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

A Risk Profile of Dairy Products in Australia

Food Standards Australia New Zealand

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EXECUTIVE SUMMARY

The Risk Profile of Dairy Products in Australia brings together information on microbiological and chemical risks that may be associated with dairy products. The risk profile was undertaken within the framework of existing dairy regulations and risk management practices in Australia and comprises two separate parts:

- **Part A: Microbiological Risk Profile**
- **Part B: Chemical Risk Profile**

The purpose of this risk profile is to inform the development of the Primary Production and Processing Standard for dairy products. To this end, the two risk profiles provide an objective analysis of scientific data and information to identify the public health and safety risks arising from microbiological and chemical hazards associated with dairy products.

The scope of the Risk Profile includes the identification and examination of risks along the dairy supply chain from milk production through to consumption of dairy products.

The key findings of the **Microbiological Risk Profile (Part A)** can be summarised as:

- Australian dairy products have an excellent reputation for food safety, and this is supported by the lack of evidence attributing food-borne illness to dairy products;
- A wide range of microbiological hazards may be associated with raw milk and dairy products, but these do not represent a problem under current management practices which:
  - control animal health;
  - ensure adherence to good milking practices;
  - require effective heat treatment e.g. pasteurisation; and
  - have controls to prevent post-pasteurisation contamination in the dairy processing environment.

The key findings of the **Chemical Risk Profile (Part B)** can be summarised as:

- There are extensive regulatory and non-regulatory measures in place along the dairy industry primary production chain resulting in minimal public health and safety concerns regarding the use or presence of chemicals in dairy products.
- Extensive monitoring of chemical residues in milk over many years has demonstrated a high level of compliance with the regulations.
- There are a number of areas where further research or monitoring would assist in providing further reassurance that the public health and safety risk is low.
- Continuation of the current management practices, particularly monitoring programs for chemicals along the primary production chain, will ensure that the dairy industry continues to maintain a high standard of public health and safety.

Part A - The Microbiological Risk Profile

The microbiological risk profile encompasses a detailed discussion of the following elements:

- Microorganisms that may be associated with dairy products including key attributes of each organism and its public health impact;
- Epidemiological data related to the consumption of dairy products and illness;
- An examination of prevalence and concentration data on microbiological hazards found in dairy products and along the entire dairy food chain; and
- A description of the dairy production, dairy processing, distribution and consumption chain and a description of factors that impact on the public health and safety risks associated with microbiological hazards in dairy products.

The safety of dairy products relies on the quality of raw materials, correct formulation, effective processing, the prevention of recontamination of product, and maintenance of temperature control during distribution, retail sale and storage of the product in the home.

There is relatively little data on the presence of pathogens in raw milk in Australia although it is well established that raw milk may be contaminated with pathogenic microorganisms.

Raw milk has a mixed microflora, which is derived from several sources including the interior of the udder, exterior surfaces of the animals, environment, milk-handling equipment, and personnel. In general, there are two means by which pathogens contaminate raw milk. Contamination may occur when microorganisms are shed directly into raw milk from the udder as a result of illness or disease, or through contamination from the external surface of the cow and the milking environment. Primary production factors that impact on these routes of contamination and the microbiological quality of the raw milk include:

- animal-related factors e.g. animal health, herd size, age and production status;
- environment-related factors e.g. housing, faeces, feed, soil, and water; or
- milking and operation of milking equipment factors e.g. cleanliness of equipment and lines.

Some of these primary production factors can be managed to reduce the risk of contamination of raw milk by pathogens, while management of others will have limited impact on the final microbiological status of raw milk.

Following milking, milk is transferred to the dairy processing facility where it subsequently undergoes a series of processes that transform liquid milk into a wide range of dairy products, many of which may be classified as ready-to-eat. The majority of these processes involve a heat-processing stage, typically pasteurisation or an equivalent process. Further steps involve physical processes such as separation, aeration, and homogenisation and product transformation by drying, churning, acidification, etc.

Pasteurisation represents the principal process for rendering dairy products safe for consumption. However, the effectiveness of pasteurisation is dependent upon the microbiological status of the incoming raw milk. Control of risk factors on-farm will minimise the opportunity for microbiological hazards to contaminate raw milk and reduce the likelihood and concentration of these hazards.

A survey of Australian dairy manufacturers determined that all respondents met the minimum time and temperature standards prescribed in the Code for the HTST (high temperature short time) pasteurisation of milk and cream. In many cases, milk was heated to a temperature and/or a time in excess of the prescribed minimums. For the majority of dairy products, pasteurisation also represents an initial treatment before specific processes are used to transform raw milk into various manufactured products.

Dairy products containing elevated levels of fat or solids such as ice-cream mixes, cream and yoghurt, warrant higher time/temperature combinations than those currently specified in the *Australia New Zealand Food Standards Code* (the Code) to compensate for the protective effect of fat and solids on microorganisms.

Post-pasteurisation contamination however, is an ongoing management issue for manufacturers in the provision of safe dairy products. Contamination may result from the environment, including equipment, personnel or contamination of finished product with raw materials. Rigorous control over hygiene, cleaning and sanitation, and product handling is therefore necessary to ensure safety of the final product post-heat treatment.

As many dairy products do not undergo a further pathogen reduction step prior to consumption, prevention of contamination and control over bacterial growth, storage time and temperature is of particular importance in minimising potential exposure to pathogens. Most dairy products have a relatively short shelf-life, especially milk (10-16 days under optimum storage conditions) thus storing dairy products according to manufacturer instructions and following good hygiene and handling practices in the home is also important.

Microbiological survey data for pasteurised dairy products in Australia show a very low incidence of hazards of public health significance in these products. Overseas data demonstrates that pathogens are frequently isolated from raw milk and raw milk products. Pathogens were detected in raw milk in 85% of 126 surveys identified in the literature. In surveys of raw milk cheese pathogens were rarely detected. Pathogens are found infrequently in pasteurised milk and pasteurised milk products.

In Australia, illness from dairy products is rare. Between 1995-2004, there were only eleven reported outbreaks directly attributed to dairy products, eight of which were associated with consumption of unpasteurised milk. In other Australian outbreaks, dairy products were an ingredient of the responsible food vehicle identified as the source of infection. However, dairy products are a component of many foods and it is often difficult to attribute the cause of an outbreak to a particular food ingredient. Microbiological survey data for pasteurised dairy products in Australia show a very low incidence of hazards of public health significance in these products.

While commercial dairy products have rarely been identified as sources of food-borne illness in Australia, there have been a number of reports of outbreaks associated with consumption of dairy products internationally. Unpasteurised dairy products are the most common cause of these dairy-associated outbreaks of illness.

The microbiological risk profile has identified a range of microbiological hazards potentially associated with the Australian dairy supply chain.

The majority of these hazards pose little or no threat to public health because under current risk management conditions they are unlikely to be present in high numbers in raw milk, and the pasteurisation step effectively eliminates all but the spore-forming bacteria. This is supported by the lack of food-borne illness attributed to dairy products in Australia.

While there is a lack of evidence showing food-borne illness attributing illness to pasteurised dairy products, the following organisms can be summarised as the most significant to public health and safety for the dairy industry due to their association with reported incidents of food-borne illness from dairy products and/or their potential to contaminate dairy products post-pasteurisation.

Pathogens	**Significance in dairy products**
Salmonella	*Salmonella* is destroyed by pasteurisation, however it can be present in the environment and can gain access to product after heat treatment. Initial source is often birds and rodents, although occasionally present in the raw milk. Non-dairy ingredients can be an important source of contamination.
Listeria monocytogenes	*L. monocytogenes* is destroyed by pasteurisation. Its presence in heat-treated products is due to post-pasteurisation contamination. *L. monocytogenes* is a concern to the dairy industry as it can grow at 0ºC (refrigeration temperatures).
Staphylococcus. aureus	*S. aureus* is destroyed by heat-treatment, however its toxins are heat stable, thus control of growth of this organism prior to heat treatment is essential. However, *S. aureus* does not grow well at low temperatures (i.e. refrigeration).
Bacillus cereus	Vegetative cells of *B. cereus* do not survive pasteurisation, however spores will survive heat treatments. *B. cereus* is rapidly outgrown by gram-negative psychrotrophs at refrigeration temperatures, but in their absence, *B. cereus*, if present, may then be able to grow to high levels. This is a concern with extended shelf-life chilled products, such as desserts.
Escherichia coli	*E. coli* is found in cattle and may enter milk through faecal contamination, however *E. coli* is heat-sensitive and does not survive pasteurisation.
***Campylobacter* spp.**	*Campylobacter* spp. is destroyed by pasteurisation and its presence in milk products is due to environmental contamination after heat treatment. *Campylobacter* spp. are fragile organisms unable to grow in foods.
Yersinia enterocolitica	*Y. enterocolitica* is destroyed by pasteurisation and its presence in heat-treated milk products is due to environmental contamination after heat treatment. *Y. enterocolitica* is able to grow in dairy products held at refrigeration temperatures and therefore may be considered as a hazard in prolonged shelf-life products.
Enterobacter sakazakii	*E. sakazakii* will not survive pasteurisation. Recontamination of powdered infant formulae during manufacture is a risk. *E. sakazakii* cannot grow in a dry substrate, but it can survive a long period of time and is a potential hazard when the powder is reconstituted and held for long periods of time at favourable temperatures. Contamination and subsequent growth may occur during reconstitution and preparation.

