. Even if eggs were stored under conditions that do not permit the growth of *Salmonella* spp., the risk of illness if consumed raw was estimated to be approximately 4 cases per one million serves.
- The quantitative model did not consider the potential for cross-contamination during food preparation or multiple serves of uncooked food containing raw egg such as raw egg-containing sauces, desserts etc. These practices would increase the predicted rate of salmonellosis cases.
- Raw egg pulp is often contaminated with *Salmonella* spp. and there is a potential for growth if stored at temperatures > 7°C.
- Current pasteurisation requirements for liquid whole egg resulted in a large predicted inactivation of *Salmonella* (>80-$\log_{10}$ reduction), with much less for liquid albumen and yolk (10.5-$\log_{10}$ and 4.1-$\log_{10}$ respectively).

Given the data available for the review of chemical hazards in eggs, the current regulatory measures outlined in the Code, in combination with relevant state and territory legislation and industry codes of practise adequately protect public health and safety with respect to chemical hazards in eggs and egg products in Australia. The following is a summary of the main conclusions from the chemical risk assessment for eggs and egg products.

- Although dioxins, PCBs and PBDEs have been detected in Australian eggs, an analysis of the consumption of eggs and egg products by the general population indicated that exposure to these contaminants in food is low. On the basis of the available data it can be concluded that the Australian public health risk arising from exposure to dioxins, PCBs and PBDE in food, including eggs, is low.
- Exposure of Australian consumers to heavy metal contaminants (*e.g.* cadmium, lead and mercury) through food is within safe levels and eggs are a minor contributor to this exposure. However it was identified that the routine consumption of eggs from a contaminated site may pose a risk to consumers, particularly in relation to children exposed to lead.
- There is a lack of data on both the total dietary exposure of Australian consumers to plant toxins, mycotoxins and bacterial toxins from all foods, and the presence or absence of these toxins in eggs and egg products. However the data, where available, indicates that exposure to these toxins by Australian consumers is generally low, and that eggs are a negligible source of exposure in most cases.
- Results from recent surveys of residues of agricultural and veterinary chemicals in eggs indicate that they are either absent or within safe levels and are unlikely to pose a risk to public health and safety.

- Some feed additives, such as the carotenoid pigments used to enhance yolk colour, appear to be unregulated, as they do not meet the definition of either a veterinary medicine or a food additive. However, this is not considered to pose a risk to public health and safety as these carotenoids are naturally found in eggs when laying hens are fed a diet containing particular plant foods (*e.g.* corn and lucerne). These same carotenoids are approved food colours in a range of foods in Australia and New Zealand. The use of sudan red dyes in duck feed to colour the eggs, which has been reported to occur overseas, would be of some concern, however the presence of sudan dyes in food in Australia is not permitted.
- The reported use of lead oxide as a processing aid or food additive in alkali-cured eggs is of concern. The use of lead compounds in food is not permitted in the Code, and enforcement action has been taken in New South Wales to eliminate this practice.
- The monitoring of chemical residues in eggs over recent years has demonstrated a high level of compliance with the regulations

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APPENDIX 1 Summary of commercial egg laying production systems

There are three main types of egg production systems currently used in Australia; cage (or intensive cage systems), barn and free range which are briefly described below.

Cage

Cage egg production systems represent apporximately 75% of all egg production in Australia[14]. Birds in these systems are continuously housed in cages within a shed. Depending on the size of the cage facility, cages may be arranged in multiply layers within the shed and house over 30,000 birds per shed. Cages are designed to allow eggs to roll clear from the birds for collection, either manually or more commonly via conveyer belts. Feed is supplied by automated delivery systems that run the length of the layer cages. Drinking water is provided via nipple drinkers with cups or drip channels.

Systems designed for the removal of faeces in cage facilities vary in sophistication, from simple drop-through methods which collect faeces below the cage, through to automated conveyer belts which sit below the cages and removes faeces to a collection point outside of the shed.

Modern cage production systems are often housed in fully enclosed, temperature-controlled sheds with tunnel ventilation. Alternatively, cages can be housed in open sheds which permit natural ventilation. In this type shed facility, “foggers” are sometimes used whereby a fine mist of water is sprayed into the shed to assist cooling during periods of high temperature.

Barn

In barn egg production systems, also known as deep litter systems, birds are housed in sheds and generally have access to roosting areas, nesting boxes and litter. A portion of the shed is raised and covered with plastic or wooden slats to form a manure pit. Feeders and drinkers are placed above the manure pit as this is the area where the majority of manure is deposited.

Birds have access to laying nests during the day. Eggs that are not laid in the nests (floor eggs) have a greater potential to be exposed to faecal material and contaminated litter. At night the nests are either closed off or raised to prevent soiling. In medium and large barn egg production systems, eggs are collected automatically daily via a conveyer belt. In some small-scale barn systems, eggs may be collected manually.

Free range

Free range egg production systems include both an enclosed shed (barn facility) for protection, roosting, feeding, drinking and laying and as well as access to a fenced outdoor area. Some free-range production systems use small, portable hutches that can be moved around the property.

[14] AECL (2008) *Industry overview 2007/08*. Australian Egg Corporation Limited. http://www.aecl.org.au/. Accessed on 10 August 2009.

APPENDIX 2 Import conditions for eggs and egg products[15]

Product	Specified treatment	Restrictions
Spray dried egg white	Spray dried and then hot boxed in its final packaging to a minimum core temperature of: 70°C for 7 days; or 62°C for 10 days	Processing plant must be approved by AQIS.
Spray dried whole egg and egg yolk powder	Heated to a minimum core temperature of not less than 70°C for 120 minutes	USA, Denmark, Belgium, Canada and New Zealand only. Processing plant must be approved by AQIS or: USDA, EU, Agriculture Canada or New Zealand Food Safety Authority.
Pasteurised egg products - whole egg, egg yolk and egg white products	Products were processed as follows: Liquid whole egg: 64°C for a min of 2.5 minutes Liquid egg yolk: 60°C for a min of 3.5 minutes or 60.5°C for a min of 3 minutes Egg white: 55°C for a min of 9.5 minutes	New Zealand only
Whole boiled eggs	Heat processed so that a minimum core temperature of 80°C was reached or the product was cooked in water where the water maintained a temperature of at least 97°C for at least 17 minutes.	New Zealand only
Canned/retorted egg products	During the canning/retorting process, the product was heated to a minimum core temperature of 100°C, obtaining an F_0 value of at least 2.8	
Egg pasta or noodles (up to 20% egg)	Cooked by a process sufficient to raise the core temperature of the noodles to at least one of the following temperatures: 87°C for 2 minutes 30 seconds; or 75°C for 15 minutes; or 60°C for 5 hours; or 60°C for 30 minutes followed by 54°C for 5 hours	
Egg waffles	Baked at 250°C for at least 140 seconds	
Mooncakes with egg content	Immersed in solution of 1 kg salt per 2 litres water for a period not less than 20 days; and Yolks removed from eggs and oven cooked at 180°C for a period of not less than 15 minutes; and Cooked yolks and other ingredients moulded to form the cakes which are to be baked in an oven at not less than 180°C for a period of not less than 30 minutes.	

[15] Import conditions can be found at www.aqis.gov.au/icon32/asp/ex_querycontent.asp.

APPENDIX 3 Avian Influenza Risk Assessment

HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1

AN ASSESSMENT OF RISK TO CONSUMERS AND HANDLERS OF POULTRY PRODUCTS

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Executive Summary

Ongoing outbreaks of highly pathogenic H5N1 avian influenza virus in poultry in Asia, Europe and Africa, have raised concern about the possibility of the virus spreading to Australia, and the subsequent implications for food safety.

As of the 12 October 2007, there have been 331 laboratory confirmed cases of human H5N1 avian influenza infection worldwide, resulting in 202 deaths (WHO, 2007) since the start of the current outbreak in 2003. HPAI H5N1 first appeared as a human pathogen during a major poultry outbreak in Hong Kong during 1997.

A risk assessment undertaken by Food Standards Australia New Zealand concluded that there have been no epidemiological data suggesting handling or consumption of cooked poultry meat or egg and egg products contaminated with highly pathogenic avian influenza (HPAI) H5N1 virus has lead to human illness. Although it is not always possible to identify the mechanism of human infection, almost all human cases occurred in people believed to have been involved in handling live or dead infected birds, or who have had close contact with infected birds and their excretions. A small number of human cases have been infected from family members, but human to human transmission of infection has not been sustained.

The HPAI H5N1 avian influenza virus has not been detected in Australia. In addition, poultry meat and egg products imported into Australia must be heat-treated, which would inactivate any avian influenza virus present. There is therefore a negligible risk of transmission of HPAI H5N1 virus to humans via the consumption of poultry meat or egg and egg products in Australia.

The rapid evolution of HPAI H5N1 viruses in poultry species, and the severity of illness in humans mean that the potential public health risk of HPAI H5N1 viruses in poultry products must be continually reviewed and updated.

Introduction and Scope of Risk Assessment

The recent spread of highly pathogenic avian influenza (HPAI) H5N1 in a number of Southeast Asian, European and African countries has resulted in renewed concern regarding the source and spread of avian influenza and the risk of transmission of HPAI viruses to humans from various exposures, including the possibility of foodborne exposure (Capua and Alexander, 2006).

Avian influenza is a viral disease that affects many species of birds including chickens, ducks, turkeys, pheasants, partridges, quail, pigeons, geese, guinea fowl, ostriches as well as water fowl and sea birds. Influenza viruses are of three types (A, B, and C), of which Influenza A is the most important for humans. It is a zoonotic infection, for which aquatic birds are the reservoir hosts. When the virus infects other birds it has the potential to evolve rapidly and in some cases increases in virulence. There are a number of subtypes and strains of avian influenza viruses, each having differing pathogenic potential. For instance, some avian influenza virus strains (known as low pathogenic avian influenza, LPAI) cause subclinical infection or mild clinical infection even in highly susceptible species such as chickens or turkeys, whereas other strains cause highly lethal disease in chickens and turkeys, with a high proportion of birds dying within a few days, or even hours, of infection (known as highly pathogenic avian influenza, HPAI). Influenza A viruses can also evolve to infect mammals, including humans, but the virus normally has to undergo substantial genetic change before it can transmit effectively between people, and become a human rather than an avian virus.

In 1996, a new strain of avian influenza virus (HPAI subtype H5N1) was found in a sick goose in southern China. In 1997, this virus was detected in Hong Kong, initially in poultry and later in people. Eighteen people were infected by the virus, and six subsequently died. For the first time, a true avian virus caused serious disease and infected several people resulting in a high mortality rate. Beginning in 2003, outbreaks of avian influenza virus subtype H5N1 in birds and humans occurred apparently simultaneously in a number of Southeast Asian countries. Since that time, HPAI H5N1 has been found in countries, across Asia, Europe and Africa. To date, there have been no reports of HPAI H5N1 avian influenza infection of either birds or humans in Australia, although outbreaks of poultry disease due to avian influenza viruses, other than subtype H5N1, have occurred in poultry during the past 30 years (Appendix 1).

The purpose of this risk assessment is to determine the extent of food safety risk associated with avian influenza virus subtype H5N1 resulting from the preparation and consumption of poultry products including poultry meat and egg products.

The assessment of food safety risk addresses the following areas:

- The risk to human health due to exposure to HPAI H5N1 viruses associated with the consumption of poultry meat.
- The risk to human health from exposure to HPAI H5N1 viruses associated with the consumption of eggs/egg products.
- The risk to food handlers and consumers associated with handling and preparing poultry meat and eggs infected by HPAI H5N1 viruses.

While some of the discussion in this assessment considers highly pathogenic avian influenza viruses in general, the focus of this assessment is on HPAI H5N1 whenever possible. The information in this risk assessment will be used to inform the development of appropriate measures or strategies to manage any food safety risk associated with HPAI H5N1 viruses, if, when and where appropriate.

Hazard Identification

The first recorded incident of the highly pathogenic form of avian influenza known as 'fowl plague' occurred in Italy around 1878 (Swayne and Halvorson, 2003). The causative agent was eventually isolated in 1902, making it the first documented isolation of an influenza virus (HPAI subtype H7N7). Non-pathogenic and mildly pathogenic influenza viruses occur worldwide.

Influenza viruses belong to the *Orthymyxoviridae* family of RNA viruses. There are five genera in the *Orthomyxovirdae* family: *Influenzavirus A*, *Influenzavirus B*, *Influenzavirus C*, *Thogotovirus* and *Isavirus.* Only influenza A viruses are known to infect birds, with varying degrees of infectivity, morbidity and mortality (OIE, 2004).

Morphology

Avian influenza virus is a pleomorphic enveloped single-stranded RNA virus. The complete genome contains 10,000-14,600 nucleotides and is segmented into 8 sections. Each segment of the genome is encapsidated in a separate nucleocapsid. The nucleocapsids are surrounded by a host cell derived envelope.

Virions (or virus particles) are spherical with club-shaped projections comprising of hemagglutinin and neuraminidase glycoproteins. These projections, along with M2 proteins, play a key role in the identification and cell entry mechanisms of the virus (Horimoto and Kawaoka, 2001). Virions are 80-120nm in diameter and consist of an envelope, matrix protein, nucleoprotein complex, nucleocapsid and a polymerase complex.

Subtypes of influenza A viruses

Influenza A viruses are classified into subtypes based on the combination and arrangement of surface glycoproteins; hemagglutinin (H) and neuraminidase (N) (OIE 2004). Influenza A viruses have 16 H subtypes, each of which can have up to 9 N subtypes, resulting in potentially 144 different subtypes of the virus. Within influenza virus subtypes there are also different strains, which can have different physical, chemical and biological properties. All H and N subtypes have been found in birds, indicating their role as the main reservoir, but only a limited range of subtypes have been shown to circulate in humans as seasonal influenza (Advisory Committee on the Microbiological Safety of Food, 2005).

In addition to the classification of avian influenza viruses based on surface proteins, they can be classified as either low pathogenic (LPAI) or high pathogenic (HPAI) strains, with this classification being based on their genetic features and pathogenicity for avian species. The Office International des Epizooties (OIE) has developed detailed criteria for classifying avian influenza as either LPAI or HPAI, based on laboratory tests on the pathogenicity of the virus to birds (OIE, 2007).

New strains of influenza virus are constantly generated either via genetic mutation or recombination of genetic material with other influenza viruses that are co infecting a bird (particularly wild waterfowls), humans, or other mammals e.g. swine (Webster *et al*., 2006).