The factors along the Australian dairy supply chain that have the most significant impact on the safety of processed dairy products are:

- the quality of raw materials;
- correct formulation;
- effective processing (pasteurisation in particular);

- the prevention of recontamination of a product; and
- maintenance of temperature control during distribution, retail sale and storage of the product in the home.

The quality of raw milk is dependent on animal health, exposure to faecal contamination, environmental contamination and temperature control.
The key risk factors that may affect the quality of raw milk on-farm can be summarised as follows:

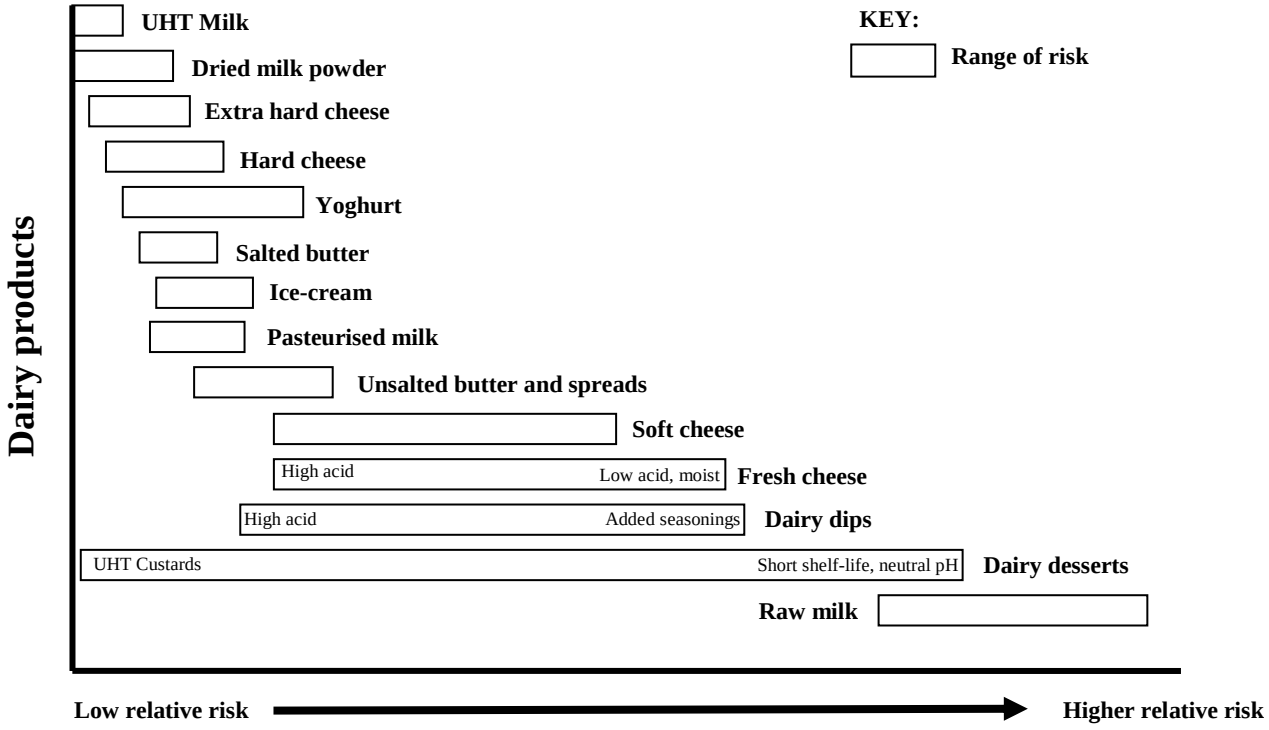
Risk factor	**Effect**
Animal health	Disease in, sickness of, and carriers in milking animals can increase shedding of pathogens directly into raw milk, or in animal faeces.
Herd size	Herd size may have some effect on the prevalence of some pathogens.
Age/production status	Calves have an increased susceptibility to infection.
Housing	Intensive housing practices may increase risk of contamination of udders.
Faeces	Faeces contaminate the udder and may introduce pathogens into raw milk.
Effluent	Effluent can contaminate pasture and the animal.
Feed	Contaminated feed can lead to shedding of pathogens into faeces.
Water-stock drinking	Water sources may be contaminated with cud and/or faecal material
Milking	Poor milking practices *i.e.* dirty teats; inadequate maintenance, sanitation and cleaning of equipment; and poor personal hygiene can lead to contamination of raw milk.
Water use during milking	Potential source of contamination during washing of teats and cleaning of milking equipment.
Storage	Poor temperature control of milk after milking can lead to growth of pathogens.
Transport	Poor temperature control of milk during transport can lead to growth of pathogens. Poor maintenance, sanitation and cleaning of tankers can lead to contamination of milk.

The formulation of dairy products, effective processing, and prevention of recontamination of product all contribute to the level of risk a dairy product poses. Those dairy products, which are prone to contamination after final heat treatment and provide a benign environment, may be categorised as being of higher risk to public heath and safety, than products that don't provide a favourable environment for pathogens. The intrinsic properties of the product *i.e.* the impact of water activity, pH, salt concentration, etc., influence pathogen survival and growth as does the storage environment. The following table provides a relative rating for selected dairy products, based on the inherent stability of the product and therefore the degree of risk they may pose to the consumer.

Risk	**Dairy product**
Higher risk	Soft cheeses Dairy desserts
Intermediate risk	Unsalted butters
Low risk	Yoghurts Salted butters

Risk	Dairy product
	Extra hard cheeses

Qualitative objective methods of describing the relative risk to public health associated with dairy products is extremely difficult. The relative risk from dairy products, based on intrinsic properties of the product, may also be expressed graphically as a continuum:



Part B - The Chemical Risk Profile

The chemical risk profile identifies and examines chemicals introduced along the dairy primary production and processing chain, from the farm environment through to retail dairy produce. Through this whole-chain analysis, an overall picture of the current regulations and controls for chemicals associated with dairy products has been assembled and any inadequacies identified. Issues may however be identified which go beyond the backdoor of retail, and hence are not in the scope of the Dairy Primary Production and Processing Standard.

The profile considers the following:

- Agricultural and veterinary (Agvet) chemicals used in primary production;
- Environmental contaminants, including heavy metals, organic contaminants and micronutrients;
- Naturally-occurring chemicals found in plants or in fungi or bacteria associated with plants;
- Food processing by-products;
- Food additives, processing aids, and those chemicals that may migrate from packaging.

Chemicals used in primary production
Chemicals are used intentionally at the primary production stage for a number of purposes, including pest and weed control, animal health and equipment sanitization. The agricultural chemicals to which cattle are exposed may potentially leave residues. However, the Australian Milk Residue Survey (AMRA) showed that there were no detections of agricultural chemical residues above the maximum residue limit (MRL) in milk in over 33,382 analyses during the seven years of the survey. No residues of agricultural chemicals were found in milk and milk products in the Australian Total Diet Survey (ATDS) either. The low incidence of agricultural chemical residues in cattle is supported by the results of the National Residue Surveys.

Veterinary chemicals administered to dairy cattle are mainly antimicrobials and endo- and ectoparasiticides. Other veterinary chemical uses include reproductive therapy use and use of anti-inflammatory drugs or anaesthetics. During the 1998-2005 period of the AMRA surveys, 89,121 analyses were carried out for antimicrobials with 99.997% compliance with the MRL.

In order to comply with hygienic production and manufacturing practices, cleaning and sanitising agents are utilised throughout the whole production process to ensure that the products remain free from microbial or physical contamination.
The water used on-farm for both agricultural and for cleaning purposes was found to be of high quality and free from chemical contamination.

In addition to the current regulatory and non-regulatory measures in place for chemicals used in primary production, there are areas of uncertainty which have been identified where further data may be necessary to better characterise any potential public health and safety risks (summarised in the Table below):

- Colostrum, which is collected 1-2 days after the birth of a calf for the specific purpose of therapeutics manufacture, may potentially have higher concentrations of agricultural or veterinary chemical residues if critical withholding periods (WHP) before milking are not observed. Monitoring data on Agvet chemical residues would assist in addressing this issue.
- Standard dairy industry practice does not include the first eight milkings (containing colostrum) and Quality Assurance (QA) programs are in place to ensure that colostrum does not enter the milk collection and processing streams.
- There is evidence of substantial off-label usage of veterinary therapeutics for goats, in particular for antihelmintics, which could lead to residues in the milk if the incorrect WHP is used. Monitoring data in goat, sheep and buffalo milk would assist in addressing this issue.
- Sanitizers are an integral part of the dairy production processes, but have the potential to contaminate milk and other dairy products if QA programs fail. Monitoring of valves and manufacturing practices in conjunction with increased training would assist in addressing these concerns.