Host range of avian influenza viruses

Aquatic birds such as waterfowl are the natural reservoir for influenza A virus, with the virus being circulated via the faecal-oral route. In wild ducks, influenza viruses replicate preferentially in the cells lining the intestinal tract. This usually does not cause disease in the host bird, but the virus is excreted in high concentrations in the faeces (Webster *et al*., 1978). The virus may persist in bodies of water for variable periods of time, from 9-100 days (Stallknecht *et al*., 1990).

Domestic birds, including poultry, may become infected via direct contact with infected wild birds (e.g. ducks, geese, gulls and shorebirds), or through faecal contamination of water or feed supplies (Swayne and Suarez, 2000). The virus may also be spread between farms horizontally by contaminated people, fomites or aerosols, as well as through the movement of birds.

Migratory birds, such as waterfowl, can introduce LPAI viruses into poultry flocks across a wide geographic area. These viruses may then mutate to highly pathogenic forms within domestic flocks. Mutation may occur within only a few months. Highly pathogenic viruses have been isolated from migratory birds, but only on rare occasions. Recent events, however, indicate that it is likely that some migratory birds are directly spreading the HPAI H5N1 virus to new areas (WHO, 2005a; Webster *et al*., 2006)

Some strains of avian influenza virus, including HPAI H5N1, also have the ability to infect humans and other mammals leading to illness, including pigs, horses, tigers, leopards, domestic cats, dogs, rats, ferrets, rabbits, civets, and seals (Hinshaw *et al*., 1981; Hinshaw *et al*., 1984; Shortridge *et al*., 1998; Fouchier *et al*., 2004; Maines *et al*., 2005; Amonsin *et al.,* 2007). Leschnik *et al*. (2007) demonstrated that under natural conditions, infection of cats with influenza virus H5N1 may occur after contact with infected birds or their excrement without inducing clinical disease. In this study, horizontal transmission between cats was not observed and there was no evidence that cats are responsible for transmitting the virus to humans.

Mode of transmission

Transmission of avian influenza virus occurs primarily through exposure to nasal and/or oral secretions and faeces of infected birds (Koopmans *et al*., 2004; European Centre for Disease Prevention and Control (ECDC), 2005; European Food Safety Authority (EFSA), 2006). The virus can be transmitted in aerosol form and through environment contamination by bird faeces (Englund and Hard af, 1998). Transmission may also occur via indirect contact with fomites such as dust, followed by self-inoculation of the upper respiratory tract or conjunctival mucosa (Hayden and Croisier, 2005).

Investigation of human H5N1 cases in several Asian countries and Turkey has identified close contact with live infected poultry as the main, if not exclusive, risk factor for transmission of the virus (WHO 2005a; Webster *et al*., 2006). Evidence currently available favours the view that a high proportion of confirmed human cases acquired infection during the slaughtering and subsequent handling of diseased or dead birds prior to cooking. The evidence for this view is circumstantial rather than direct, and is supported by the fact that infected poultry meat must have been eaten by many people, without any cases clearly attributable to properly cooked poultry meat. In some cases, a history of exposure to a likely source of infection cannot be elicited and for many cases exposure histories are unavailable.

The exact entry route of the HPAI H5N1 virus in humans is not known however it is thought that the conjunctival and/or respiratory tissues would be the most likely entry portal (European Food Safety Authority (EFSA), 2006), with a study from Southeast Asia confirming that the lungs were the major site of avian influenza viral pathology in humans (Ungchusak *et al*., 2005).

The gastrointestinal tract and the nervous system have also been postulated as possible sites of viral entry in humans and their involvement in human infection cannot be excluded. However viral antigen-positive intestinal cells as well as any virologically confirmed infection of the gastrointestinal tract have not been identified in human infections with HPAI H5N1 (European Food Safety Authority (EFSA), 2006).

Suitable receptors need to be present in order for the virus to gain entry and infect a cell. Host specificity, tissue tropism and potential virulence of the influenza virus is controlled by a variety of viral genes; the HA (haemagglutinin) of the virus molecule mediates the binding of the virus to receptors on the host cell, with the NA (neuraminidase) responsible for breaking the bond between HA and the host cell receptor and allowing for the release of new viral progeny particles (European Food Safety Authority (EFSA), 2006).

The receptor on a host cell recognised by the HPAI H5N1 virus consists of a sialic acid (either N-acetylneuraminic acid or N-glycolylneuraminic acid) attached to a galactose molecule by either an α-2,3 or an α-2,6 linkage (Suzuki *et al*., 2005), with the ability of the virus to replicate in a specific host cell being influenced by the sialic acid type and the linkage type between the virus and the host cell in addition to the viruses HA's receptor binding site (European Food Safety Authority (EFSA), 2006). The majority of avian influenza viruses will by preference bind to a N-acetylneuraminic acid - α-2,3 – galatose linkage on sialyloligosaccharides, whilst the influenza viruses common in human influenza infections prefer the N-acetylneuraminic acid - α-2,6 – galatose linkage (Ito *et al*., 1998).

The α-2,6 linked sialic acids predominate in the human respiratory tract (Baum and Paulson, 1990), however no information is available on the types of receptors, if any, which may be present in human intestinal cells. Additionally, if ingested, viable viral particles must be able to pass through the hostile environments of the stomach and intestines if the gastrointestinal track were to serve as a potential site of infection and it is likely that very high virus quantities would be required to make up an effective infectious dose. However the possibility of infection occurring via ingested food coming into contact with the upper respiratory tract or oropharyngeal tissues during eating cannot be discounted (European Food Safety Authority (EFSA), 2006).

There is no epidemiological information suggesting that HPAI can be readily transmitted to humans via food (Thomas and Swayne, 2007; EFSA, 2006). Some cases of human illness in Vietnam were suspected to have occurred following consumption of contaminated fresh duck blood and undercooked poultry products (Hayden and Croisier, 2005), although it is not clear that other potential exposures to diseased birds or their excreta did not occur in these cases. Consumption of raw poultry products infected by avian influenza may therefore be a means of transmitting the virus to humans and other mammals. For example, experimental feeding of infected raw chickens to domestic cats resulted in transmission of the virus (Kuiken *et al*., 2004), the consumption of wild birds by domestic cats and stone marten caused illness in Germany (WHO, 2006) and the feeding of raw infected chickens to tigers led to outbreaks of in zoos in Thailand (Thanawongnuwech *et al.,* 2005 ;Keawcharoen *et al*., 2004).

HPAI has been shown to contaminate the surface of egg shells and may have the ability to contaminate the egg contents (WHO, 2005b). There have been reports of lesions occurring in the ovaries and oviducts of HPAI infected egg-laying chickens and hence it is possible that virus particles present may be directly deposited into the egg contents as the egg is formed (Swayne and Beck, 2004). Eggs from H5N1-infected hens that are externally contaminated with faecal material may represent a greater risk of transmission via cross-contamination compared with visibly clean eggs (DAFF, 2008; Swayne and Beck, 2004). However, whilst raw eggs have been shown to contain viral particles, there has been epidemiological evidence indicating raw or undercooked eggs as the source for human HPAI infection (WHO, 2005b; Swayne and Beck, 2004).

It is also biologically plausible that filter feeding molluscs such as oysters, cultivated in water contaminated by excreta from infected poultry and waterfowl, may be able to absorb and concentrate avian influenza viral particles and provide a potential route of human exposure. There are plans to explore this mode of exposure in the future (Dr Potter, 2006 Pers con).

Cats (and other domestic animals) have also been considered as a potential vectors for avian influenza viruses; however no cat-to-human or cat-to-bird transmission has been documented (Kuiken *et al*., 2004; Amonsin *et al*., 2007). Experimentally infected cats have been shown to transmit infection to other cats (Thiry *et al.,* 2007; Rimmelzwaan *et al.,* 2006)

To date, sustained human-to-human transmission has not been documented for HPAI H5N1, although limited human-to-human transmission may have occurred. With continued circulation and geographic spread, the potential exists for this strain to mutate and re-assort with other influenza strains to gain the ability to pass easily from human-to-human, leading to pandemic human influenza (Webster *et al*., 2006).

Inactivation and survival of avian influenza virus

Avian influenza viruses in general appear to be highly sensitive to heat, lipid solvents, non-ionic detergents, formaldehyde, and oxidising agents. The resistance of the virus to physical and chemical action is summarised in Table 1.

Table 1. Effect of physical and chemical treatment on avian influenza virus (OIE 2006; Swayne, 2006; Thomas *et al*., 1982).

Treatment	Impact on Avian Influenza Virus
Temperature	Inactivated by 56°C for 3 hours or 60°C for 30 minutes (OIE, 2006); or 70°C for 5 seconds (Swayne, 2006)
pH	Inactivated by acid pH; but remain viable between pH 5-8 Some variability in acid tolerance between strains
Chemicals	Inactivated by oxidising agents *e.g.* sodium dodecyl sulphate, lipid solvents, β-propiolactone
Disinfectants	Inactivated by formaldehyde and iodine compounds
Irradiation	Reduces infectivity (Thomas *et al*., 1982) but high doses may be required

A study by Swayne (2006), identifying the heat inactivation of experimentally HPAI H5N1 contaminated chicken meat, showed this virus is effectively inactivated when the meat reached an internal temperature of 70°C for 5 seconds, even in heavily infected samples. Information regarding strain to strain variation for other HPAI strains is not currently available (OIE 2006). A more recent study by Thomas and Swayne (2007) measured the thermal inactivation of H5N1 in naturally infected chicken thigh and breast meat with the 95% confidence interval for the 70°C D-value being 0.28 – 0.50 seconds. Results confirmed that

reaching an internal temperature of 73.9°C or ensuring a 70°C internal temperature for 5 seconds inactivates H5N1 virus even in heavily contaminated meat samples.

Avian influenza virus will remain viable for long periods in tissues, faeces and in water (Department of Agriculture Fisheries and Forestry (DAFF), 2006). Studies have shown that the infectivity of avian influenza virus can be maintained for several weeks at 4°C under laboratory conditions; for 30-35 days at 4°C in faeces; and for 7 days at 20°C in the field (Easterday *et al*., 1997). Avian influenza viruses are stable at low temperatures, and are able to be stored at -20°C or -196°C for at least 42 months without loss of potency (Fatunmbi *et al*., 1993).

The ability of the avian influenza virus to resist inactivation in the environment is greatly enhanced by the presence of organic material. For example, it has been shown that avian influenza viruses inoculated on the skeletal muscle and other organs of experimental chickens remained infective at room temperature for 30-40 days and on bone marrow remained infective for 60 days at room temperature (Vrtiak and Kapitancik, 1967).

Distribution of avian influenza viruses in infected poultry

Unlike other strains of avian influenza, which are predominantly found in the respiratory and gastrointestinal tract of infected birds, experimental studies have shown that HPAI H5N1 can spread to virtually all parts of the bird including the organs, blood and muscle (Webster *et al*., 2006). A summary of studies that have investigated the distribution of HPAI H5N1 virus in infected poultry tissue is provided in Appendix 2. Because of the limited tissue distribution of LPAI strains in infected poultry and egg products (i.e. virus only infecting respiratory and digestive tissue) (Swayne and Beck, 2004; Swayne and Beck, 2005), food safety concerns related to AI in poultry meat and egg products are limited to the HPAI strains, like the HPAI H5N1 viruses currently circulating in various parts of the world.

Incidence and outbreak of human influenza due to HPAI H5N1 infection

The first confirmed case of human infection by avian influenza virus subtype HPAI H5N1 occurred in 1997 in Hong Kong. In this initial outbreak, a total of 18 people were infected, of which 6 died (WHO 2005a). The human cases occurred while the poultry population in Hong Kong was experiencing an extended outbreak of avian influenza due to H5N1 infection. Culling of Hong Kong's entire poultry population, estimated to be approximately 1.25 million birds, reduced the potential for further direct transmission to humans. This action may have averted an influenza pandemic (WHO, 2004) because while H5N1 viruses continued to mutate while circulating within avian populations, the strain responsible for the 1997 Hong Kong outbreak was successfully contained and additional human exposure was minimised.

In February 2003, two new cases of human H5N1 infection were reported in Hong Kong (WHO 2004), and one of the infected cases subsequently died. Since that time, human cases of H5N1 avian influenza virus infection has occurred in a number of countries including China, Cambodia, Vietnam, Indonesia, Thailand and Turkey (see the WHO website for a detailed timeline of events[16]).

[16] http://www.who.int/csr/disease/avian_influenza/timeline.pdf

As of the 12 February 2008, the total number of confirmed human cases resulting from H5N1 infection has reached 360, of which 226 have died. The number of confirmed human cases in the recent (2003 onwards) outbreaks is summarised in Table 2.

Table 2. Human cases of avian influenza H5N1 reported to WHO (12 February 2008).

Country	2003		2004		2005		2006		2007		2008		Total	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths
Azerbaijan	0	0	0	0	0	0	8	5	0	0	0	0	8	5
Cambodia	0	0	0	0	4	4	2	2	1	1	0	0	7	7
China	1	1	0	0	8	5	13	8	5	3	0	0	27	17
Djibouti	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Egypt	0	0	0	0	0	0	18	10	25	9	0	0	43	19
Indonesia	0	0	0	0	20	13	55	45	42	37	10	8	127	103
Iraq	0	0	0	0	0	0	3	2	0	0	0	0	3	2
*Lao**	0	0	0	0	0	0	0	0	2	2	0	0	2	2
Myanmar	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Nigeria	0	0	0	0	0	0	0	0	1	1	0	0	1	1
Pakistan	0	0	0	0	0	0	0	0	1	1	0	0	1	1
Thailand	0	0	17	12	5	2	3	3	0	0	0	0	25	17
Turkey	0	0	0	0	0	0	12	4	0	0	0	0	12	4
Viet Nam	3	3	29	20	61	19	0	0	8	5	1	1	102	48
Total	4	4	46	32	98	43	115	79	86	59	11	9	360	226

* Lao People's Democratic Republic

Chains of human-to-human transmission have been short and most are limited to relatives in close contact with an infected family member. Increasingly the view of the international community is that HPAI H5N1 is poorly adapted to humans and is primarily a disease of poultry. The humans that are infected have been shown to be exposed to high doses of virus (WHO, 2005b).

Hazard Characterisation

Severity of human H5N1 infection

Available data on HPAI H5N1 infection in humans demonstrates the severity of the disease. WHO examined 205 confirmed cases up until April 2006 and found that: 90% of cases were in people aged <40 years; the overall case-fatality rate was 56%; the median interval from illness onset to hospitalization was 4 days; and the median duration from illness onset to death was 9 days (WHO, 2006) .

There is however, an indication that the fatality rate could be somewhat lower as there is a likely under-reporting of human HPAI H5N1 infections as opposed to deaths (Thorson *et al*., 2006).