Chemical Class	Current regulatory and non-regulatory measures chemicals associated with milk	Areas where further measures (regulatory or non-regulatory) may be necessary
Agricultural chemicals (pesticides, herbicides and sanitisers)	• Registration and control of use legislation • Monitoring for residues in milk • Good manufacturing practice/HACCP-based food safety plans	• Monitoring of colostrum (for therapeutics) for Agvet chemicals. • Monitoring of valves and automated processes to ensure removal of sanitizers and cleaning agents
Veterinary chemicals (Ecto- and Endo-parasiticides; other veterinary chemicals)	• Registration and control of use legislation. • Monitoring for residues in milk	• Monitoring of colostrum (for therapeutics) for veterinary chemicals • Monitoring of residues resulting from off-label use for minor dairy species (goat, sheep and buffalo)

Environmental contaminants

Environmental contaminants such as heavy metals and organic chemicals may enter the dairy production chain through stockfeed or though the direct consumption of soil. Stockfeed is an integral factor in dairy production, which may impinge on the quality of milk produced. Stockfeed contamination may also result from the presence of endogenous plant toxicants or mycotoxins, or environmental chemicals.

The available data on arsenic, cadmium, mercury and lead indicate that milk is a very small contributor to the overall dietary intake of these metals and, at the current levels found in milk, there are no public health and safety concerns. Overall, the data suggests that stockfeed and soil do not significantly contribute to heavy metal contamination of milk.

The levels of the micronutrients iodine, selenium and zinc in milk have been examined and do not raise any public health and safety concerns. In the past, iodine content in milk has increased, but the use of iodine has declined with the implementation of alternative, more efficient sanitisers. Selenium and zinc supplementation does not significantly change the micronutrient content of milk. Milk is considered to be an important source of these three micronutrients and has a role in preventing deficiencies for these micronutrients in the community.

Dioxins can occur naturally in the environment although the major source is from industrial practices; the major source of exposure is through the diet. Because of the lipid solubility of dioxins, dairy products can be a significant source of dietary exposure. Although the results of the recent National Dioxin Program indicated that the dietary contribution from dairy products was significant, the overall dietary exposure to dioxins was low and did not raise any public health and safety concerns. PCBs are not naturally occurring but are found at low levels in the environment as a result of industrial activity. PCBs have not been detected in milk in the AMRA survey or in the ATDS.

Plant, fungal or bacterial toxins are potential contaminants in stockfeed. These include aflatoxin, ochratoxin, trichothecene toxins, zearalenone, fumonisin, cyclopiazonic acid, corynetoxins, pyrrolizidine alkaloids, lupin alkaloids, phomopsins and ergot alkaloids. Of these, only aflatoxin M1 is regularly monitored in milk. While earlier data from the Australian Mycotoxin Data Centre survey showed some milk samples with aflatoxin residues, the more recent surveys have not detected any aflatoxin residues in milk.

One area was identified where further data may be necessary to better characterise any potential public health and safety risks (summarised in the Table below):

- In relation to plant, fungal or bacterial toxins, while the information available does not raise any particular public health and safety concerns, additional monitoring of milk would address some of the uncertainty in the current information relating to these toxins.

Chemical class	Current regulatory and non-regulatory measures for chemicals associated with stockfeed	Areas where further measures (regulatory or non-regulatory) may be necessary
Agricultural and veterinary chemicals	• Registration and control of use legislation • Monitoring for residues in stockfeed	
Environmental chemicals Heavy metals, organic chemicals, aflatoxin	• Sound primary production practices • Monitoring for residues in stockfeed	
Plant, fungal and bacterial toxins	• Sound primary production practices	• Monitoring of stockfeed for residues

Chemicals used in processing

At the processing end of the dairy production chain, food additives and processing aids are used in the manufacture of a wide range of dairy products. Food additives may be added to achieve a technological function, such as preservation or colouring, and are present in the final food, whereas processing aids fulfil a technological function during processing, but are not present in the final food.

The use of food additives and processing aids is regulated by the maximum permitted use levels in *the Australia New Zealand Food Standards.* There have been no recorded violations of the Code regarding the use of food additives or processing aids in dairy products. However, there is anecdotal evidence of the use of hydrogen peroxide as a preservative to prolong the shelf-life of cream. Further auditing of adherence to a Food Safety Program would address this potential concern.

Chemical class	Current regulatory and non-regulatory measures	Areas where further measures (regulatory or non-regulatory) may be necessary
Food Additives	• Approval and control of use legislation. • Sound manufacturing practices	• Additional monitoring for unapproved use of hydrogen peroxide as a preservative
Processing Aids	• Approval and control of use legislation. • Sound manufacturing practices	

Chemicals in dairy produce formed during or as a result of processing

Chemicals can be formed within dairy products due to processing or microbiological activity. The levels of biogenic amines and fungal toxins is variable although these toxins would probably only be produced in cheeses under circumstances where the microbial load was imbalanced, and temperature control and storage was not optimal. There is some data from case studies that indicates that there is potential for public health and safety concern for some individuals.

Polycyclic aromatic hydrocarbons (PAH) are by-products of cooking processes and have been found in small quantities in smoked cheeses, although exposure to PAHs through dairy products is considered to be low.

At the end of the production chain, packaging may also lead to the unintentional migration of chemicals from the packaging material into dairy produce. There is a paucity of data on the levels of migration of chemicals from packaging materials into foods in general, although in most cases, the levels are expected to be very low. Because of the high lipid content of dairy products, migration of some plasticizers may be of concern.

Three areas were identified where further data may be necessary to better characterise any potential public health and safety risk (summarised in the Table below):

- Further research is needed in relation to the public health and safety risks associated with biogenic amines. Research is also needed into the factors that influence biogenic amine formation. Further monitoring of levels in food would assist in characterising the potential public health and safety risk.
- Further monitoring of PAHs in smoked cheeses would assist in characterising the potential public health and safety risk. FSANZ is currently carrying out a survey (22nd ATDS) on dietary exposure to PAH in foods.
- Further monitoring of the level of migration of chemicals from packaging would assist in characterising the potential public health and safety risk.

Chemical class	Current regulatory and non-regulatory measures	Areas where further measures (regulatory or non-regulatory) may be necessary
Biogenic amines	• Good manufacturing practice • HACCP-based food safety plans	• Further information in relation to hazard identification and characterisation • Potential for intolerance reaction in certain individuals • Monitoring of levels in food
Fungal by-products	• Good manufacturing practice • HACCP-based food safety plans	• Monitoring of levels in food
Polycyclic aromatic hydrocarbons	• Good manufacturing practice • HACCP-based food safety plans	• Monitoring of levels in food
Chemicals which migrate from packaging	• Good manufacturing practice • HACCP-based food safety plans	• Further information in relation to hazard identification and characterisation • Monitoring of levels in foods

Conclusions from the Risk Profile of Dairy Products in Australia

High quality dairy produce has been produced in Australia for many years. This is due, in the main, to adherence to regulatory measures, good agricultural and manufacturing practices, and the maintenance of hygienic practices along the dairy primary production chain.

Microbiological risk profile

A wide range of microbiological hazards may be introduced into milk during primary production and processing. Raw milk has a mixed microflora, which is derived from including the interior of the udder, exterior surfaces of the animals, the environment (including faeces), milk-handling equipment, and personnel. In addition, the milking procedure, subsequent collection, storage of milk and processing milk into various dairy

products carry the risks of further contamination or growth of intrinsic pathogens. Importantly, the composition of many milk products makes them good media for the growth of many pathogenic micro-organisms.

The safety of dairy products is due to the use of heat treatment and a combination of management and control measures along the entire dairy supply chain. Control of animal health, adherence to good milking practices, and control over milking parlour hygiene have been important in reducing the microbial load in raw milk entering Australian dairy processing facilities.

In addition, there have been few reported failures *i.e.* food-borne illness attributed to dairy products in recent years. While dairy products have been the vehicles in some outbreaks, the cause is often multifactorial involving contaminated non-dairy ingredients, post-process (post-pasteurisation) contamination, and poor hygiene practices.

The almost universal use of pasteurisation in milk processing in Australia has resulted in the marketing of dairy products with an excellent reputation for safety and product quality. The dairy industry has introduced significant measures to ensure product safety, including the adoption of codes of practice, adherence to *Listeria* and *Salmonella* control protocols, and the extensive use of HACCP-based Food Safety Programs supported by laboratory verification.

Notwithstanding the above, there is need for ongoing vigilance and further development of safety control measures. Over the past twenty years we have seen the emergence of new pathogens and the re-emergence of traditional pathogens in various foods. These organisms often occupy specific environmental niches and may arise through changing technologies, methods of food handling and preparation, dietary habits and population. Post-processing contamination in-plant and the maintenance of control over contamination and storage conditions during transport, retail display and home use remain major factors impacting on the safety of dairy products.

Chemical risk profile

There are extensive regulatory and non-regulatory measures in place to ensure that chemicals used or present in dairy products present a very low public health and safety risk.

The Chemical Risk Profile has identified two major findings. Firstly, the extensive monitoring of chemical residues in milk over many years has demonstrated a high level of compliance with the regulations. Secondly, the regulations and control measures currently in place along the dairy industry primary production chain have resulted in minimal public health and safety concerns regarding the use or presence of chemicals in dairy products.

The Chemical Risk Profile has also identified a number of areas where further research or monitoring would assist in providing further reassurance that the public health and safety risk is low. These have been summarised above.

Continuation of the current management practices, particularly monitoring programs for chemicals along the primary production chain, will ensure that the dairy industry continues to maintain a high standard of public health and safety.

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I BACKGROUND

Food Standards Australia New Zealand (FSANZ) has responsibility for protecting the health and safety of consumers through the development of food standards. The development of through-chain Primary Production and Processing (PPP) standards requires the thorough assessment of risk[1] to public health and safety.

FSANZ uses a number of tools to assess risks to public health and safety, including risk profiling[2], quantitative and qualitative risk assessments[3] 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 the assessing risks to public health and safety for dairy products are used by FSANZ to inform risk management decisions.

The assessment of risks to public health and safety from microbiological hazards in milk and milk products has been undertaken in the form of a **Microbiological Risk Profile (Part A)**. It provides a broad overview of risks associated with consumption of dairy products in Australia and includes a description of the current status of pasteurisation in Australia and an evaluation of alternative processes to pasteurisation for the production of milk and milk products. The risk profile identifies key food safety hazards and assesses where in the primary production and processing supply chain these hazards might be introduced, increased, reduced or eliminated.

The assessment of risks to public health and safety from chemicals associated with milk and milk products has been undertaken in the form of a **Chemical Risk Profile (Part B)**. This

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1 Codex defines the term risk as 'a function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) in food'

2 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'.

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

risk profile identifies and examines where chemicals may enter the dairy supply chain (both intentionally and unintentionally) from the primary produce to processed foods. It also considers the relevant inputs (*e.g.* feed, water, etc) into the dairy primary production and processing chain.

Hazards of a physical nature associated with dairy products have not been considered.

II SCOPE AND PURPOSE

The purpose of the risk profile in dairy products is to provide an objective analysis of relevant scientific data and information to identify the public health and safety risks associated with dairy products. This will enable risk managers to consider the risks associated with dairy products and the reductions in risk that may be achieved with various production and process control options. The risk profile may also identify the need for more detailed microbiological risk assessments for specific dairy commodities[4]; requirements for further monitoring of chemicals or manufacturing practices, or further information in relation to hazard characterisation of chemicals.

The **Microbiological Risk Profile (Part A)** was undertaken to gather the following information:

1. What microbiological hazards are associated with the Australian dairy supply chain, under the current regulatory system, and what is the likelihood that these hazards pose a risk to public health and safety?

2. What are the factors along the Australian dairy supply chain that have the most significant impact on public health and safety risks?

The microbiological risk profile identifies and examines hazards along the dairy supply chain from milk production through to consumption of dairy products and has considered the relevant inputs (*e.g.* feed, water, etc) into the dairy primary production and processing chain. The risk profile encompasses the following elements:

- Identification and description of the micro-organisms that may be associated with dairy products including key attributes of each organism and its public health impact (hazard identification/hazard characterisation);
- Examination of epidemiological data (domestic and international) related to the consumption of dairy products;
- Examination of prevalence and concentration data on potential hazards from products along the entire dairy food chain; and
- Description of the dairy production, processing, distribution and consumption chain and what is currently known of the impact of these factors on public health and safety risks.

The **Chemical Risk Profile (Part B)** was undertaken to gather the following information:

1. To identify those chemicals associated with dairy products which may potentially impact on public health and safety in Australia;

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4 There is interest from the Australian dairy industry for FSANZ to consider technological innovations and the production of selected raw milk products. Currently, raw milk and raw milk products are not permitted to be sold in Australia, unless expressly permitted by a State or Territory or a specific exemption has been given as the result of an assessment process *e.g.* the sale of raw goat milk is permitted in some States. In addition, some specific raw milk cheeses are permitted where an assessment process has shown that they can be produced to an equivalent level of safety as cheeses made from heat-treated milk. To date the assessment of raw milk cheeses has been done on a case-by-case basis for selected imported cheeses

2. To assess the potential public health and safety risks associated with these chemicals, in the context of the current regulatory system;

3. To identify any areas in the current regulatory system which require further attention in relation to addressing potential public health and safety risks associated with chemicals in dairy products.

The chemical risk profile identifies and examines where chemicals may enter the dairy supply chain (both intentionally and unintentionally) from the primary produce to processed foods. It also considers the relevant inputs (*e.g.* feed, water, etc) into the dairy primary production and processing chain. The report considers the following:

- Agricultural and veterinary chemicals used in primary production;
- Environmental contaminants, including heavy metals, organic contaminants and micronutrients;
- Natural chemicals found in plants, fungi or bacteria associated with plants;
- Food processing by-products;
- Food additives, processing aids and those chemicals that may migrate from packaging.

The microbiological and chemical risk profiles were undertaken within the framework of existing management and regulations in Australia.

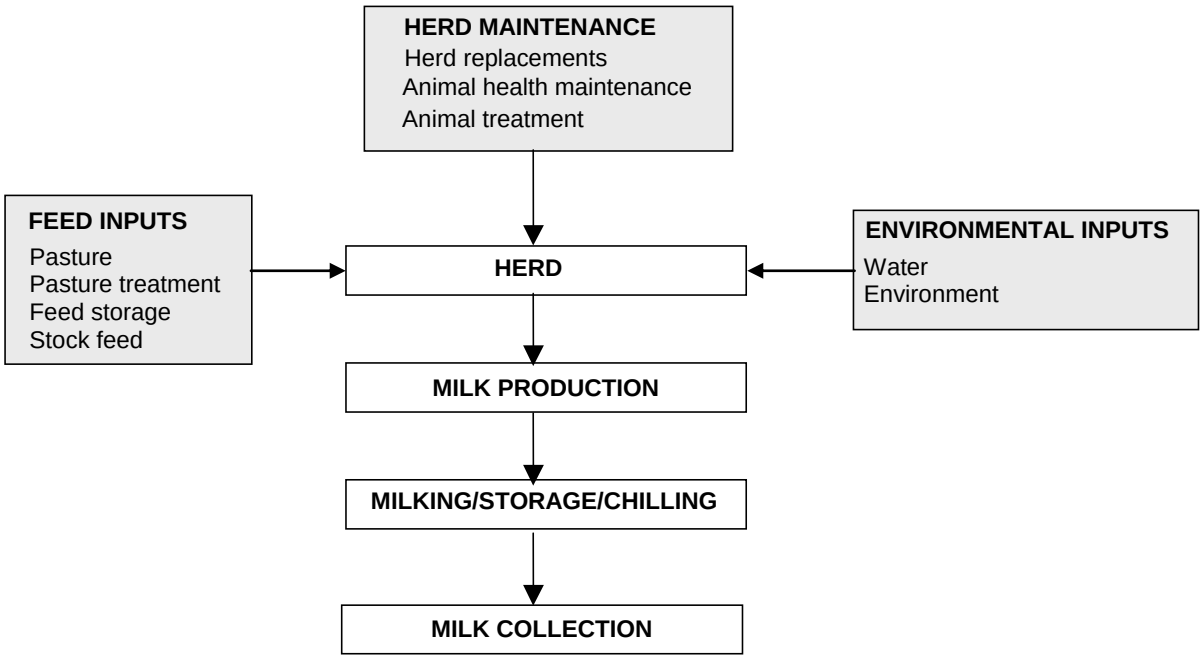
The risk profiles consider microbiological and chemical hazards associated with milk and milk products produced from the main commercial dairy species, including cows, goats, sheep, buffalo and camels.

The Australian dairy industry is predominantly based on bovine species, however other animal species are milked commercially in small operations/enterprises e.g. goats, sheep and buffalo. The bulk of goat milk production is utilised in the cheese making industry, but some is also used for cream, yoghurt and drinking milk. Sheep milk is utilised in cheese and yoghurt making, as is the milk obtained from buffaloes. Whilst there is a camel farm established near Alice Springs, it has not yet commenced commercial milk production.

The focus of this profile is on bovine species. It is assumed that on-farm and processing practices relating to other milking species other than bovine animals are largely the same as those for bovine species. Where practices are different for other species and where there is appropriate available data, specific information is provided. Dairy products from bovine animals represent the great majority of milk and dairy products sold in Australia.