Human death due to HPAI H5N1 infection is a result of primary viral pneumonia; secondary bacterial pneumonia of virus-damaged lungs; and acute respiratory distress-like syndrome possibly associated with overwhelming immune responses (Taubenberger and Morens, 2006). Other causes of death have included multi-organ failure and the exacerbation of serious chronic diseases such as diabetes, renal diseases, and congestive heart failure. Gastrointestinal symptoms (vomiting, diarrhoea and abdominal pain) have also been noted in some infections predominantly in children (de Jong and Hien, 2006). HPAI viruses are capable of invading a wide range of body cells, whereas less pathogenic influenza viruses specifically infect respiratory and occasionally gastrointestinal tract cells.

The incubation period of influenza virus subtype H5N1 in humans has been estimated to be approximately 3 days (Koopmans *et al*., 2004; Fouchier *et al*., 2004; Ungchusak *et al*., 2005).

Dose-response

There is an absence of data on the dose-response relationship for human infection with HPAI H5N1 avian influenza virus, however epidemiologic characteristics of identified human cases of infection with the Asian strain of H5N1 suggest very high levels of exposure may be necessary for human infection to occur (WHO 2004).

At risk groups

In the recent outbreaks, the majority of human cases have been previously healthy children and young adults (WHO 2004). Few data are available to ascertain which sub-populations are most susceptible to H5N1 infection and the serious consequences of this infection. Reported human illnesses resulting from H5N1 infection have generally been associated with close contact with infected birds during domestic poultry production, or through occupational exposure at poultry slaughtering facilities.

The WHO has reported a skewed age distribution of confirmed cases of H5N1 infection towards young children and young adults (WHO, 2006). Smallman-Raynor and Cliff (2007) analysed data on the confirmed human cases of infection reported to the WHO up until 4 July 2006. Age-related information was available for 169 of the 229 cases reported. The mean age of these cases was 19.8 years (median 18.0; range 0.3 – 75.0). The age distribution was 0–9 years, 26.0%; 10–19 years, 29.0%; 20–29 years, 23.1%; 30–39 years, 16.0% and ≥40 years, 5.9%. These data, however, may not represent at-risk groups because not every group of the population had been subject to the same level of exposure.

Exposure Assessment

Poultry meat and egg and egg products in Australia

Poultry meat, specifically chicken, is one of the most commonly consumed foods in Australia. The annual rate of consumption of chicken meat is approximately 36 kg per person (Australian Bureau of Agricultural and Resource Economics (ABARE), 2003). Approximately 80% of poultry produced in Australia is either fresh or frozen raw whole bird or chicken pieces, with the remainder ready to cook or fully cooked. For turkey and duck meat, the annual consumption is estimated to be 1.6 kg and 0.5 kg per person respectively (Australian Bureau of Agricultural and Resource Economics (ABARE), 2003).

Poultry meat consumed in Australia is primarily derived from domestic production. Only a minimal amount of cooked poultry meat products are imported, representing less than 1% of total poultry meat consumption. Imported poultry meat products are limited to canned meat and meat based flavours. Uncooked or fresh poultry meat is not permitted to be imported into Australia. Fertilised chicken, turkey and duck eggs are imported into Australia under strict quarantine for commercial poultry production. For hatched chickens and turkeys, the hatched birds remain in quarantine facilities and monitored for infection status for at least nine weeks[17]. The post arrival quarantine period for hatching eggs of domestic ducks is 12 weeks, although this is currently under review.

[17] AQIS conditions for importation of fertile hen eggs into Australia (2005). http://www.aqis.gov.au/icon32/asp/ex_querycontent.asp accessed 13 February 2007

The annual egg consumption in Australia is estimated to be 168 eggs per person (Australian Egg Corporation Limited (AECL), 2005). Egg products include whole shell eggs, chilled or frozen liquid egg, dried egg and pasteurised whole eggs. Egg products can also be incorporated into a wide range of other products including cakes, confectionary, bakery products and pasta.

All whole shell eggs consumed and sold in Australia are domestically produced. Imported food products containing greater than 10% egg (dry weight) are required to be heat treated under time/temperature conditions that would inactivate avian influenza viruses. Currently, only a small number of products containing more than 10% egg can be imported, as approval must be gained from Biosecurity Australia following an Import Risk Analysis. A summary of current import conditions for eggs and egg products is provided in Appendix 3.

There are no restrictions on the entry of products with less than 10% egg, except for the need for a manufacturer's declaration or Government certification stating that the product contains less than 10% whole egg. The processing (heat-treatment) provisions of Standard 1.6.2 - Processing Requirements and Standard 2.2.2 - Egg and Egg Products (Australia New Zealand Food Standards Code) would apply to these products. These provisions are compared to the OIE guidelines in Table 3.

Table 3. Comparison of heat treatment requirements under the Australia New Zealand Food Standards Code and the OIE Guidelines

Egg product	Australia New Zealand Food Standards Code	OIE Guidelines[18]
Whole egg	64ºC, 150 sec	60ºC, 188 sec
Whole egg blends	64ºC, 150 sec	60ºC, 188 sec 61.1ºC, 94 sec
Liquid egg white	55ºC, 570 sec	55.6ºC, 870 sec 56.7ºC, 232 sec
Liquid egg yolk	60ºC, 210 sec	Not stated
10% salted yolk	60ºC, 210 sec	62.2ºC, 138 sec
Dried egg white	Heat treatment equivalent to pasteurisation	67ºC, 0.83 days 54.4 ºC, 21.38 days

In summary, only eggs and egg products that do not pose a risk of introducing exotic diseases, including avian influenza, are permitted entry into Australia.

Contamination of poultry meat products with avian influenza

Due to the systemic nature of HPAI H5N1 infection, and the severity of illness in humans, infected birds should be prevented from entering the human food chain. Poultry meat products may be contaminated with avian influenza viruses due to either the bird being infected, or as a result of cross-contamination during processing (see Appendix 4). Ante-mortem inspection plays an important role in preventing poultry infected with HPAI virus from entering the human food chain. HPAI viruses such as HPAI H5N1 are notifiable diseases under the OIE list.

[18] Terrestrial Animal health code (2007) http://oie.int/eng/normes/MCode/en_chapitre_3.6.5.htm accessed 13 October 2007

The H5N1 virus has been isolated from duck meat imported into South Korea (Tumpey *et al*., 2002) and Japan (Mase and Kawaoka, 2005) from China. The isolated virus was viable as determined by chicken and mouse bioassay. Further bioassay experiments showed that the virus could be detected in breast and thigh muscle from chickens with severe clinical disease (Tumpey *et al*., 2003), and the thigh muscle from asymptomatic ducks (Tumpey *et al*., 2002). These experiments highlight the differences in viral pathogenesis in different avian species.

Numerous studies have shown that the viraemia and subsequent systemic infection caused by HPAI viruses in poultry allow for the spread to muscle tissue leading to the presence of viruses within the edible meat (Wood *et al*., 1995; Lu *et al*., 2003; Swayne and Beck, 2005; Kwon *et al*., 2005; Capua and Alexander, 2006). Additionally internal organs of chickens and turkeys such as the spleen, pancreas, heart, liver, kidney, muscle and skin tissue have been found to contain H7 HPAI virus (Starick and Werner, 2003). In a separate study, Swayne and Beck (2005) demonstrated that a H5N1 strain of virus isolated from breast and thigh meat of infected chickens could transmit disease to other chickens via the oral route.

Although these data indicate that viable virus could be present on poultry meat products and eggs, current epidemiological evidence does not suggest that foodborne exposure is an important source of human infection and disease. For instance, in spite of the widespread and extensive outbreaks of the H5N1 avian influenza virus in birds, there have only been two clearly identified cases of human infection due to transmission through food. In these cases the two Vietnamese men infected with the H5N1 strain were potentially exposed to the virus from a shared meal that included raw duck blood and organs, however other routes of contamination, such as handling during the processing of the poultry products or through faecal contamination, was not discounted (Hayden and Croisier, 2005; WHO 2005b).

Contamination of eggs and egg products

Avian influenza virus has been isolated from the surface of eggs, as well as from yolk and albumen during an outbreak of naturally occurring HPAI (Cappucci, Jr. *et al*., 1985). The virus on the surface of the egg is most likely from faecal contamination. HPAI viruses have been found to survive in faeces for at least 35 days at 4ºC and 6 days at 37°C (WHO 2005b). It is plausible that such external contamination may lead to transmission of HPAI viruses to egg handlers.

Avian influenza virus antigen has also been detected in the ovaries of chickens, and this may lead to contamination of the yolk and albumen (Nakatani *et al*., 2005). Promkuntod *et al*. (2006) recovered HPAI H5N1 form the internal contents of eggs and the oviducts of natural infected Japanese quail.

Diseased birds will usually stop producing eggs, but eggs laid in the early stages of sub-clinical disease may be contaminated (Samadieh and Bankowski, 1970). It is also possible that sub-clinically infected birds may lay infected eggs. This could pose a greater risk with duck eggs, as ducks may develop asymptomatic infection with some strains of avian influenza virus that induce severe disease in other poultry (Webster *et al*., 1992).

The detection of avian influenza viruses in the internal contents of eggs poses a potential risk to consumers if the egg is not properly cooked prior to consumption. Due to the relatively heat sensitve nature of the H5N1 virus, cooking conditions generally recommended for chicken meat and eggs to prevent bacterial contamination (e.g. *Salmonella*) appear to provide a high level of protection against HPAI H5N1 infection (Thomas and Swayne, 2007; OIE, 2006).

Liquid egg products are pasteurised, which should inactivate avian influenza virus. Swayne and Beck (2004) examined the time and temperature requirements for the inactivation of avian influenza viruses in different egg products (Table 4). They concluded that avian influenza viruses would be inactivated under the current industry practice of egg pasteurisation.

Table 4. Time to reduce virus titre by one log in egg products (Swayne and Beck, 2004)

Temperature	Homogenised whole egg	Liquid egg white	10% salted yolk	Dried egg white
55ºC	643.8 sec	256.7 sec	20.3 sec	2.2 days
57ºC	268.5 sec	22.9 sec	<20 sec	1.4 days
59ºC	22.3 sec	<19 sec	<20 sec	1.3 days
61ºC	<19 sec	<19 sec	<20 sec	1.0 days
63ºC	<19 sec	<19 sec	<20 sec	0.2 days

Where eggs are inadequately cooked in the home *i.e.* soft or liquid yolks, the presence of HPAI H5N1 virions may present a risk of exposure to consumers.

Appendix 5 provides a summary of the key stages of egg production and the likely impact of these stages on the avian influenza virus.

Primary production of poultry and migratory birds

The main identified mechanisms of spread of avian influenza virus between poultry are via the movement of infected poultry and poultry products, and contact with infected wild birds (Webster *et al*., 2006). Therefore good biosecurity measures at the farm level are vital for preventing HPAI H5N1 from entering the flock. These measures also prevent transmission of HPAI H5N1 from farm to farm by mechanical mechanisms i.e. contaminated vehicles, equipment, personnel, rodents, feed, water, clothing and shoes. Strict adherence to preventative measures such as industry biosecurity Codes of Practice (AECL, 2005; ACMF, 2003) would be the first defence in preventing the spread of H5N1 into Australian poultry flocks, in the event that H5N1 appeared in Australia.

The HPAI H5N1 virus is spread via migratory birds. The East Asian-Australasian flyway and the West Pacific Flyway of winter migratory birds include Australia, and while it is possible that H5N1 could be brought to Australia by migratory birds (Webster *et al*., 2006) it is unlikely as only shorebirds tend to travel to Australia. Each year over 3 million migratory shorebirds spend the southern summer in Australia, however, the birds principally associated with the spread of avian influenza in Asia and Europe are migrating waterfowl such as ducks, swans and geese. In Australia, most species in this group are not migratory, and there is probably only limited interaction with similar birds to the immediate north of Australia. Monitoring data from northwest Australia over the past 25 years suggest the incidence of avian diseases (including influenza, encephalitis and Newcastle disease) in migratory shorebirds is low. No birds with the HPAI H5N1 strain of avian influenza have ever been found in Australia (Webster *et al*., 2006).

While there has been no reported presence of HPAI H5N1 in migratory birds in Australia, it is necessary to maintain appropriate strategies/measures to monitor and report HPAI H5N1 should it appear in Australia. Australian veterinary infrastructure and accumulated experience in handling large-scale poultry disease outbreaks by Australian poultry farmers (for example the Newcastle disease in late 1990s) would facilitate the early detection and establishment of prevention and control measures if HPAI H5N1 appeared in Australia. Non-H5N1 HPAI has been detected in Australia in the past and on each occasion it was successfully eradicated from poultry flocks. The last reported case was in 1997 in Tamworth, NSW. Prior outbreaks occurred in commercial poultry farms in Victoria (1976, 1985 and 1992) and Queensland (1994) (Capua and Alexander, 2004).

In Asia, HPAI H5N1 has been transmitted to humans through close contact with infected poultry *e.g.* backyard poultry flocks, live poultry markets and home slaughter of poultry (Webster *et al*., 2006). Poultry handling in Australia is markedly different, with the home slaughter of poultry uncommon, and there are very few live poultry markets. Furthermore, the Australian commercial poultry industry has rigorous biosecurity control measures in place. However, exposure may be more likely to occur outside the mainstream commercial industry.

Illegal imports

A potential means of HPAI H5N1 viruses entering Australia is via smuggled birds, illegal entry of untreated poultry products, or through infected food products accompanying people travelling into Australia. The risk is considered low as is the indirect transmission to commercial poultry flocks. This is because effective border control measures are maintained by the Australian Quarantine and Inspection Service (AQIS) (Geering *et al*., 1995; Nunn, 1997).

Hypothetical Modelling scenario

In order to assess the extent to which Australian consumers may be exposed to poultry meat or eggs produced by HPAI infected birds, the following scenarios consider how much product could potentially enter the marketplace (Table 5). These scenarios mimic a worst-case scenario where a flock demonstrates clinical illness after eggs have been harvested or broilers have been sent for slaughter, and estimates the amount of product that may potentially enter the marketplace before recalls and other food safety mechanisms are initiated.

With HPAI, birds rapidly become viraemic and significant mortalities occur within 24-48 hours. Hence there is only a narrow timeframe where sub-clinically infected birds may be producing eggs or despatched for slaughter. Eggs from infected commercial flocks are unlikely to reach the marketplace because of the time taken to process, transport and market eggs. Meat from wild-caught poultry may represent a greater risk as the disease status of the bird at slaughter is rarely known.