III DAIRY FARMING IN AUSTRALIA

The key stages in the primary production of raw milk are shown diagrammatically as follows:



The dominant dairy cattle breed in Australia is the Holstein-Friesian, accounting for approximately 70% of all dairy cattle. Other important breeds in Australia include the Jersey, the Holstein/Jersey cross, and the Illawarra. The average herd size has been increasing in Australia and was estimated at 195 cows in 2002/03. However there are some very large farm operations where a single property may support up to ten individual herds of 500-1,000 dairy cattle.

The Australian dairying industry is predominantly pasture-based, with approximately 80% of cattle feed requirements coming from grazing. Feedlot-based dairying remains unusual, although the use of feedpads for the provision of supplementary feed, such as hay, silage and grains, is common. Pastures such as white clover, strawberry clover, perennial ryegrasses, paspalum and kikuyu are the major feed sources for cattle. Large quantities of pasture are conserved as hay and silage for feeding during periods of low pasture growth or feed availability. Concentrates, particularly mixed grains, which are commonly based on cereal grains such as barley and may include other ingredients such as lupins are fed as a supplement to pasture.

Grazing systems are either set/continuous or rotational. Animals have continuous access to one paddock all year round in set stocking or continuous grazing, whereas in rotational systems animals move around a series of paddocks in a strictly controlled way.

Australian milk production is strongly seasonal, reflecting the pasture based nature of the industry. Milk production is seasonally more pronounced in southern states such as Victoria and Tasmania where cattle graze on pasture year round. Peak milk production occurs in October/November and lowest production in May/June.

In Australia, approximately 20% of cows from milking herds are lost each year through poor health, calving problems, mastitis, infertility, death or culling for old age or poor milk production. These cows need to be replaced to maintain a constant number of lactating cows each year. Milk quality decreases and mastitis incidence increases with ageing of animals. Farmers therefore need to replace these animals through herd replacement purchases and/or calf rearing. Cattle are mostly bred by artificial insemination, however the adoption of embryo transfer technology is increasing.

Calves receive their first feed of colostrum within the first six hours of birth. Milk or milk replacers are offered to calves until at least 5-6 weeks of age. Weaning usually is based on live weight rather than age, to ensure the calf is sufficiently well developed to deal with bulky roughage feeds such as pasture. It may take 6-12 weeks for calves to reach target weights for weaning. Once calves are trained to drink they can be transferred to a separate calf paddock.

As the young female calf grows and matures she is called a heifer, and it takes about 15 months for her to grow to a size at which she can be mated resulting in a first calving at an age of about 24 months. After parturition, the cow will give milk for about 300 days (termed the lactation period). The average amount of milk produced in a complete lactation period is approximately 5,000 litres. More milk is produced at the start of lactation (average 17 litres per day) than at the end of lactation period (5-10 litres per cow).

Cows are mated about 60 days after calving, so the cow will have a calf to initiate milk production in 9 months from mating or next lactation. After about 300 days, cows are dried off and rested from milking for about 2 months before calving again and repeating the cycle.

In Australia, the milking process is largely mechanised. Animals are milked by suction and the milk transferred to refrigerated farm vats. Types of milking systems include herringbone, rotary, and fully automated. The herringbone-type system is the most popular style in Australia, with cows positioned along each side of a pit. The rotary dairy is the fastest and most efficient way of milking large numbers of cows and consists of a rotating milk platform, which allows a constant flow of cows to enter and leave the platform individually.

Robotic milking is a fully automatic modular milking system capable of milking all herd sizes. The technology is highly advanced, and only one robotic dairy currently operating in Australia. The robotic dairy's automated identification system recognises each cow, directs laser-sensing guides for application of teat cups, and records milk volume and colour. Milk discolouration due to blood or mastitis is recognised and the system will automatically discard this milk. Once milking is completed, a disinfectant is applied, usually as a spray. The cow is then released to the exiting area.

Milk is initially cooled after it leaves the udder most commonly by passage through a heat exchanger (plate cooler), prior to entering the milk vat (bulk milk storage tank). The milk is filtered before it enters the bulk milk storage tank and this provides a safeguard to ensure sediment or other extraneous matter is removed from the milk prior to storage. The milk is further cooled by the refrigeration system in the milk vat.

For a significant part of the year in Australia, milk is collected on alternate days or every 36 hours, depending on the milk vat capacity of individual farmers. The milk is then transported to milk processors in insulated bulk milk tankers.

IV REGULATION OF DAIRY PRODUCTS IN AUSTRALIA

Australia currently has State-based regulations for the dairy sector that cover on-farm activities, milk collection and dairy product manufacture. For most jurisdictions this includes the requirement for HACCP-based food safety programs for on-farm and dairy processing activities. The Authorities responsible for maintaining and implementing these requirements are:

- NSW Food Authority;
- Safe Food Queensland;
- Dairy Authority of South Australian;
- Tasmanian Dairy Industry Authority;
- Dairy Food Safety Victoria;
- Health Department of WA.

There are no dairy farms in the ACT or Northern Territory however, milk processing and packaging is conducted in these jurisdictions. These activities are covered by the requirements of the Food Acts in those jurisdictions. A summary of State legislative requirements is provided in the table below.

Summary of State legislative requirements

State	Legislation	Food Safety Programs(requirement outlined in)
NSW	Food Act 2003 (& Food Standards Code) Food Production (Dairy Food Safety Scheme) Regulation 1999	NSW Dairy Manual
QLD	a) Food Production (Safety) Act 2000 & Food Production (Safety) Regulations 2002 (SFQ) b) Food Act 1981 (QLD Health Dept)	a) FPS Act b) To be implemented under revisions to the Food Act
SA	Primary Produce (Food Safety Schemes) Act 2004 Primary Produce (Food Safety Schemes) (Dairy Industry) Regulations 2005	Dairy Authority of South Australia Code of Practice for Dairy Food Safety
TAS	Dairy Industry Act 1994	Tasmanian Code of Practice for Dairy Food Safety
VIC	Dairy Act 2000	Victorian Code of Practice for Dairy Food Safety
WA	Health Act 1911 Health (Food Hygiene) Regulations 1993 Food Safety Standards	Code of Practice for Dairy Food Safety (Under development)

A technical group known as the Australia New Zealand Dairy Authorities Committee (ANZDAC), comprising of representatives from the each State jurisdiction, the New Zealand Food Safety Authority and the Australian Quarantine Inspection Service (AQIS), ensures that the statutory responsibilities of each jurisdiction with respect to the legislative requirements for dairy premises and products is applied in a uniform and consistent way across Australia.

On-farm requirements

As outlined in the table above, on-farm food safety programs are required and implemented through licensing arrangements by regulators in New South Wales, Queensland, Victoria, South Australia and Tasmania. They are under development in Western Australia. The elements covered by these programs include:

- animal health;

- environmental hygiene;
- animal feeds, agricultural and veterinary drugs;
- areas and premises for milk and milk production, milk storage and milking equipment;
- pest control;
- hygienic milking, and
- milk storage

Animal health
All regulations require milking animals to be free from diseases and verified through record keeping requirements in relation to the health of the animal to be milked. NSW has an additional requirement for animals to be free of Enzootic Bovine Leucosis (EBL).

Environmental hygiene
On-farm requirements include the management of effluent to minimise contamination from this source, particularly to the use of reclaimed water to irrigate dairy pastures. Additional requirements to those set out in Dairy regulations include:

- Environmental Guidelines for the use of reclaimed Water, (EPA, 2001);
- Reclaimed water on dairy farms – General Information and Requirements for Users, (VDIA, 1999) – Victoria;
- Managing Dairy Farm Effluent in Tasmania – Code of Practice; and
- Guidelines for the Use of Reclaimed Water in Tasmania

Animal feeds, agricultural and veterinary chemicals
An outcome based requirement of State legislation for animal feeds (which includes pasture) is that feeds should not present a risk of introducing hazards into the milk. The use of agricultural and veterinary chemicals is also controlled by State legislation- only registered chemicals should be used and in accordance with instructions for use. Agricultural and veterinary chemicals are assessed as part of a pre-market evaluation and approval process and generally residues in milk are specified in the Australia New Zealand *Food Standards Code*[5]. Record keeping and vendor declarations should be used to verify appropriate controls are in place.

Areas and premises for milk and milk production, milk storage and milking equipment
State legislation covers the requirement for dairy premises for milk production and storage to be designed, constructed and maintained in order to prevent/minimise contamination of the milk. In NSW, additional guidelines are provided in the Code of Practice for Dairy Buildings.

Pest Control
Pests should be controlled on-farm so that they do not contaminate milk through their activities. The pest control method (such as the use of pesticides) should also not result in contamination.

[5] Standard 1.4.2 Maximum Residue Limits (Agricultural and Veterinary Checmials)

Hygienic Milking,
Requirements for hygienic milking include the exclusion of personnel from milking activities if they are ill with, or are carriers of, an infectious disease. Additionally, people undertaking milking activities must employ good sanitary practices to prevent contamination of the milk.
Milk storage
All State requirements specify temperature controls for the storage of milk. In general milk must be cooled within a specified period (3.5 hours) to a temperature of less than 5°C (less than 4°C in NSW).

Milk collection and transport requirements
All States have food hygiene requirements for the collection and transporting of milk from the farm to processing centres. Temperature requirements are specified such that milk must be collected at a temperature of less than 5°C (less than 4°C in NSW). In NSW, there is a Code of Practice for Milk Collection from Dairy farms.

Dairy manufacturing requirements
The requirements of Standard 3.2.2 Food Safety Practices and General Requirements and Standard 3.2.3 Food Premises and Equipment of the *Food Standards Code* apply to manufacturing premises. These are referenced by State and Territory Food Acts. State legislation also requires dairy manufacturers to have food safety HACCP-based food safety programs in place. The elements covered by regulations include:

- chemical, microbiological, physical contamination (from premises, equipment and personnel);
- cleaning and sanitising;
- temperature control; and
- personnel competencies.

State legislation also requires dairy manufacturing establishments to comply with the:

- Australian Manual for the Control of Salmonella in the Dairy Industry published by Australian dairy Authorities Standards Committee (ADASC), and,
- Australian Manual for the Control of Listeria in the Dairy Industry published by ADASC.

Milk for manufacture must be heat-treated in accordance with Standard 1.6.2 – Processing Requirements, of the *Food Standards Code*. Food additives and processing aids used in the manufacture of milk and dairy products undergo pre-market evaluation and approval and are specified in the *Food Standards Code*.[6] Sanitisers are also assessed before use and regulated by the Australian Pesticides and Veterinary Medicines Authority (APVMA).

In addition the Food Standards Code specifies Microbiological Limits[7] and maximum levels for contaminants[8] in various dairy products

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[6] Standard 1.3.1 Food Additives
Standard 1.3.3 Processing Aids
Standard 1.3.4 Identity and Purity
Standard 1.4.3 Articles and Materials in Contact with Food

[7] Standard 1.6.1 Microbiologica Limits for Food

[8] Standard 1.4.1 Contaminants and Natural Toxicants

Distribution of milk and milk products requirements
Dairy distributors or depots are also covered by State dairy legislation. In NSW the requirements emphasise temperature control and record keeping and reference is made to the Code of Practice for Dairy Depots.

In the Queensland Food Production (Safety) Regulation 2002, there are requirements relating to temperature control environmental conditions to avoid contamination.

The requirements of the Code of Practice for Dairy Food Safety currently implemented in Victoria, South Australia and Tasmania, and under development in Western Australia, dairy distributors must have a food safety program based on the Codex HACCP principles

V CONSUMPTION OF DAIRY PRODUCTS IN AUSTRALIA

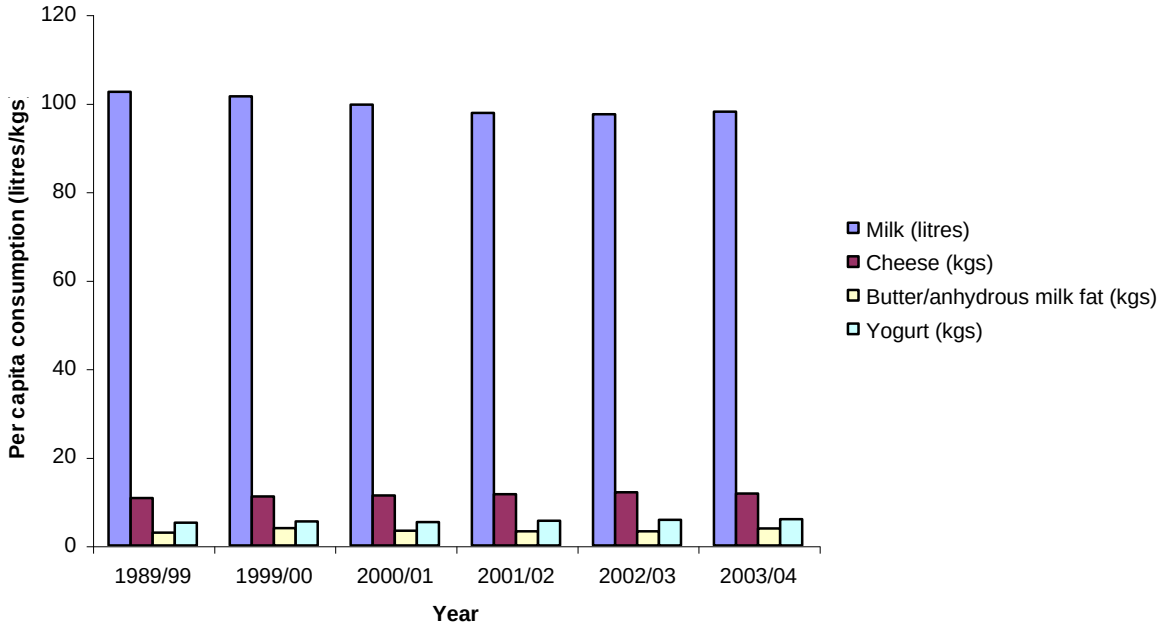
Milk and milk products are a significant component of the diet for the majority of the Australian population. Milk is an extremely valuable food for human nutrition as it contains all the basic components required for human life (Spreer, 1998).

The four major Australian consumer dairy products are drinking milk, cheese, butter and dairy blends, and yoghurt (Dairy Australia, 2004). Consumption trends over the past two decades vary quite significantly by individual product. These reflect changes in consumer tastes and preferences in response to a multitude of variables such as the multicultural influences on the foods we eat; health perceptions of dairy products and manufacturers' responses (such as low fat variants); new product development; flavour and packaging innovations; competitive category offerings; distribution and availability of product.

Consumption data can be calculated from food production statistics or food consumption surveys. Food production statistics provide an estimate of the amount of specific food commodities available to the total population. This type of data may include national statistics on per-capita food production. Consumption surveys (such as national nutrition surveys) provide detailed information regarding the types and amounts of foods consumed by individuals or households and sometimes the frequency with which the foods are consumed.

Consumption data for dairy products from food production statistics

The following Figure illustrates the Australian per capita consumption figures for major dairy products from 1989 - 2004.



Per capita milk consumption has slowly declined since the mid-1990s to an estimated level of 98 litres per head in 2003/04. Milk consumption has been decreasing due to concerns over excess fat in diets and the increased availability of substitute non-dairy products such as soya bean-based drinks. Patterns of milk consumption have also been steadily changing from

regular whole milk to modified milk types, such as reduced and low-fat milks and fortified specialty milks. Flavoured milks are also increasing being consumed in place of regular milk (Dairy Australia, 2004). The annual consumption of cream in 2001 was 4.05 kg per head (Datamonitor, 2002).

Cheese consumption is estimated at nearly 12 kilograms per head per annum. Almost all cheese consumed is cheddar and cheddar types, however, there is an trend to consume increased amounts of non-cheddar cheese types (Dairy Australia, 2004).

Annual consumption of butter in Australia is about 3 kg per head. There has been a decline in butter consumption over the past three decades as consumers have sought to reduce their saturated fat intake. However there has been a slight increase in consumption of these products since 1996 with the introduction of butter and vegetable oil-based dairy blends (Datamonitor, 2002).

Annual consumption of yoghurt has significantly increased over the past decade with 6.46 kg/head being consumed in 2001 (Datamonitor, 2002). Low-fat and diet varieties account for more than 60% of the yogurt market (Dairy Australia, 2004). The consumption of dairy desserts in Australia is 1.27 kg per head/year in 2001 (Datamonitor, 2002). Consumption of dairy desserts is growing with products such as mousses, crème caramels and fromage frais marketed for adults and fromage frais flavoured custards marketed towards children.

Consumption of ice cream has slowly been increasing. In 2001, annual consumption was 16.34 kg per head compared to 14.41 kg per head in 1996 (Datamonitor, 2002).

Annual consumption of dried milk is static and was 0.11 kg per head in 2001 (Datamonitor, 2002).

Consumption of condensed and evaporated milks is estimated at 0.08 and 0.03 kg/head/year respectively in 2001. The consumption of these products appears to be relatively stable since 1996 (Datamonitor, 2002).

Consumption data for dairy products from the 1995 Australian National Nutrition Survey
Data from the Australian National Nutrition Survey (NNS) provides detailed information regarding the types and amounts of dairy foods Australian's are consuming. The most recent national survey was conducted during the period from February 1995 to March 1996. Approximately 13,800 people aged two years or over from urban and rural areas in all States and Territories participated in the survey.

Two approaches were used in the NNS to collect data on food and beverage intake. The daily food consumption (24-hour recall) method was used as the main indicator of food intake. All participants were interviewed by trained nutritionists who sought detailed information on all foods and beverages consumed during the day prior to the interview (from midnight until midnight). A sample of approximately 10% of the NNS participants also provided intake data for a second 24-hour period. A Food Frequency Questionnaire was used to assess usual frequency of intake for those aged 12 years or more.