Table 5. Hypothetical scenarios

Industry Sector	Production throughput	Product handling	Notes
Layer hen eggs	Large commercial operations collect eggs daily, but will hold eggs for periods of 3-15 days before dispatch to the retail sector	Sophisticated traceability arrangements are in place for many large commercial operations however source information from the retail environment may be problematic	With HPAI, there is rapid onset of symptoms, and illness is very likely to be clinically obvious before eggs enter the marketplace, and they can be withdrawn **Outcome**: Unlikely that HPAI infected material enters the marketplace
	More rapid entry of product into the market from small farms		**Outcome**: Small volumes may enter the marketplace before clinical symptoms become apparent
Broiler chickens	Harvest of two loads (4,500-6,000 broilers) from two sheds per day result in a maximum of 18,000-24,000 birds/day from a single grower	Dressed product sent from processor to retail sector approximately 24 hours after dispatch from grower. Traceability in place	**Outcome**: A maximum of 216,000 serves of HPAI infected material enters the marketplace (assuming 9 portions/chicken carcass and all chickens viraemic). Some cross-contamination may also occur

It is more difficult to estimate precise figures for broiler chickens as the extent of depopulation and the size of the farm influence the amount of poultry meat that enters the food supply chain.

While it is plausible that infected poultry meat could enter the marketplace, chicken is not consumed raw and the virus is heat sensitive. Handling of contaminated poultry meat presents a potential risk, but infection has typically only originated from close contact with live or dead infected birds and their faeces and secretions.

Risk Characterisation

Consumers in Australia have a negligible risk of exposure to the H5N1 virus as it has not been detected in Australia to date. Additionally there is a low likelihood of introduction of H5N1 from migratory birds as the geographic flight paths of birds known to carry the virus do not include Australia. This, in combination with active HPAI surveillance programs, reporting systems, and biosecurity controls limits the likelihood of the virus entering and/or spreading in Australia.

Extensive controls limit the import of poultry meat and eggs into Australia. Only cooked or heat treated poultry meat and egg products are permitted for importation into Australia, so they do not appear to present a risk of introducing the H5N1 avian influenza virus into the country.

The H5N1 virus is inactivated when poultry meat and/or eggs are thoroughly cooked (at temperatures recommended to inactivate bacterial pathogens). If the virus was present in poultry flocks, proper cooking would ensure consumers are protected.

To date, there has been no epidemiological evidence suggesting handling during the preparation and consumption of properly cooked poultry meat or egg and egg products has lead to human infection by HPAI viruses and illness. Available evidence indicates that H5N1 transmission occurs through the handling of live or dead infected birds, or through close contact with infected birds and their excretions. There has been very limited evidence identifying the possibility of human-to-human transmission of H5N1 avian influenza virus.

The ongoing spread of H5N1 viruses in poultry species across the globe, and the severity of illness in humans, mean that the potential public health risk of H5N1 influenza viruses during the primary production and processing of poultry meat and eggs, food preparation, handling and consumption must be continually reviewed and updated.

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APPENDIX 3A: PREVIOUS OUTBREAKS OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN AUSTRALIAN POULTRY FLOCKS

There are no current reports of avian influenza, either in birds or humans, in Australia. However, in the recent past there have been five outbreaks of HPAI in commercial poultry flocks in Australia. The details of these are as follows:

Outbreak No.	Year	Strain	Details
1.	1976	H7N7	Involved three adjacent poultry farms in a Melbourne suburb (Victoria)
2.	1985	H7N7	Involved a poultry complex near Bendigo (Victoria)
3.	1992	H7N3	Occurred near Bendigo (Victoria)
4.	1994	H7N7	Occurred near Lowood (south-eastern Queensland)
5.	1997	H7N4	Reported in three properties at Tamworth (NSW)

During each outbreak, a 'stamping-out' policy based on slaughter, disinfection and movement controls was applied and all outbreaks were eradicated before any significant spread occurred.

Eradication was confirmed by serological surveys. In none of these cases was the source of the virus confirmed, although it was suspected that wild waterbirds were directly or indirectly implicated.

APPENDIX 3B: VIRUS TISSUE DISTRIBUTION AND TITRE IN POULTRY INFECTED WITH HIGHLY PATHOGENIC H5N1 STRAINS OF AVIAN INFLUENZA.

Virus strain	Host animal	Tissues with avian influenza virus antigen / infectivity	Reference
A/chicken/Korea/ES/2003	Chickens	Infectivity detected in breast muscle.	(Swayne and Beck, 2005)
H5N1	Naturally infected layer hens	Virus antigen detected in: liver, spleen, heart, intestine, gizzard, proventriculus, oviduct, brain, kidney, pancreas and ovary. Virus antigen mainly detected in capillary endothelium and parenchymal cells. Virus antigen was not detected in the lung or the trachea. Infectivity of tissues not examined.	(Nakatani *et al*., 2005)
H5N1: 4 different strains: Ck/Yamaguchi/04 Dk/Yokohama/03 HK/483/97 Tn/SA/61	Domestic ducks	Infectious virus found in: trachea, lungs, kidneys, brain, blood, liver, and colon. The different strains varied in their tissue distribution and pathogenicity for ducks	(Kishida *et al*., 2005)
DK/Anyang/AVL-1/01 (isolated from duck meat imported into Korea from China)	Chickens	High titres of avian influenza virus was found in the: brain, lung, oropharynx kidney, and thigh tissue. Low titres were found in: breast tissue and cloaca. Varying levels of virus antigen was found in vascular endothelium throughout most of the visceral organs.	(Tumpey *et al*., 2002) (Tumpey *et al*., 2003)
H5N1: 4 different strains: DK/Anyang/AVL-1/01 (isolated from duck meat imported into Korea from China) CK/HK/220/97 Env/HK/437-6/99 CK/HK/317.5/01	Pekin white ducks	Infectious virus was detected in: lungs, kidneys, brain, muscle, cloaca and oropharynx. The different strains varied in their tissue distribution and pathogenicity.	(Tumpey *et al*., 2002) (Tumpey *et al*., 2003)

APPENDIX 4 Reported prevalence of *Salmonella* spp. in shell eggs

Table 1 Prevalence of *Salmonella* in shell eggs

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
Argentina	Shell	122	0	0.00		(Favier *et al*., 2001)
Australia	**Cracked eggs**	**336**	**0**	**0.00**		**(Lake *et al*., 2004)**
Australia	**Upgraded shell eggs - Caged**	**2160**	**0**	**0.00**		**(Daughtry *et al*., 2005)**
	Upgraded shell eggs - Free range	**1200**	**0**	**0.00**		
	Upgraded shell eggs - Barn	**1200**	**0**	**0.00**		
	Graded shell eggs - Caged external	**6476**	**0**	**0.00**		
	Graded shell eggs - caged internal	**20000**	**0**	**0.00**		
Austria	Not stated	223	3	1.35	*Salmonella*, S. Enteritidis	(Anonymous, 2003)
Canada	Grade cracked eggs	94	2	2.13		(D'Aoust *et al*., 1980)
Canada	Chicken egg	16560	10	0.06		(Poppe *et al*., 1992)
Canada	Whole egg (half dozen)					(Lake *et al*., 2004)
	Layer hatching:					
	Surplus	126	2	1.6	S. Typhimurium PT66 and PT3	
	Early	126	1	0.8	S. Heidelberg PT8	
	Culled	126	9	7.1	S. Typhimurium PT66 and PT193, S. Heidelberg PT8	
	Broiler hatching:					
	Surplus	42	0	0		
	Early	42	0	0		
	Culled	42	0	0		
	Layer table:					
	Regular	168	0	0		
	Early	84	1	1.2	S. Agona	

Table 1 continued

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
Canada	Grade crack eggs (not leaking)	299	39	13.0	S. Infantis (41.5%), S. Montevideo (15.4%), S. Schwarzengrund (10.0%), S. Bareilly (10.0%), S. Oranienburg (10.0%), S. Heidelberg (5.4%), S. Cerro (5.4%), S. Typhimurium (5.4%), S. Alachua (5.4%), S. London (5.4%)	(D'Aoust *et al*., 1980)
Denmark	Egg shell	9820	6	0.06	S. Enteritidis	(Anonymous 2003)
	Contents	1480	10	0.68	S. Enteritidis	
EU non UK	Pooled raw shell eggs	1433	29	2.02	*Salmonella*	(Little *et al*., 2007) from ACMSF, 2004
		1433	18	1.26	S. Enteritidis	
		1433	2	0.14	S. Enteritidis PT4	
Europe, USA, unknown country	Pooled raw shell eggs	2101	86	4.09	*Salmonella*	(Little *et al*., 2007)
		2101	82	3.90	S. Enteritidis	
		2101	3	0.14	S. Enteritidis PT4	
Germany	Chicken egg	70	2	2.80	SE	(Buchner *et al*., 1992)
		349	5	1.40		
		630	0	0.00		
		1070	3	0.30		
		309	4	1.30		
		30	1	3.30		
Germany	Not stated	11435	69	0.60	*Salmonella*, S. Enteritidis	(Anonymous 2003)
Ireland	Egg shell	5018	2	0.04	S. Infantis and S. Montevideo	(Murchie *et al*., 2007)
	Egg contents	5018	0	0.00		
Italy	Shell	360	0	0.00	S. entertidis PT4	(Mawer *et al*., 1989)
	Contents	360	1	0.28	S. entertidis PT4	
Italy	Not stated	590	4	0.68	*Salmonella*, S. Enteritidis	(Anonymous 2003)
Japan	Contents	284715	22 (6 SE)	0.01	Off farm	(Shirota *et al*., 2001)
	Egg shell and contents	855	22	2.57	On farm	
Republic of Ireland	Contents	1169	0	0.00		(Anonymous 2003)

Table 1 continued

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
Spain	Whole eggs associated with outbreaks	372	5	1.34	S. Enteritidis (60%) and S. Typhimurium PT96 (40%)	(Perales and Audicana, 1989)
Spain	Whole eggs associated with outbreaks	998	6	0.60	S. Enteritidis (100%)	
Spain	Not stated	305	15	4.92	S. Enteritidis	(Anonymous 2003)
New Zealand	Internal contamination External contamination	N/S N/S	N/S N/S	0 14	*Salmonella* spp.	(Lake *et al*., 2004)
Northern Ireland	Half dozen packs	2090 (half dozen packs)	9 (1 internal contents)	0.4	S. Enteritidis PT4 (22.2%), S. Enteritidis PT1 (11.1%) (the internal contaminant) , S. Infantis (22.2%), S. Mbandaka (11.1%), S. Monetvideo (11.1%), S. Typhimurium DT104 (11.1%), S. Kentucky (11.1%)	(Wilson *et al*., 1998)
UK	Imported half dozen packs	1433 (half dozen packs)	29	2.0	S. Enteritidis PT21 (34.4%), S. Enteritidis PT6 (10.3%), S. Enteritidis PT11 (10.3%), S. Enteritidis PT4 (6.9%), S. Taksony (17.2%), S. Livingstone (6.9%), S. Braenderup (6.9%), S. Virchow PT2 (3.4%), S. Infantis (3.4%)	(ACMSF, 2001)
UK	Domestic half dozen packs	13970 (half dozen packs)	138	1.00	S. Enteritidis PT4 (59.4%), S. Enteritidis PT7 (8.0%), S. Enteritidis PT8 (4.3%), S. Enteritidis PT6 (1.4%), other S. Enteritidis PT (13.0%), S. Typhimurium DT 104 (3.6%) other S. Typhimurium (0.7%), S. Mbandaka (2.9%), S. Livingstone (3.6%), S. Kimuenza (1.4%), S. Indiana (1.4%), S. Virchow (1.4%), S. Infantis (3.4%) S. Braenderup (0.7%), other (1.4%)	
UK	Retail half dozen packs	7730 (half dozen packs)	17 (9 egg surface, 8 internal contents)	0.2	S. Enteritidis (94.1%) of which 76.5% were PT4. N.B. 4 samples exceeded 104 *Salmonella*/ml egg contents after 5 weeks storage at 21oC. Three were S. Enteritidis PT4, one S. Enteritidis PT1A	(de Louvois, 1994)
UK	Shell and egg contents	28518 (4753 half dozen packs)	9	0.34		(FSA, 2004)
UK	Internal contents of eggs Whole egg mixed including shell			0.3 6.0	Experimentally infected chickens, 10^8 cells S.E	(Barrow and Lovell, 1991)
UK	Raw shell eggs - Collected from catering premises	5686 lots of 6 eggs	17	0.30	15 of these were SE	(Elson *et al*., 2005)

Table 1 continued

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
UK	Pooled raw shell eggs	7045	65	0.92	*Salmonella* (S.E)	(de Louvois, 1993)
		7045	47	0.67	S. Enteritidis	
		7045	33	0.47	S. Enteritidis PT4	
Imported into UK	Pooled raw shell eggs	8630	138	1.60	*Salmonella*	(de Louvois, 1993)
		8630	19	0.22	S. Enteritidis	
		8630	16	0.19	S. Enteritidis PT4	
UK	Pooled raw shell eggs	13970	138	0.99	*Salmonella*	(Little *et al*., 2007) from (ACMSF, 2001)
		13970	119	0.85	S. Enteritidis	
		13970	82	0.59	S. Enteritidis PT4	
UK	Pooled raw shell eggs	4753	14	0.29	*Salmonella*	(Little *et al*., 2007) from (FSA, 2004)
		4753	7	0.15	S. Enteritidis	
		4753	3	0.06	S. Enteritidis PT4	
UK	Retail eggs	4753	9	0.19	*Salmonella*	(Murchie *et al*., 2007)
UK and other EU	Pooled raw shell eggs	726	7	0.96	*Salmonella*	(Little *et al*., 2007)
UK and other EU	Pooled raw shell eggs	5686	17	0.30	*Salmonella*	(Little *et al*., 2007)
		5686	15	0.26	S. Enteritidis	
		5686	4	0.07	S. Enteritidis PT4	
UK	Shell	68	5	7.35	Experimental infection S. entertidis PT4	(Humphrey *et al*., 1989)
	Shell	194	10	5.15	Experimental infection S. entertidis PT4	
UK	Contents	2412	24	1.00	Free range, S. entertidis	(Humphrey, 1994)
		2489	10	0.40	Battery farm, S. entertidis	
		1120	1	0.09	Layer breeder, S. entertidis	
UK	Contents	83820	18 (16 SE)	0.02	Retail	(Wall and Ward, 1999)
	Shell	83820	120 (103 SE)	0.14	Retail	