A summary of consumption of dairy products from the 1995 Australian National Nutrition Survey is outlined in the following Table**(Australian Bureau of Statistics and Department of Health and Family Services, 1997)**. Detailed results from the 1995 Australian National Nutrition Survey for the various dairy products are presented in Appendix 4.

Product	Average no. people surveyed consuming product (%)	Average amount product consumed per day (g)
Milk and other liquid milk products	84	347
All Cheese types	41	34
Very hard cheese	2.3	8
Hard cheese	27.7	32
Semi soft cheese	1.6	23
Soft cheese	5.3	33
Processed cheese	9.4	30
Ice cream	15	112
Butter	14	13
Yoghurt	9	177
Cream	7.7	29
Dairy based dips	1.5	43
Dairy based desserts	4.7	148
Dried milk	1.26	17
Goats milk	1	248
Concentrated milk	<1	57

Milk and other liquid milk products are consumed in significant quantities, with 84% of those people surveyed during the 1995 Australian National Nutrition Survey consuming an average amount of 347 g/day with the quantity ranging from 215–536 g/day. Less than 1% of those surveyed consumed goats milk.

A relatively high proportion of people surveyed consumed cheese, with consumption by 41% of the population. The average daily consumption varied from 25 g/d (65^+ years females) to 50 g/d (19-24 year males). Of the various cheese types available, hard cheese was the cheese most commonly consumed with 27.7% of people surveyed consuming this product. The average amount of hard cheese consumed was 32 g/day. This was followed by processed cheese with consumption by 9.4% of the population surveyed, predominantly by children. The average amount of processed cheese consumed was 30 g/day. Soft cheese was consumed by 5.3% of the population, with consumption greatest among females aged 45-64 years. Only 1.6% of the population consumed semi-soft cheese, with the principal consumers being people aged 45^+ for both genders.

Fifteen percent of people surveyed consumed ice-cream. The greatest consumers of ice cream were females aged from 12-15 years, however at least 10% of all age groups consumed ice-cream. Average amounts eaten varied between 59 g/day for girls aged 2-3 years and 217 g/day for boys aged 16-18 years.

Butter was consumed by 14% of the people surveyed. The greatest consumers of butter were males aged 65^+. The average amount of butter consumed by this age group was 20 g/day.

Yoghurt and cultured dairy products were consumed by 9% of the people surveyed and the greatest consumers were females aged from 45-64 (consuming 137g/day) and the lowest consumers where males aged 12-15 and 19-24.

Dried milk was only consumed by 1.26% of the people surveyed. The greatest consumers were males and females aged from 45-64 and 65^{+}. The average amount of dried milk (after reconstitution) consumed by these age groups was 220 ml. Dried milk probably replaces liquid milk in the diet. In Australia, a significant proportion of manufactured dried milk is exported or used as an ingredient in other foods.

Concentrated milks are consumed by less than 1% of the people surveyed, the greatest consumers were males aged from 45. The average amount of concentrated milks consumed by this age group was 37 g/day.

Dairy based dips and dairy based desserts were consumed by 1.5% and 4.7% of the people surveyed respectively. Of those who consumed dairy based dip products, the greatest consumers were females aged from 16-18 (consuming 46 g/day) and the lowest consumers where females aged 12-15. Of those who consumed dairy based dessert products, the greatest consumers were children aged from 2-7 (consuming between 103-157 g/day).

Although some data is available for whey consumption in Australia, whey products are normally used as functional ingredients in other foods.

VI RISK PROFILES

PART A: MICROBIOLOGICAL RISK PROFILE

1. Introduction

This risk profile identifies the microbiological public health and safety risks associated with dairy products in Australia.

In compiling the risk profile, a wide range of scientific literature, data and information from Australia and overseas was reviewed and evaluated. The risk profile provides a broad overview of microbiological risks associated with consumption of dairy products in Australia and includes a description of the current status of pasteurisation in Australia and an evaluation of alternative processes to pasteurisation for the production of milk and milk products.

Dairy Australia has funded the development of a quantitative microbiological risk assessment model to assess factors influencing the fate of hazards along the dairy supply chain. Dairy Australia permitted FSANZ to access the results and to utilize the model to inform the risk profile. The work was performed by the Australian Food Safety Centre of Excellence (AFSCoE) who modelled the fate of selected microbial pathogens in milk from primary production until after pasteurisation.

FSANZ also engaged external experts to review the status of pasteurisation and other technologies in Australia to:

- Define the effect of pasteurisation on pathogenic micro-organisms in milk;
- Determine how current pasteurisation practices compare with regulatory requirements;
- Describe what alternative technologies are currently being investigated for use in the dairy industry worldwide, and what is known about their ability to destroy pathogens;
- Consider processes and challenges in validating these alternative technologies;
- Determine the opportunities for, and limitations on, the use of these technologies; and
- Describe the level of interest in such technologies within the Australian dairy industry.

1.1 Grouping of dairy commodities

For the purpose of this profile, dairy commodities were grouped into broad categories as follows:

- Milk and cream
- Cheese
- Dried milk powders
- Infant formulae
- Concentrated milk products
- Butter and butter products
- Ice-cream
- Cultured and fermented milk products
- Dairy deserts

- Dairy-based dips
- Casein, whey products and other functional milk derivatives
- Colostrum

These categories are based primarily on those foods that are currently included in microbiological standards or guidelines in the *Australia New Zealand Food Standards Code* (the Code). Additional dairy commodities were considered where they are relatively new to the market or little is known of their microbiological status.

1.2 Microbiological hazards associated with dairy products

A wide range of microbiological hazards may be introduced into dairy products during the primary production and processing stage.

Raw milk has a mixed microflora which is derived from several sources including the interior of the udder, exterior surfaces of the animals, the environment, milk-handling equipment, and personnel. Milking animals may carry a wide range of micro-organisms, some of which are human pathogens and these may contaminate raw milk.

In addition, the milking procedure, subsequent collection, storage of milk and processing milk into various dairy products carry the risks of further contamination or growth of intrinsic pathogens. Importantly, the composition of many milk products makes them good media for the outgrowth of many pathogenic micro-organisms.

A broad range of micro-organisms were considered in this assessment. The micro-organisms are representative of those that may be present in raw milk, either directly transmitted via the mammary gland or via faecal/environmental contamination. In addition, micro-organisms that originate from the milking environment and/or post-pasteurisation contamination were also considered. Table 1 provides a brief summary of the micro-organisms, the severity of associated illness and the availability of epidemiological data.

Table 1: Summary of micro-organisms considered in the risk profile

Organism	Shed directly in milk[#]	Contaminant of raw milk[##]	Survives pasteurisation	**Severity of illness[§]**	Dairy/dairy products implicated in food-borne illness
Aeromonas spp.	×	✓	×	Serious	+
Bacillus cereus	×	✓	✓	Moderate	++
Brucella spp.	✓	✓	×	Severe	+
Campylobacter jejuni/coli	×	✓	×	Serious	++
Clostridium botulinum	×	✓	✓*	Severe	+
Clostridium perfringens	×	✓	✓	Moderate	+
Corynebacterium spp.	✓	✓	×	Serious	+
Coxiella burnetii	✓	✓	×	Serious	+
Cryptosporidium	×	✓	×	Severe	+
Enterobacter sakazakii	×	✓	×	Severe^	++
Pathogenic *E. coli*	×	✓	×	Severe	++
Listeria monocytogenes	✓	✓	×	Severe^	++
Mycobacterium avium subs. *paratuberculosis*	×	✓	×	–	–
Mycobacterium bovis	✓	✓	×	Severe	+
Salmonella spp	×	✓	×	Serious	++
Shigella spp.	×	✓	×	Serious	+
Staphylococcus aureus	✓	✓	×**	Moderate	++
Streptococcus spp.	✓	✓	×	Serious	+
Yersinia enterocolitica	×	✓	×	Serious	+

[#] transmission through udder; mastitis etc
[##] via faeces, the environment etc
* neurotoxin is heat labile
** enterotoxin is heat stable
^ for vulnerable populations
[§] based on ICMSF (2002) severity ranking[9]
– No data/unknown
\+ Reported, but rare
++ More commonly associated with food-borne illness

When examining each dairy commodity category, only those potential pathogens relevant to the commodity being evaluated were assessed *i.e.* only those micro-organisms relevant to the particular dairy commodity category were considered. A detailed characterisation of potential hazards is attached as Appendix 5.

[9] The estimate of the severity of adverse health effects caused by a food-borne agent is based on the ranking scheme for food-borne pathogens and toxins described by the International Commission on Microbiological Specifications for Foods {ICMSF, 2002 1843 /id}. The ICMSF ranking scheme categorises hazards by the severity of the threat they pose to human health, taking into consideration the: likely duration of illness; likelihood of death; and potential for ongoing adverse health effects.