Table 1 continued

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
USA	Shell (pre processing)	90	7	7.78	S. Heidelberg (77.3%) and S. Montevideo (22.7%)	(Jones *et al*., 1995)
	Shell (after processing)	90	1	1.11	S. Heidelberg	
	Egg contents	180	0	0.00		
USA	Shell (unwashed)	1400	3	0.21	S. Typhimurium	(Baker *et al*., 1980b)
USA	Not stated	1200	12	1.00	S. Heidelberg	(Lake *et al*., 2004)
USA	Contents	140000	73 (63 SE)	0.05	Off farm	(Saeed, 1998)
USA	Contents	647000	198 (178 SE)	0.03	Off farm	(Schlosser *et al*., 1999)
USA	Internal shell egg contents	180	0	0.00		(Curtis *et al*., 1994)
USA (Hawaii)	Egg surface	106 dozen	10	9.43	S. Braenderup, S. Oranienburg, S. Mbandaka, S. Cerro, S. Ohio, S. Havana, S. Montevideo, S. Livingstone	(Ching-Lee *et al*., 1991)
USA (Arkansas)	Whole egg contents	100 dozen	0	0		(Lake *et al*., 2004)

Table 1 continued

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
USA	Shell (Clean) surface	222	3	1.35	Surface pathogens included; S. Oranienburg, S. Montevideo, S. Tennessee, S. Bareilly, S. Typhimurium, S. Derby, S. Essen, S. Worthington, S. Pullorum	(Solowey *et al*., 1946) from (Lake *et al*., 2004)
	Shell (Dirty) surface	232	11	4.74		
	Shell (washed dirty) surface	123	6	4.88		
	Duck shell surface	85	4	4.71		
	Guinea shell surface	16	1	6.25		
	Turkey shell surface	18	0	0.00		
	Shell membranes (clean)	37	0	0		
	Shell membranes (dirty)	33	3	9.11		
	Shell membranes (washed dirty)	39	6	15.4		
	Whole liquid (clean)	58	0	0		
	Whole liquid (dirty)	55	2	3.6		
	Whole liquid (washed dirty)	30	1	3.3		
	Yolk (clean)	8	0	0		
	Yolk (dirty)	8	1	12.5		
	Yolk (washed dirty)	11	0	0		
	White (clean)	8	0	0		
	White (dirty)	8	0	0		
	White (washed dirty)	11	0	0		
	Duck shell	17	1	5.9		
	Guinea hen shell	8	0	0		
	Turkey shell	4	1	25.0		
	Goose shell	8	0	0		
	Duck contents	22	0	0		
	Guinea hen contents	8	0	0		
	Turkey contents	7	0	0		
	Goose contents	8	0	0		

Table 2 Prevalence of *Salmonella* in egg products

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
Albania	Pooled liquid egg and shells	79 lots of 10 eggs (representing 22945520 eggs)	1 lot (representing 275000 eggs)	1.27	*Salmonella* group C (non S. Enteritidis)	(Telo *et al*., 1999)
Australia	**Bulk unpasteurised liquid egg**					
	Export	**524**	**114**	**21.8**	**S. Typhimurium (69.3%), S. Anatum (7.8%), S. Singapore (4.6%), S. Hessarek var 27 (3.7%), S. Oranienburg (3.7%), S. Chester (2.8%), S. Adelaide (1.8%), S. Havana (1.8%), S. Bovis-morbificans (0.9%), S. Bredeny (0.9%), S. Give (0.9%), S. Kottbus (0.9%), S. Pullorum (0.9%), S. Senftenberg (0.9%), S. Taxony (0.9%),**	**(Peel, 1976) from (Lake *et al*., 2004)**
	Local	**622**	**154**	**24.8**	**S. Typhimurium (56.9%), S. Singapore (8.5%), S. Saint-paul (5.6%), S. Anatum (5.2%), S. Oranienburg (4.0%), S. Adelaide (2.4%), S. Derby (2.0%), S. Tennessee (2.0%), S. Bredeny (1.2%), S. Havana (1.2%), S. Ondestepoort (1.2%), S. Senftenberg (1.2%), S. Birkenhead (0.8%), S. Give (0.8%), S. Hessarek var 27 (0.8%), S. Kottbus (0.8%), S. Newbrunswick (0.8%), S. Newington (0.8%), S. Newport (0.8%), S. Potsdam (0.8%), S. Rubislaw (0.8%), *Salmonella* untypable (0.8%)**	
	Bulk pasteurised liquid egg					
	Export	**5088**	**2**	**0.04**	**S. Typhimurium**	
	Local	**560**	**0**	**0**		
	Whole egg contents	**847**	**0**	**0**		
	Bulk unpasteurised liquid egg (whole, yolk and albumen)	**1031**	**326**	**32.0**		
Australia QLD	**Individual farm unpasteurised egg products; egg yolk, egg white and whole egg**	**1031**	**326**	**32.0**		
		1031	**7**	**0.7**		
	Pooled unpasteurised whole egg pulp	**110**	**105**	**95.5**	**25% S.Singapore, 23% S.Mbandaka and 19% S.Cerro**	**(Cox *et al*., 2002)**

Table 2 Continued

Country	Product	No sampled	No positive	% prevalence	Other info	Reference
England	Bulk liquid egg from 11 farms	9	5	55.6		(Chapman *et al*., 1988)
Japan	Unpasteurised frozen liquid egg					
	Plant A	60	7	11.7	S. Cerro (72.8%), S. Braenderup (14.8%), S. Thompson (6.1%), S. Infantis (3.9%), S. Mbandaka (1.7%), S. Senftenberg (0.7%)	(Suzuki *et al*., 1981)
	Plant B	44	37	84.1		
	Plant C	19	3	15.8		
	Plant D	30	0	0		
USA	Unpasteurised liquid eggs	40	4	10.0		(Garibaldi *et al*., 1969)
		100	54	54.0		
		29	15	51.7		
		80	19	23.8		
		18	10	55.6		
USA	Unpasteurised liquid eggs	1002	130	13.0		(Mason, 1994)

Table 3 Prevalence of *Salmonella* in poultry flocks

Country	Species	Organism	No sampled	No positive	% prevalence	Other info	Reference
Switzerland	Chicken	SE PT4	37	10	16.0	Naturally infected	(Hoop and Pospischil, 1993)
European Union	Poultry flocks	SE	5007	1486	30.8		(EFSA, 2006b)
			5007	986	20.4		
		S.E and/or S.T ONLY					
USA	Poultry flocks	SE			Estimated at 3%		(Whiting and Buchanan, 1997)

Table 4. Prevalence of Salmonella spp. in non-chicken eggs (from Daughtry *et al.*, 2005).

Non-chicken avian species	No. of eggs tested	% Pos for *Salmonella*	*Salmonella* serotype and phage type	Comment	Country	Reference
Duck Egg - Shell surface	1128	12.4	23 including *S* Typhimurium *S*. Cerro *S*. Tennessee *S*. Amsterdam *S*. Agona *S*. Infantis	retail markets	Thailand	(Saitanu *et al*., 1994)
	50	14.0	not stated	source unknown	India	(Ghosh *et al*., 2002)
	15	0.0	not applicable	domestic	Croatia	(Miokovic *et al*., 2003)
	100	8.0	*S*. Typhimurium *S*. Montevideo	free range	Iraq	(Shareef *et al*., 1997)
	102	4.9	*S*. Anatum *S*. Oranienburg *S*. Paratyphi B	source unknown	India	Chowdhury *et al* 1976
	544	5.1	*S*. Enteritidis *S*. Hadar	breeder farms	United States	(Baker *et al*., 1985)
Duck Egg - Content	1128	11.0	as for shell surface	retail markets	Thailand	(Saitanu *et al*., 1994)
	15	0.0	not applicable	domestic	Croatia	(Miokovic *et al*., 2003)
	90	4.3	not stated	clean eggs	Bangladesh	(Ali *et al*., 1987)
Quail Egg - Content	1152	0.6	*S*. Typhimurium *S*. Hadar	includes dead in-shell embryos and infertile eggs	Egypt	(Fatma *et al*., 2001)
	123	5.7	*S*. Enteritidis	not stated	Turkey	(Erdourul *et al*., 2002)

APPENDIX 5 Summary of international outbreak data associated with egg and egg products

Year	Country	Product	Agent	Cases	Deaths	Reference
1973-2001	USA	Eggs	*Salmonella* Heidelberg	3		(Chittick *et al*., 2006)
1973-2001	USA	Egg containing food	*Salmonella* Heidelberg	17		(Chittick *et al*., 2006)
1973-2001	USA	Eggs and Poultry	*Salmonella* Heidelberg	8		(Chittick *et al*., 2006)
1976	Spain	Egg salad	S. Typhimurium	702	6	(D'Aoust, 1994)
1977	Sweden	Mustard dressing	S. Enteritidis PT4	2,865	0	(D'Aoust, 1994)
1981	Netherlands	Salad base	S. Indiana	600	0	(D'Aoust, 1994)
1982	USA	Home made ice cream	S. Typhimurium	8	1	(Taylor *et al*., 1984)
1985	USA	Eggs used for breakfast	S. Heidelberg	91		(CDC, 1986)
1987	China	Egg drink	S. Typhimurium	1,113	NS	(D'Aoust, 1994)
1988	Japan	Cooked eggs	*Salmonella* spp.	10,476	NS	(D'Aoust, 1994)
1988	UK	Mayonnaise	S. Typhimurium Type 49	120		(Mitchell *et al*., 1989)
1989	UK London	Sandwiches containing mayonnaise	S. Typhimurium DT 4	68		(Ortega-Benito and Langridge, 1992)
1989	USA (New York)	Baked pasta dish containing raw egg	*Salmonella* Enteritidis	21		(CDC, 1990)
1989	USA (Pennsylvania)	Egg based custard pies	*Salmonella* Enteritidis	12		(CDC, 1990)
1989	USA (Tennessee)	Hollandaise or Béarnaise sauce made with heated but not cooked eggs	*Salmonella* Enteritidis	27		(CDC, 1990)
1989	USA	Chocolate moose made with raw chicken eggs from family farm	*Salmonella* Enteritidis PT4	5		(Mawer *et al*., 1989)
1991	USA	Macaroni cheese pasta containing egg	*Salmonella* Enteritidis PT8	28		(Luby and Jones, 1993)
1992	Spain	Eggs	S. Enteritidis and S. Typhimurium	100	16	(Arnedo *et al*., 1998)
1993	France	Mayonnaise	S. Enteritidis	751	0	(D'Aoust, 1994)
1992-2002	UK	Raw shell eggs	*Salmonella* Enteritidis PT4	37		(Gillespie *et al*., 2005)
1992-2002	UK	Eggs and egg products	*Salmonella* Enteritidis PT4	27		(Gillespie *et al*., 2005)
1994	USA	Hollandaise sauce containing egg	*Salmonella* Enteritidis PT8	56		(CDC, 1996)

APPENDIX 5 continued

Year	Country	Product	Agent	Cases	Deaths	Reference
1996	Spain	Omelette using cracked eggs	*Salmonella* Enteritidis PT1	>18		(Furtado *et al*., 1997)
1996-1997	USA	Runny egg	*Salmonella* Heidelberg	10		(Hennessy *et al*., 2004)
1996-1997	USA	Fried egg outside home	*Salmonella* Heidelberg	5		(Hennessy *et al*., 2004)
1996-1997	USA	Scrambled egg outside home	*Salmonella* Heidelberg	10		(Hennessy *et al*., 2004)
1997	France	Egg mayonnaise	*Salmonella* Typhimurium	>12		(Carraminana *et al*., 1997)
1997	USA	Cheesecake containing lightly cooked eggs	*Salmonella* Enteritidis PT4	13		(CDC, 2000)
1997	USA	Lasagne containing egg	*Salmonella* Enteritidis PT8	43		(CDC, 2000)
1998	Italy	Icing on cake produced with raw egg whites	*Salmonella* Enteritidis	36		(D'Argenio *et al*., 1999)
1998	USA (Hawaii)	Cooked eggs from the same farm	*Salmonella* Enteritidis PT4	18		(Burr *et al*., 2005)
1998	Southern Italy	Home made dessert using raw chicken and duck eggs	*Salmonella* Enteritidis PT4	6		(Nastasi *et al*., 1998)
1999	Canada	Ice cream pie dessert with whole shell eggs	*Salmonella* Typhimurium	4		(Canada Communicable Disease Report, 2000)
2002	Spain	Hard pasty with Vanilla cream (Pasty made with raw egg)	*Salmonella* Enteritidis	1435		(Camps *et al*., 2005)
2003	Canada (Oregon)	Hard boiled egg product - salad kit	*Salmonella* Typhimurium	18		(Canada Communicable Disease Report, 2005)
2003	UK	Sandwiches containing egg	*Salmonella* Bareilly	186		(Cowden *et al*., 2003)
2005	Austria	Spatzle (traditional pasta like side dish containing eggs)	*Salmonella* Enteritidis PT4	35		(Schmid *et al*., 2007)
2006	UK	Tiramisu made with raw shell eggs	*Salmonella* Enteritidis PT4	15	1	(Calvert *et al*., 2007)

APPENDIX 6 Foodborne disease outbreaks associated with eggs, Australia

Foodborne Disease Associated with Eggs in Australia, 2001–June 2005

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Introduction

Eggs are a commonly consumed food in Australia with approximately 193 million dozen eggs laid each year.[1] In any given week, 60.4% of people report eating one or more dishes containing eggs, although this probably underestimates consumption as eggs are widely used ingredients of many foods.[2] Food vehicles containing eggs are often implicated in foodborne disease outbreaks internationally and in Australia.[3,4,5,6]

While dishes containing eggs have been implicated as vehicles in outbreaks of foodborne disease, investigators often have difficulty pinpointing specific foods or ingredients as the original source of contamination. Poor recognition and recall of brands and products by consumers, and widespread distribution of products make it difficult for public health agencies to implicate commonly consumed foods, such as dairy products, chicken meat and eggs. Many outbreaks occur where small numbers of people are affected, which makes analytical studies difficult to conduct. Foods, such as eggs, may be responsible for cases of apparently sporadic foodborne disease due to infrequent and low levels of contamination.

Salmonella is the main pathogen of concern for public health agencies investigating egg-associated disease.[7] In many countries egg-laying flocks infected with *S.* Enteritidis have caused large persistent outbreaks of salmonellosis, although Australian layer flocks are currently free from this serotype.[8] Contamination of eggs with *S.* Enteritidis in some countries have resulted in public advice to only eat thoroughly cooked eggs. *S.* Enteritidis is more likely to infect the internal contents of eggs through trans-ovarian transmission from infected hens than other serotypes of *Salmonella*.[9]

The contamination rate of the surface or internal contents of eggs with *Salmonella* serotypes is often extremely low. In a recent survey in the United Kingdom *Salmonella* was isolated from only 0.34% (9/4753) of egg samples.[10] A similar egg survey in Australia found that *Salmonella* was not detected from pooled samples of 20,000 caged graded egg contents or the surfaces of 6,476 caged graded eggs and 4,560 ungraded eggs.[11] However, the sensitivity of testing in this Australian survey puts the upper 95% confidence limits at between 0.02–0.3% prevalence of *Salmonella* for the different categories of eggs. Despite the findings from these surveys, testing of unpasteurised egg pulp and lower quality eggs reveal that they are commonly contaminated with *Salmonella* serotypes that may infect humans.[12]

This report summarises outbreaks of human illness implicating eggs or egg-based products reported to State and Territory health departments in Australia between the years 2001–2005. In this report, we also consider historical information about egg-associated outbreaks from 1980–2000.

Methods

OzFoodNet—Australia's national system of foodborne disease surveillance—established a register of foodborne disease outbreaks in all Australian States and Territories in 2001. In Australia, public health investigators fill in an OzFoodNet form summarising the key features for all foodborne or diarrhoeal disease outbreaks. OzFoodNet epidemiologists in each jurisdiction compile these summary reports and enter them into an Microsoft Access 2003 ™ database. Every three months, data are aggregated nationally in a register of outbreaks. The register also houses information on outbreaks of disease that are spread by waterborne, zoonotic and person-to-person modes of transmission.

To identify outbreaks that were potentially associated with eggs we extracted reports from the outbreak register database relating to the following food vehicles: eggs, dessert, sauce, cake, ice cream, and dressings. Data were collected between January 2001 and June 2005. We also searched the 'Comments' field of all foodborne disease outbreaks in the Register to identify outbreaks where the food vehicle was listed as 'unknown', but eggs were mentioned as the suspected source. In these instances, we made a new variable that recoded these outbreaks as 'suspected eggs'.

We reviewed all records to include only outbreaks where the majority of cases arose from exposure to eggs. We excluded outbreaks that were thought to be due to food handler contamination, or toxin-related outbreaks due to poor food preparation. We cleaned data and recoded individual records to provide consistent categories for data fields, including aetiological agents and food vehicles.

Enhanced data collection

OzFoodNet sites were asked to review outbreaks of foodborne salmonellosis associated with eggs and provide information not included in the OzFoodNet Outbreak Register. A questionnaire was developed by OzFoodNet central staff and results entered into a web-based database constructed using Net Epi ™ (http://www.health.nsw.gov.au/public-health/epi/open_source_tools.html) that was later downloaded into Microsoft Excel 2003™.

We examined summary data for each outbreak and enhanced data supplied by Sites to assess the strength of evidence that eggs were the cause of the outbreak (Table 1). The criteria we considered as evidence supporting eggs as the cause of the outbreak were:

- an analytical epidemiological study (case control or cohort) implicating eggs;
- implicated or suspected vehicle contained raw eggs;
- isolation of the specific infecting *Salmonella* subtype from the food vehicle containing eggs;
- isolation of the specific infecting *Salmonella* subtype from the eggs, shells, or egg packaging materials;
- trace back of implicated eggs to a specific farm; and

- isolation of the specific infecting *Salmonella* serotype or phage type on the egg farm.

Table 1: Levels of evidence for outbreaks implicating eggs.

Level of evidence	Interpretation	Criteria
Strong	Definitely due to contaminated eggs	≥3 sources of evidence
Moderate	Some evidence implicating eggs	2 sources of evidence
Weak	Associated with consumption of eggs, but causal role of eggs not established	≤1 sources of evidence

Data Analysis

We analysed data in Microsoft Excel 2003™ to summarise the number of people ill and hospitalised, different settings for outbreaks, mode of transmission and pathogen. Data were cross-tabulated to explore the relationship between reported outbreaks and other variables of interest. Where multiple food handling errors were listed as causing outbreaks, we only considered the terminal event in food handling in this analysis.

Literature Review

To summarise historical information about outbreaks of egg-associated illness we conducted a limited search of the literature using the terms 'foodborne', 'disease', 'outbreaks' and 'Australia', and searched within references for information relating to outbreaks caused by eggs. This search strategy was also used for non-foodborne routes of transmission by substituting the term '*Salmonella*' for 'foodborne'.

Results

OzFoodNet epidemiologists reported a total of 441 outbreaks of foodborne or suspected foodborne disease between the years 2001–June 2005, which represented 24.1% (441/1828) of all outbreaks reported. We excluded 5 outbreaks where eggs were mentioned as components of the food vehicle, but the presumed causes were due to infected food handlers or intoxications from poor food handling. The pathogens responsible for these excluded outbreaks were unknown aetiology (2 outbreaks), norovirus (1), *Bacillus cereus* (1) and *Staphylococcus aureus* (1).

In total, we reviewed data for 7% (31/441) of outbreaks that were related to the consumption of dishes containing egg. All of these outbreaks were due to various serotypes and phage types of *Salmonella*.

In total, there were 31 outbreaks potentially associated with eggs, affecting at least 689 people, with 128 people hospitalised and three deaths. The median number of people affected in these outbreaks was 12 people (range 3–213). The largest number of egg-associated outbreaks in a single year was 9 outbreaks in 2002, although 2005 data was only available to June (Appendix 6A).

The majority of egg-associated outbreaks occurred in association with food served at restaurants (10 outbreaks, 32%) and private homes (26%), with bakeries (16%) and aged care settings (13%) the next most common (Table 2). High hospitalisation and case fatality rates were associated with outbreaks in aged care settings. Three outbreaks associated with

bakeries were due to Vietnamese pork or chicken rolls where eggs were specifically mentioned in the summary report.

Table 2: Number of outbreaks associated with eggs or suspected eggs showing the setting where food was prepared and numbers of persons affected, OzFoodNet, 2001—June 2005 (*n*=31).

Setting	No. Outbreaks	No. Ill	No. Hospitalised	No. Deaths
Aged care	4	84	27	2
Bakery	5	286	33	1
Child care	1	12	0	0
Commercial caterer	1	14	6	0
Institution	1	43	10	0
Primary produce	1	13	5	0
Private residence	8	59	20	0
Restaurant	10	178	27	0

There were 26% (8/31) of outbreaks were associated with mixed dishes containing eggs. 16% (5/31) of outbreaks were associated with desserts containing raw or partially cooked eggs. The food vehicle for 13% (4/31) of outbreaks was a sauce made from raw eggs. In 35% (11/31) of outbreaks, investigators were unable to definitively identify the food vehicle responsible for the outbreak, but nominated that they suspected egg-based products as the cause.

Three outbreaks were attributed to Vietnamese pork rolls, which contain a mixture of high-risk ingredients including butter containing raw egg. It is difficult to establish the role of different ingredients when these food vehicles are implicated. Investigations of these outbreaks in the past have often revealed widespread cross contamination in the premises.[3]

Of the 31 potentially egg-related outbreaks, 81% (25/31) were caused by *S.* Typhimurium, followed by other serotypes of *Salmonella*. There was one outbreak caused by *S.* Enteritidis phage type 26 var, which occurred in an aged care facility. *Salmonella* serotypes causing single outbreaks included: Hadar, Heidelberg, Hessarek, Potsdam and Saintpaul. There were 12 outbreaks of *S.* Typhimurium phage type 135 (and local variants), 6 of phage type 126, 4 of phage type 9 and 3 of phage type 170/108, and a single outbreak due to phage type 197.

In 17 outbreaks, investigators relied on descriptive epidemiology only, while there were 8 cohort and 4 case control studies conducted. In two outbreaks, no formal epidemiological study was conducted.

Levels of Evidence

OzFoodNet Sites supplied enhanced data for 52% (16/31) of outbreaks, although some information indicating the level of evidence was available from the summary record of the remaining outbreaks. The level of evidence for 16% (5/31) of outbreaks was considered to be strong, while 42% (13/31) of outbreaks were considered to be moderate. The remaining 13 outbreaks were considered to have only weak evidence implicating eggs.

The levels of evidence varied depending on the type of food vehicle under investigation, with raw egg sauces and dressings having stronger evidence (Table 3). Outbreaks where the number of people affected tended to be small or patient recall specifically about brands of eggs purchased was poor were categorised as having less evidence implicating eggs.

Table 3: Number of outbreaks by category of food vehicle and the level of evidence implicating eggs, OzFoodNet, 2001—June 2005 (*n*=31).

Food Vehicle Category	Evidence			Total
	Strong	Moderate	Weak	
Eggs		2	1	3
Mixed dish containing eggs	1		4	5
Raw egg dessert	1	3	1	5
Raw egg sauce/dressing	2	2		4
Suspected raw egg dessert	1		3	4
Suspected eggs		3	1	4
Suspected raw egg sauce/dressing		1	2	3
Vietnamese pork/chicken rolls		2	1	3
Total	**5**	**13**	**13**	**31**

In 22% (7/31) of outbreaks, the suspected food vehicles were unable to be sampled, particularly for outbreaks having weak evidence. In 26% (9/31) outbreaks, investigators identified the specific pathogen in the implicated food, which was more likely to be for outbreaks with moderate or strong evidence (p=0.02).

Enhanced Data

Where OzFoodNet Sites provided enhanced data for egg-associated outbreaks, 75% (12/16) of outbreaks occurred where foods containing uncooked eggs were eaten. In these, 31% (5/16) used whole egg in the suspected food vehicle, while 44% (7/16) used egg yolk only. The components were unknown for the remaining 4 outbreaks. Investigators reported that 31% (5/16) of the eggs in these outbreaks came from a non-commercial source, such as a backyard supplier.

Investigators were able to trace-back the source of the eggs in 63% (10/16) of outbreaks. Trace-back identified a specific farm in 4 instances and a specific shed on a farm in 4 instances. For the 8 of the trace back investigations were a farm was identified, environmental sampling was undertaken. On 63% (5/8) of occasions *Salmonella* was identified in the farm environment. Environmental samples included: drag swabs, boot covers, chicken feed, chicken faeces, egg rinsing and farm and packaging equipment.

Historial Information

Crerar *et al.* published a summary of foodborne gastroenteritis outbreaks from 1980 to 1995. This 16 year summary identified 128 outbreaks, an average of eight outbreaks per year with six deaths in 15 years.[3] The summary identifies 3 outbreaks attributed to eggs, however, no further detail was provided.

Dalton *et al.* collected data on outbreaks from 1995 through to 2000 and identified 214 outbreaks of gastroenteritis of foodborne origin resulting in 8,124 cases of illness.[2] A food vehicle was implicated in 81% (173/214) of outbreaks, with eggs implicated in 4% (9/214) outbreaks. These 9 outbreaks affected 773 people with a mean of 36 persons (Range: 7–500 persons). Of the egg related outbreaks 2 occurred in restaurants, 4 through commercial caterers and 2 in hospital/aged care facilities, 1 outbreak does not identify the setting. However, when all outbreaks containing foods in which egg was the main high-risk ingredient were collated, a total of 16 potentially egg-associated outbreaks were identified. *Salmonella* was the aetiological agent for 14 of the 16 potentially egg-associated outbreaks.

The literature search identified various reports of egg-related outbreaks of salmonellosis in Australia, although these were all included in the summaries prepared by Crerar *et al.*, Dalton *et al.* and the data presented in this current summary. For these reasons we have not reviewed individual reports of outbreaks here.[3,4]

In addition, there were two reports of non-foodborne disease outbreaks of salmonellosis in the literature where young children had hatched eggs at childcare centres and a pre-school.[13,14]

Discussion

This review summarises foodborne disease outbreaks associated with eggs in Australia and highlights some foods and pathogens that are common causes of these outbreaks. We found that eggs may be responsible for 7% of all foodborne disease outbreaks. *Salmonella* was the predominant cause of these outbreaks, and particularly serotype Typhimurium. The majority of *S.* Typhimurium outbreaks were associated with phage type 135, which has been one of the most common phage types isolated from both humans and chicken environments for several years in Australia.[2,12]

In many other countries, egg-associated outbreaks are very common due to trans-ovarian transmission of *S.* Enteritidis. *S.* Typhimurium reportedly has a lower potential for trans-ovarian transmission in layer flocks than *S.* Enteritidis, meaning that outbreaks in Australia may be occurring from surface contamination of eggs or at very low rates of trans-ovarian transmission.[8] The non-Typhimurium serotypes we identified as a cause of outbreaks in Australia were also commonly reported as a cause of human illness associated with eggs overseas.[6,15]

Surveillance of outbreaks is important for identifying emerging causes of disease in both humans and animal reservoirs. For egg-related disease, public health agencies carefully monitor outbreaks to identify the incursion of *S.* Enteritidis into egg laying flocks. Currently, *S.* Enteritidis is not endemic in Australian layer flocks and phage types that are commonly associated with trans-ovarian transmission are rarely isolated in Australia. This was confirmed in our summary with only one outbreak of *S.* Enteritidis phage type 26 var reported in an aged care facility where consumption of eggs from an *S.* Enteritidis phage type 26 infected flock was suspected as the cause.

Outbreak investigators are increasingly using trace back to identify the original source of infection.[16,17] These trace back investigations are very difficult, particularly where the food items are common and specific brands are difficult for patients to recall.[17] It is not always possible to identify batches through the food production chain. In our summary, 63% of outbreaks were able to trace back the sources of eggs, although in only 8 instances were investigators able to identify a specific farm. Approximately one third of outbreaks related to non-commercial supplies of eggs where there may be a higher risk of *Salmonella* contamination from cracked or dirty eggs and no quality assurance is applied. However, it is important to note that outbreaks relating to smaller production systems, specialty brands and eggs produced at home are much easier to recognise.

As mentioned previously, it can be very difficult to identify key ingredients responsible for illness, or critical factors contributing to an outbreak. This is particularly true with egg related outbreaks, when they are used as an ingredient in many dishes and often in a raw or partially cooked form. This was highlighted in this review by the large number of outbreaks that investigators "suspected" to be due to eggs. Several outbreaks were included in this review where the investigators implicated eggs only in the comments summarising the individual outbreak.

To account for this we attempted to ascribe levels of evidence to individual outbreak investigations. In many respects the individual criteria we used were a marker for how thoroughly outbreaks were able to be investigated. Approximately 42% of outbreaks had only weak evidence implicating eggs. The criteria we used may not have been ideal to identify outbreaks with higher quality evidence, as this was a retrospective collection of data. In the future, outbreak investigators need to specifically attempt to obtain standard information about outbreaks. The attribution of lower categories of evidence did not mean that public health agencies did not investigate well, but reflect the difficulty of identifying a specific ingredient as the cause of an outbreak rather than a food vehicle, and being able to conduct trace back.

It is important to recognise that there are considerably more sporadic cases of salmonellosis than those associated with outbreaks. An estimated 81,000 cases of foodborne salmonellosis occur each year in Australia; the majority of which do not visit a doctor or receive a laboratory-confirmed diagnosis of *Salmonella* infection. The majority of notified cases are not part of recognised outbreaks. In 2004, there were 7,842 cases of salmonellosis reported to the National Notifiable Diseases Surveillance System compared with 679 cases reported as part of 36 outbreaks to OzFoodNet, which shows the large number of potentially sporadic cases.[13,19] Outbreak related cases only represent a small fraction of all cases, despite improved recognition and reporting of outbreaks since the establishment of OzFoodNet. For these reasons, there is a need to exercise caution when generalising about the causes of community-acquired *Salmonella* infections from the summary results of outbreak investigations.

Conclusions

Approximately 7% of foodborne disease outbreaks in Australia during 2001–June 2005 were potentially caused by egg-based foods. *Salmonella* was the most common pathogen causing these outbreaks. It is important that public health agencies and departments of primary industry recognise the zoonotic basis for these outbreaks, which often have a complex pathway via food to humans. While some disease may be due to preparation errors, such as cross contamination, there are certain foods containing eggs that present a higher risk to the public. Of the egg-containing foods associated with foodborne illness, sauces and desserts containing raw or lightly cooked eggs were frequently reported. Improved surveillance at the primary production level and targeted interventions may reduce the likelihood of infections occurring in the community.

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Appendix 6A: Outbreaks of salmonellosis associated with eggs or suspected eggs in Australia, 2001–June 2005.

State	Year	Setting	Ill	Hospitalised	Food Vehicle	Food Vehicle Category[19]	Epidemiological Methods	Aetiology	Comments	Evidence	Trace back
VIC	2002	Bakery	20	0	Pork Rolls	Vietnamese pork/chicken rolls	Case Series	*S.* Typhimurium 135	Isolate had resistance to ampicillin. Other community acquired cases occurring.	Weak	Not Supplied
	2002	Private Residence	6	6	Hedgehog - Eggs Cookie Dough Raw	Raw egg dessert	Case Series	*S.* Typhimurium 170	Left over hedgehog was positive for *S.* Typhimurium 170	Moderate	Not Supplied
	2002	Restaurant	3	1	Unknown	Suspected raw egg sauce/dressing	Case Series	*S.* Hessarek	Raw egg mayonnaise common food consumed	Weak	Not Supplied
	2002	Restaurant	4		Unknown	Suspected raw egg sauce/dressing	Case Series	*S.* Typhimurium 135	Four cases linked to the same food premises. No common food, but 2 cases consumed raw egg mayonnaise.	Weak	Unknown
	2003	Bakery	213	22	Pork Rolls	Vietnamese pork/chicken rolls	Case Series	*S.* Typhimurium 135	Raw egg butter ingredient positive for *S.* Typhimurium 135, along with other ingredients.	Moderate	Unknown
	2003	Restaurant	54	4	Raw Egg Dish	Eggs	Point Source Cohort	*S.* Typhimurium 170	Randomised cohort study to sample from >800 people exposed	Moderate	Yes
	2004	Primary Produce	13	5	Eggs	Eggs	Case Series	*S.* Typhimurium 126	8 cases consumed the same brand of organic free range eggs. Farm testing negative for *Salmonella*. Two other cases shared a raw egg smoothie	Moderate	Yes
	2004	Restaurant	8	1	Hollandaise Sauce	Raw egg sauce/dressing	Case Series	*S.* Typhimurium 9	All 8 cases ate eggs with hollandaise sauce	Moderate	Yes
	2005	Private Residence	5	1	Suspected Chocolate Mousse	Suspected egg dessert	Case Series	*S.* Typhimurium 126	All cases ate the chocolate mousse	Weak	Yes
	2005	Restaurant	13	5	Hollandaise Sauce	Raw egg sauce/dressing	Case Series	*S.* Typhimurium 9	Trace back to farm where *S.* Typhimurium 9 was isolated	Strong	Yes

[19] 'Food vehicle category' was decided based on review of 'food vehicle' and the 'comments' fields in the OzFoodNet outbreak register. Where comments field mentioned that investigators suspected eggs and the food vehicle was 'unknown' we coded the 'food vehicle category' as 'suspected eggs'.

State	Year	Setting	Ill	Hospitalised	Food Vehicle	Food Vehicle Category[19]	Epidemiological Methods	Aetiology	Comments	Evidence	Trace back
	2005	Aged Care	7	2	Suspect Eggs	Suspected eggs	Case Series	*S.* Enteritidis 26 var	Two cases positive for *S.* Enteritidis 26 var. A food source for the outbreak not identified as patients were demented. Trace back of eggs to company with positive farm	Moderate [20]	Not Supplied
	2005	Commercial Caterer	14	6	Suspected Chocolate Mousse	Suspected egg dessert	Point Source Cohort	*S.* Typhimurium 9	Children's cooking class. Trace back to farm where *S.* Typhimurium 9 was isolated	Strong	Yes
SA	2001	Private Residence	11	2	Tiramasu	Raw egg dessert	Case Control Study	*S.* Typhimurium 135a	Tiramisu made with raw egg. Eggs obtained from friend	Strong	Not Supplied
	2001	Bakery	16	3	Pastry Custard Tart With Strawberries & Jelly Glaze	Raw egg dessert	Case Control Study	*S.* Typhimurium 126	Unable to identify original source of infection	Moderate	Not Supplied
	2001	Aged Care	18	3	Meat Based Potato Pie, Rice Pudding (Both Containing Raw Eggs)	Mixed dish containing eggs	No Formal Study	*S.* Typhimurium 135	*S.* Typhimurium 135 detected in patient's and staff faeces, food vehicle, and chickens from flock supplying eggs.	Strong	Not Supplied
	2004	Private Residence	8	1	Boiled Eggs	Eggs	Point Source Cohort	*S.* Saintpaul	Unboiled eggs negative for *Salmonella*.	Weak	Not Supplied
	2004	Private Residence	5	1	Homemade Icecream	Raw egg dessert	Case Series	*S.* Typhimurium 9	Raw egg used in the ice cream	Weak	Not Supplied
	2004	Private Residence	8		Potato Bake, Lemon Meringue, Chicken Patty	Mixed dish containing eggs	Point Source Cohort	*S.* Typhimurium 108	Eggs used in lemon meringue and chicken pattie were negative for *Salmonella*.	Weak	Not Supplied
QLD	2001	Aged Care	12	6	Unknown	Suspected eggs	Point Source Cohort	*S.* Heidelberg 1	Eggs used in raw egg flips suspected. Food and environmental samples negative for *Salmonella*	Weak	Unknown
	2002	Restaurant	3	0	Asparagus Egg Surprise Dish	Mixed dish containing eggs	Case Series	*S.* Hadar 22	No source identified.	Weak	Unknown

[20] Evidence categorised as 'moderate' due to the rarity of *S.* Enteritidis 26 infections in Southern Australian States combined with consumption of eggs from interstate where a company had reported a *S.* Enteritidis 26 flock, which is also an extremely rare strain in commercial layer flocks.

State	Year	Setting	Ill	Hospitalised	Food Vehicle	Food Vehicle Category[19]	Epidemiological Methods	Aetiology	Comments	Evidence	Trace back
	2002	Private Residence	10	8	Salmon/Egg/Onion/Rice Patties	Mixed dish containing eggs	Case Series	*S.* Typhimurium 135a	Salmon/egg/onion/rice patties probably undercooked.	Weak	Unknown
	2002	Child Care	12	0	Unknown	Suspected eggs	Case Series	*S.* Typhimurium 135	Sandwiches made with eggs sourced from local farm, suspected source. Eggs were not cleaned and *S.* Typhimurium 135a isolated from 2/3 poultry sheds.	Moderate	Yes
	2003	Restaurant	18	3	Not Identified	Suspected raw egg sauce/dressing	Case Control Study	*S.* Typhimurium 135	Uncooked eggs in a hollandaise sauce.	Moderate	Yes
	2003	Aged Care	47	16	Suspected Raw Egg	Suspected eggs	Case Series	*S.* Typhimurium 135a	Suspected use of raw eggs was source of illness.	Moderate	Yes
	2003	Private Residence	6	1	Unknown	Suspected egg dessert	Case Series	*S.* Typhimurium	Coffee mousse made using raw commercial eggs.	Weak	Unknown
	2004	Bakery	5	0	Custard Fruit Tart	Raw egg dessert	Case Series	*S.* Typhimurium 135a	Custard fruit tarts and apple tarts from bakery was common food among the cases. Almond sauce added to fruit tarts was the suspected source.	Moderate	Yes
NSW	2001	Restaurant	17	11	Mayonnaise, Chicken	Mixed dish containing eggs	Case Control Study	*S.* Typhimurium 126		Weak	Not Supplied
	2002	Bakery	32	8	Pork/Chicken Rolls From Bakery	Vietnamese pork/chicken rolls	No Formal Study	*S.* Typhimurium 126		Moderate	Not Supplied
	2002	Restaurant	17	2	Caesar Dressing/ Dill Mayonnaise	Raw egg sauce/dressing	Point Source Cohort	*S.* Potsdam	Point source outbreak implicating dressings, along with trace back to farm, which was negative for specific serotype.	Strong	Yes
	2003	Restaurant	41	0	Mayonnaise	Raw egg sauce/dressing	Point Source Cohort	*S.* Typhimurium		Moderate	Not Supplied
	2004	Institution	43	10	Custard	Suspected egg dessert	Point Source Cohort	*S.* Typhimurium 135		Weak	Not Supplied

APPENDIX 7 Maximum Residue Limits

Residue limits for agricultural and veterinary chemicals approved in eggs in food as of August 2009.

Chemical	Residue description	Product	MRL (mg/kg)
Schedule 1 – Maximum Residue Limits			
Acephate	Acephate	Eggs	0.2
Acetamiprid	Sum of Acetamiprid and N-dimethyl acetamiprid ((E)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyanoacetamidine), expressed as acetamiprid	Eggs	*0.01
Acibenzolar-S-methyl	Acibenzolar-S-methyl and all metabolites containing the benzo[1,2,3]thiadiazole-7-carboxyl moiety hydrolysed to benzo[1,2,3]thiadiazole-7-carboxylic acid, expressed as acibenzolar-S-methyl	Eggs	*0.02
Acifluorfen	Acifluorfen	Eggs	*0.01
Aldoxycarb	Sum of aldoxycarb and its sulfone, expressed as aldoxycarb	Eggs	0.1
Aminopyralid	Aminopyralid	Eggs	*0.01
Amoxycillin	Inhibitory substance, identified as amoxycillin	Eggs	T*0.01
Amprolium	Amprolium	Eggs	4
Azamethiphos	Azamethiphos	Eggs	*0.05
Azimsulfuron	Azimsulfuron	Eggs	*0.02
Azoxystrobin	Azoxystrobin	Eggs	*0.01
Bacitracin	Inhibitory substance, identified as bacitracin	Eggs	*0.5
Bendiocarb	Sum of conjugated and unconjugated Bendiocarb, 2,2-dimethyl-1,3-benzodioxol-4-ol and N-hydroxymethylbendiocarb, expressed as Bendiocarb	Eggs	0.05
Bentazone	Bentazone	Eggs	*0.05
Bifenthrin	Bifenthrin	Eggs	*0.05
Bitertanol	Bitertanol	Eggs	*0.01
Bromoxynil	Bromoxynil	Eggs	*0.02
Butafenacil	Butafenacil	Eggs	*0.01
Butroxydim	Butroxydim	Eggs	*0.01
Captan	Captan	Eggs	*0.02
Carbaryl	Carbaryl	Eggs	T0.2
Carbendazim	Sum of carbendazim and 2-aminobenzimidazole, expressed as carbendazim	Eggs	*0.1
Carbetamide	Carbetamide	Eggs	*0.1
Carbofuran	Sum of carbofuran and 3-hydroxycarbofuran, expressed as carbofuran	Eggs	*0.05
Carfentrazone-ethyl	Carfentrazone-ethyl	Eggs	*0.05
Chlorfenapyr	Chlorfenapyr	Eggs	*0.01
Chlorfluazuron	Chlorfluazuron	Eggs	0.2
Chlormequat	Chlormequat cation	Eggs	0.1
Chlorpyrifos	Chlorpyrifos	Eggs	T*0.01
Chlorpyrifos-methyl	Chlorpyrifos-methyl	Eggs	*0.05
Chlorthal-dimethyl	Chlorthal-dimethyl	Eggs	*0.05

Chemical	Residue description	Product	MRL (mg/kg)
Chlortetracycline	Inhibitory substance, identified as chlortetracycline	Eggs	0.2
Clodinafop-propargyl	Clodinafop-propargyl	Eggs	*0.05
Clodinafop acid	(R)-2-[4-(5-chloro-3-fluoro-2-pyridinyloxy) phenoxy] propanoic acid	Eggs	*0.1
Cloquintocet-mexyl	Sum of cloquintocet mexyl and 5-chloro-8-quinolinoxyacetic acid, expressed as cloquintocet mexyl	Eggs	*0.1
Clothianidin	Clothianidin	Eggs	*0.02
Cyclanilide	Sum of cyclanilide and its methyl ester, expressed as cyclanilide	Eggs	*0.01
Cyfluthrin	Cyfluthrin, sum of isomers	Eggs	*0.01
Cyhalofop-butyl	Sum of cyhalofop-butyl, cyhalofop and metabolites expressed as cyhalofop-butyl	Eggs	*0.05
Cyhalothrin	Cyhalothrin, sum of isomers	Eggs	*0.02
Cypermethrin	Cypermethrin, sum of isomers	Eggs	0.05
Cyproconazole	Cyproconazole, sum of isomers	Eggs	*0.01
Cyromazine	Cyromazine	Eggs	0.2
2,4-D	2,4-D	Eggs	*0.05
Daminozide	Daminozide	Eggs	0.2
2,4-DB	2,4-DB	Eggs	*0.05
Deltamethrin	Deltamethrin	Eggs	*0.01
Diafenthiuron	Sum of diafenthiuron; N-[2,6-bis(1-methylethyl)- 4-phenoxyphenyl]-N'-(1,1-dimethylethyl)urea; and N-[2,6-bis(1-methylethyl)-4-phenoxyphenyl]- N'-(1,1-dimethylethyl)carbodiimide, expressed as diafenthiuron	Eggs	*0.02
Diazinon	Diazinon	Eggs	*0.05
Dicamba	Dicamba	Eggs	*0.05
Dichlorprop-P	Sum of dichlorprop acid, its esters and conjugates, hydrolysed to dichlorprop acid, and expressed as dichlorprop acid	Eggs	*0.02
Dichlorvos	Dichlorvos	Eggs	0.05
Diclofop-methyl	Diclofop-methyl	Eggs	*0.05
Difenoconazole	Difenoconazole	Eggs	*0.05
Diflufenican	Diflufenican	Eggs	*0.02
Dimethenamid-P	Sum of dimethenamid-P and its *(R)*-isomer	Eggs	*0.01
Dimethipin	Dimethipin	Eggs	*0.02
Dimethoate	Sum of dimethoate and omethoate, expressed as dimethoate *see also* Omethoate	Eggs	*0.05
Dimetridazole	Sum of dimetridazole and its hydroxy metabolite (2-hydroxymethyl-1-methyl-5-nitroimidazole), expressed as dimetridazole	Eggs	T*0.0001
Diphenylamine	Diphenylamine	Eggs	0.05
Diquat	Diquat cation	Eggs	*0.01
Disulfoton	Sum of disulfoton and demeton-S and their sulfoxides and sulfones, expressed as disulfoton	Eggs	*0.02
Dithiocarbamates	Total dithiocarbamates, determined as carbon disulphide evolved during acid digestion and expressed as milligrams of carbon disulphide per kilogram of food	Eggs	*0.5
Endosulfan	Sum of A- and B- endosulfan and endosulfan sulphate	Eggs	0.02
Epoxiconazole	Epoxiconazole	Eggs	T*0.01
EPTC	EPTC	Eggs	*0.01
Ethametsulfuron methyl	Ethametsulfuron methyl	Eggs	*0.02
Ethephon	Ethephon	Eggs	*0.2
Etoxazole	Etoxazole	Eggs	*0.01
Fenamiphos	Sum of fenamiphos, its sulfoxide and sulfone, expressed as fenamiphos	Eggs	*0.05
Fenbuconazole	Fenbuconazole	Eggs	*0.01
Fenitrothion	Fenitrothion	Eggs	*0.05

Chemical	Residue description	Product	MRL (mg/kg)
Fenoxaprop-ethyl	Sum of fenoxaprop-ethyl (all isomers) and 2-(4-(6-chloro-2-benzoxazolyloxy)phenoxy)-propanoate and 6-chloro-2,3-dihydrobenzoxazol-2-one, expressed as fenoxaprop-ethyl	Eggs	*0.02
Fenthion	Sum of fenthion, its oxygen analogue, and their sulfoxides and sulfones, expressed as fenthion	Eggs	*0.05
Fenvalerate	Fenvalerate, sum of isomers	Eggs	0.02
Fipronil	Sum of fipronil, the sulphenyl metabolite (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl) sulphenyl]-1H-pyrazole-3-carbonitrile), the sulphonyl metabolite (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulphonyl]-1H-pyrazole-3-carbonitrile), and the trifluoromethyl metabolite (5-amino-4-trifluoromethyl-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile)	Eggs	0.02
Flavophospholipol	Flavophospholipol	Eggs	*0.02
Florasulam	Florasulam	Eggs	*0.01
Fluazifop-butyl	Fluazifop-butyl	Eggs	*0.05
Flucythrinate	Flucythrinate	Eggs	*0.05
Flumetsulam	Flumetsulam	Eggs	*0.1
Flumiclorac pentyl	Flumiclorac pentyl	Eggs	*0.01
Flumioxazin	Flumioxazin	Eggs	*0.01
Fluquinconazole	Fluquinconazole	Eggs	*0.02
Fluroxypyr	Fluroxypyr	Eggs	*0.01
Flutolanil	Flutolanil and metabolites hydrolysed to 2-trifluoromethyl-benzoic acid and expressed as flutolanil	Eggs	*0.05
Flutriafol	Flutriafol	Eggs	*0.05
Glufosinate and Glufosinate-ammonium	Sum of glufosinate-ammonium, N-acetyl glufosinate and 3-[hydroxy(methyl)-phosphinoyl] propionic acid, expressed as glufosinate (free acid)	Eggs	*0.05
Glyphosate	Sum of glyphosate and Aminomethylphosphonic acid (AMPA) metabolite, expressed as glyphosate	Eggs	*0.05
Haloxyfop	Sum of haloxyfop, its esters and conjugates, expressed as haloxyfop	Eggs	*0.01
Hexazinone	Hexazinone	Eggs	*0.05
Imazapic	Sum of imazapic and its hydroxymethyl derivative	Eggs	*0.01
Imazethapyr	Imazethapyr	Eggs	*0.1
Imidacloprid	Sum of imidacloprid and metabolites containing the 6-chloropyridinylmethylene moiety, expressed as imidacloprid	Eggs	*0.02
Indoxacarb	Indoxacarb	Eggs	*0.01
Iodosulfuron methyl	Iodosulfuron methyl	Eggs	*0.01
Isoxaben	Isoxaben	Eggs	*0.01
Isoxaflutole	The sum of isoxaflutole, 2-cyclopropylcarconyl-3-(2-methylsulfonyl-4-trifluoromethylphenyl)-3-oxopropanenitrile and 2-methylsulfonyl-4-trifluoromethylbenzoic acid expressed as isoxaflutole	Eggs	T*0.05
Kitasamycin	Inhibitory substance, identified as kitasamycin	Eggs	*0.2
Lasalocid	Lasalocid	Eggs	*0.05
Levamisole	Levamisole	Eggs	1
Lincomycin	Inhibitory substance, identified as lincomycin	Eggs	0.2
Linuron	Sum of linuron plus 3,4-dichloroaniline, expressed as linuron	Eggs	*0.05
Lufenuron	Lufenuron	Eggs	T0.05
Maldison	Maldison	Eggs	1
MCPA	MCPA	Eggs	*0.05
MCPB	MCPB	Eggs	*0.05
Mecoprop	Mecoprop	Eggs	*0.05
Mefenpyr-diethyl	Mefenpyr-diethyl	Eggs	*0.01

Chemical	Residue description	Product	MRL (mg/kg)
Mepiquat	Mepiquat	Eggs	0.05
Mesosulfuron-methyl	Mesosulfuron-methyl	Eggs	*0.01
Metalaxyl	Metalaxyl	Eggs	*0.05
Methidathion	Methidathion	Eggs	*0.05
Methomyl	Sum of methomyl and methyl hydroxythioacetimidate ('methomyl oxime'), expressed as methomyl *see also* thiodicarb	Eggs	*0.02
Metolachlor	Metolachlor	Eggs	*0.01
Metosulam	Metosulam	Eggs	*0.01
Metribuzin	Metribuzin	Eggs	*0.05
Neomycin	Inhibitory substance, identified as neomycin	Eggs	T0.5
Omethoate	Omethoate *see also* Dimethoate	Eggs	*0.05
Oxabetrinil	Oxabetrinil	Eggs	*0.1
Oxamyl	Sum of oxamyl and 2-hydroxyimino-N,N-dimethyl-2-(methylthio)-acetamide, expressed as oxamyl	Eggs	*0.02
Oxydemeton-methyl	Sum of oxydemeton-methyl and demeton-S-methyl sulphone, expressed as oxydemeton-methyl	Eggs	*0.01
Oxyfluorfen	Oxyfluorfen	Eggs	0.05
Paraquat	Paraquat cation	Eggs	*0.01
Pendimethalin	Pendimethalin	Eggs	*0.01
Permethrin	Permethrin, sum of isomers	Eggs	0.1
Phenothrin	Sum of phenothrin (+)cis- and (+)trans-isomers	Eggs	*0.5
Phorate	Sum of phorate, its oxygen analogue, and their sulfoxides and sulfones, expressed as phorate	Eggs	*0.05
Picolinafen	Sum of picolinafen and 6-[3-trifluoromethyl phenoxy]-2-pyridine carboxylic acid	Eggs	*0.01
Pinoxaden	Sum of 8-(2,6-diethyl-4-methylphenyl)-tetrahydro-pyrazolo [1,2-d][1,4,5] oxadiazepine-7,9-dione and 8-(2,6-diethyl-4-hydroxymethylphenyl)-tetrahydro-pyrazolo [1,2-d][1,4,5] oxadiazepine-7,9-dione, expressed as pinoxaden	Eggs	*0.02
Piperonyl butoxide	Piperonyl butoxide	Eggs	*0.1
Pirimicarb	sum of pirimicarb, dimethyl-pirimicarb and N-formyl-(methylamino) analogue (dimethylformamidio-pirimicarb), expressed as pirimicarb	Eggs	*0.1
Pirimiphos-methyl	Pirimiphos-methyl	Eggs	*0.05
Procymidone	Procymidone	Eggs	T*0.01
Profenofos	Profenofos	Eggs	*0.02
Propachlor	Propachlor	Eggs	*0.02
Propanil	Propanil	Eggs	*0.1
Propargite	Propargite	Eggs	*0.1
Propiconazole	Propiconazole	Eggs	*0.05
Propyzamide	Propyzamide	Eggs	*0.05
Prosulfocarb	Prosulfocarb	Eggs	*0.02
Prothioconazole	Sum of prothioconazole, prothioconazole desthio (2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)-propan-2-ol), prothioconazole-3-hydroxy-desthio (2-(1-chlorocyclopropyl)-1-(2-chloro-3-hydroxyphenyl)-3-(1*H*-1,2,4-triazol-1-yl)-propan-2-ol) and prothioconazole-4-hydroxy-desthio (2-(1-chlorocyclopropyl)-1-(2-chloro-4-hydroxyphenyl)-3-(1*H*-1,2,4-triazol-1-yl)-propan-2-ol), expressed as prothioconazole	Eggs	*0.01
Pymetrozine	Pymetrozine	Eggs	*0.01
Pyraclostrobin	Sum of pyraclostrobin and metabolites hydrolysed to 1-(4-chloro-phenyl)-1H-pyrazol-3-ol, expressed as pyraclostrobin	Eggs	*0.05
Pyraflufen-ethyl	Sum of pyraflufen-ethyl and its acid metabolite (2-chloro-5-	Eggs	*0.02

Chemical	Residue description	Product	MRL (mg/kg)
	(4-chloro-5-difluoromethoxy-1-methylpyrazol-3-yl)-4-fluorophenoxyacetic acid		
Pyrasulfotole	Sum of pyrasulfotole and (5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)[2-mesyl-4-(trifluoromethyl)phenyl]methanone, expressed as pyrasulfotole	Eggs	*0.01
Pyridate	sum of pyridate and metabolites containing 6 chloro-4-hydroxyl-3-phenyl pyridazine, expressed as pyridate	Eggs	*0.2
Pyrithiobac sodium	Pyrithiobac sodium	Eggs	*0.02
Pyriproxyfen	Pyriproxyfen	Eggs	0.05
Quizalofop-ethyl	Sum of quizalofop-ethyl and quizalofop acid and other esters, expressed as quizalofop-ethyl	Eggs	*0.02
Quizalofop-p-tefuryl	sum of quizalofop-p-tefuryl and quizalofop acid, expressed as quizalofop-p-tefuryl	Eggs	*0.02
Salinomycin	Salinomycin	Eggs	*0.02
Sethoxydim	Sum of sethoxydim and metabolites containing the 5-(2-ethylthiopropyl)cyclohexene-3-one and 5-(2-ethylthiopropyl)-5-hydroxycyclohexene-3-one moieties and their sulfoxides and sulfones, expressed as sethoxydim	Eggs	*0.05
Simazine	Simazine	Eggs	*0.01
Spectinomycin	Inhibitory substance, identified as spectinomycin	Eggs	2
Spinosad	Sum of spinosyn A and spinosyn D	Eggs	T0.05
Sulfosulfuron	Sum of sulfosulfuron and its metabolites which can be hydrolysed to 2-(ethylsulfonyl)imidazo[1,2-a]pyridine, expressed as sulfosulfuron	Eggs	*0.005
Sulphadiazine	Sulphadiazine	Eggs	T*0.02
Sulphadimidine	Sulphadimidine	Eggs	T*0.01
Sulphaquinoxaline	Sulphaquinoxaline	Eggs	T*0.01
Tebuconazole	Tebuconazole	Eggs	0.1
Tepraloxydim	Sum of tepraloxydim and metabolites converted to 3-(tetrahydro-pyran-4-yl) glutaric and 3-hydroxy-3-(tetrahydro-pyran-4-yl)-glutaric acid, expressed as tepraloxydim	Eggs	*0.1
Terbufos	Sum of terbufos, its oxygen analogue and their sulfoxides and sulfones, expressed as terbufos	Eggs	*0.01
Terbutryn	Terbutryn	Eggs	*0.05
Thiamethoxam	: Sum of thiamethoxam and N-(2-chloro-thiazol-5-ylmethyl)-N'-methyl-N'-nitro-guanidine, expressed as thiamethoxam	Eggs	*0.02
Thifensulfuron	Thifensulfuron	Eggs	*0.01
Thiometon	Sum of thiometon, its sulfoxide and sulfone, expressed as thiometon	Eggs	*0.05
Toltrazuril	Sum of toltrazuril, its sulfoxide and sulfone, expressed as toltrazuril	Eggs	T*0.05
Triadimefon	Sum of triadimefon and triadimenol, expressed as triadimefon *see also* Triadimenol	Eggs	*0.1
Triadimenol	Triadimenol *see also* Triadimefon	Eggs	*0.01
Triasulfuron	Triasulfuron	Eggs	*0.05
Trichlorfon	Trichlorfon	Eggs	*0.05
Trifloxysulfuron sodium	Trifloxysulfuron	Eggs	*0.01
Triflumuron	Triflumuron	Eggs	0.01
Trifluralin	Trifluralin		*0.05
Trimethoprim	Trimethoprim	Eggs	T*0.02
Triticonazole	Triticonazole	Eggs	*0.05
Tylosin	Tylosin A	Eggs	*0.2
Virginiamycin	Inhibitory substance, identified as virginiamycin	Eggs	*0.1

Chemical	Residue description	Product	MRL (mg/kg)
Schedule 2: Extraneous residue limits			
Aldrin and Dieldrin	Sum of HHDN and HEOD	Eggs	E0.1
BHC (other than the gamma isomer, Lindane)	Sum of isomers of 1,2,3,4,5,6-hexachlorocyclohexane, other than lindane	Eggs	E0.1
Chlordane	Sum of cis- and trans-chlordane and in the case of animal products also includes 'oxychlordane'	Eggs	E0.02
DDT	Sum of p,p '-DDT; o,p '-DDT; p,p '-DDE and p,p '-TDE (DDD)	Eggs	E0.5
HCB	Hexachlorobenzene	Eggs	E1
Heptachlor	Sum of heptachlor and heptachlor epoxide	Eggs	E0.05
Lindane	Lindane	Eggs	E0.1

'*' denotes that the maximum residue limit or the extraneous residue limit is set at or about the limit of determination.
'T' denotes that the maximum residue limit or the extraneous residue limit is a temporary maximum residue limit or extraneous residue limit.
'E' denotes an extraneous residue limit.