The severity of adverse health effects caused by a hazard is ranked as moderate, serious or severe according to the following definitions:

Severity	***Description***
Moderate	Not usually life threatening; no sequelae; normally short duration; symptoms are self-limiting; can be severe discomfort
Serious	Incapacitating but not life threatening; sequelae infrequent; moderate duration
Severe	Life threatening, or substantial sequelae, or long duration

Under the ICMSF ranking, severe hazards are further divided into those applying to the general population and those applying to specific sub-populations, that is, susceptible individuals (for example, the very young and old, the immunocompromised, and pregnant women and their unborn children). This takes into account those situations where a hazard considered to be of moderate or serious severity to the general population may cause a severe illness in certain susceptible sub-populations.

This risk profile does not consider animal health issues associated with micro-organisms other than those that specifically impact upon human health via food-borne transmission. The Australian Quarantine and Inspection Service (AQIS) and Biosecurity Australia maintain import requirements that are concerned with animal health and biosecurity issues. A quarantine permit must be obtained in order to import dairy products into Australia. These requirements must be met prior to compliance within the Code.

Bovine Spongiform Encephalopathy (BSE) is an animal and human health issue. FSANZ has undertaken a comprehensive risk assessment of the scientific data and information for BSE and Australia has developed a policy/framework (including a Standard) to manage the risk of BSE in food. Therefore this risk profile does not consider this issue.

There are no viral zoonoses shed in milk that are of concern to human health. Although Foot and Mouth disease[10] is shed in milk, it is of major concern to the dairy industry because it can be the vehicle for animal infection rather than human infection (Desmarchelier, 2001).

1.3 Use of antimicrobials

There are two public health issues arising out of the use of antimicrobial agents in the dairy industry. The first is the emergence of bacteria resistant to antimicrobial agents, which may reduce the ability of health and veterinary professionals to control infections resulting from food-borne transmission of such resistant bacteria. This may lead to an increase in morbidity and mortality and an increase in the costs associated with treatment of specific bacterial diseases. The second issue[11] is the potential for residues of the antimicrobial agent(s) to be present in food products, resulting in toxigenic or allergenic responses in some individuals. These issues are not restricted to the dairy industry. They are common to the use of antimicrobial agents in all food-producing animals. In addition, acquired antimicrobial resistance (AMR) can occur independently of the use of antimicrobial agents. However, antimicrobial agents can exert selective pressure on bacteria, increasing the rate of AMR development. A third issue specifically associated with the dairy industry is the impact on antimicrobials in cultured dairy products, where they can adversely affect starter cultures and their ability to adequately acidify products and hence assist product safety.

1.3.1 Evidence of antimicrobial resistant bacteria in milk and milk products

In general, there is a paucity of published information concerning antibiotic resistance in pathogenic bacteria from food and food-producing animals in Australia. The JETACAR report {Anon, 1999 1844 /id} contains information on diagnostic laboratory results of testing for AMR in food isolates of salmonellae. For the period 1989-1994, 9.7% (26/267)of *Salmonella* isolates from milk and milk products displayed resistance to at least one of ten antibiotics tested.

In Australia, most dairy animals are pasture fed. Antibiotics are predominantly used for therapeutic purposes, under veterinary supervision. Hence long term exposure to antibiotics through prophylactic use or as growth promotants is rare.

[10] Australia is officially free from Foot and Mouth Disease

[11] see Section IV Part B Chemical Risk Profile: subsection 5.7.2

1.4 Existing risk assessments of dairy products

In preparing the Dairy Risk Profile, previous risk assessments conducted by other scientific agencies were reviewed and evaluated (Appendix 6). There have been few assessments undertaken for dairy products, and typically they address specific pathogen:commodity pairs.

This profile considers the entire dairy supply chain, including the wide range of milk and milk products marketed in Australia. Where possible, the results of international risk assessments have been used to inform the development of the Dairy Risk Profile. Table 2 lists the major published risk assessments on dairy products.

Table 2: Existing risk assessments of dairy products

Pathogen	Risk Assessment/Comments
B. cereus	A concise risk assessment on *B. cereus* in the Netherlands predicted that 7% pasteurised milk might have levels of *B. cereus* contamination above 10^5 per ml. (Notermans *et al*., 1997).
Campylobacter	Risk profile explored potential transmission routes of *Campylobacter* in New Zealand, where dairy cows are implicated as a significant source of *Campylobacter jejuni* (Savill *et al*., 2003).
Enterobacter sakazakii	Significant resources have been devoted to assessing the food safety risk of *E. sakazakii* in powdered infant formula. Two comprehensive risk assessments have been completed by WHO/FAO (Food and Agriculture Organization of the United Nations/World Health Organization, 2004) and the European Food Safety Authority (EFSA (European Food Safety Authority) - Scientific Panel on Biological Hazards, 2004). Two risk profiles have been published separately on the topic (CCFH (Codex Committee on Food Hygiene), 2003; Iversen and Forsythe, 2004).
Listeria monocytogenes	Farber et al. (Farber *et al*., 1996) published a *Health risk assessment of L. monocytogenes in Canada*. The USDA, FDA and Centers for Disease Control and Prevention jointly developed a quantitative risk assessment on *L. monocytogenes* in RTE foods in 2003 (FDA/Centre for Food Safety and Applied Nutrition, 2003). The New Zealand Food Safety Authority (NZFSA) prepared a risk profile on *L. monocytogenes* in ice cream (2003) (Lake *et al.*, 2003). WHO/FAO developed a risk assessment on *L. monocytogenes* in ready-to-eat foods in 2004 (WHO/FAO, 2004). Specifically related to dairy products, several independent risk assessments have been published on *L. monocytogenes* in cheese made with raw milk including: • A quantitative risk assessment of human Listeriosis from consumption of soft cheese made from raw milk (Bemrah *et al*., 1998), • A risk assessment of Listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux (Sanna *et al*., 2004), and • A risk assessment of *L. monocytogenes* in Swiss Emmental cheese (Aebi *et al*., 2003). There have been other publications dealing with specific methodologies used in risk assessment for *L. monocytogenes* in foods. One refers to an animal model (Notermans *et al*., 1998) and the other refers to inactivation of *L. monocytogenes* in milk by HTST pasteurisation (Piyasena *et al*., 1998).
M. bovis	NZFSA developed a risk profile on *M. bovis* in milk in 2002 (Lake *et al.*, 2002).
Staphylococcus aureus	Lindqvist et al. (Lindqvist *et al*., 2002) prepared a risk assessment on *S. aureus* in raw milk cheese. The European Commission (European Commission, 2003) published an Opinion of the Scientific Committee on veterinary measures relating to public health on Staphylococcal enterotoxins in milk products, particularly cheeses
Others	• (Sumner, 2002) Food safety risk profile for primary industries in South Australia • Agency for Food and Fibre Science, Queensland Department of Primary Industries (2004) Queensland raw goat milk risk assessment, commissioned by Safe Food Qld (unpublished) • Centre for Food Technology (2002) Queensland dairy product risk assessment, commissioned by Safe Food Queensland (unpublished) • AgriQuality New Zealand Ltd. (2002) Risk assessment of sheep and goat milk, for Safefood NSW (unpublished)

2. Occurrence of microbiological hazards associated with Dairy products

Data from a number of sources was used to assess the occurrence of microbiological hazards in dairy products in Australia and overseas. Most data was obtained from the scientific literature, with additional data sourced from Dairy Australia, the National Enteric Pathogen Surveillance Scheme, the Dairy Authority of South Australia, Australia's Imported Food Program and FSANZ's food recall database. The data is summarised in Appendix 3.

2.1 Occurrence of microbiological hazards in dairy products in Australia

Data collated by the National Enteric Pathogen Surveillance Scheme (NEPPS) from 1983 - 2004 showed that *Salmonella* was isolated from 1,156 dairy samples, including raw cow's milk, raw goat's milk, dried milk powders, infant formula, ice cream, concentrated milk, whey powder and casein (Table 1, Appendix 3). The data showed that a large range of *Salmonella* serovars have been isolated from dairy products in Australia, but the total number of samples is not provided in the dataset so prevalence calculations cannot be made.

Analytical data was obtained from the Dairy Authority of South Australia for pasteurised milk, cheese, dip/dessert and yoghurt between 1998 and 2004 (Tables 2-3, Appendix 3). Tests were undertaken for standard plate count, coliforms, yeasts, moulds, *E. coli*, coagulase-positive *S. aureus*, *L. monocytogenes* and antimicrobial substances. The data were recorded as groups of tests, and so an analysis of results against individual tests could not be undertaken. However, generally there was a good level of compliance in the foods tested.

Analytical data was also obtained from Dairy Food Safety Victoria's product testing program for various dairy products between 2002 and 2005 (Tables 4-6, Appendix 3). Tests were undertaken for coliforms, *E. coli, L. monocytogenes,* Coagulase-positive *S. aureus* and *Salmonella*. Detections for *Coliforms, E. coli, L. monocytogenes* and Coagulase-positive *S. aureus* in various dairy products were found (Tables 4-6, Appendix 3).

Microbiological survey data from the scientific literature and Dairy Australia for pasteurised dairy products in Australia showed a very low incidence of hazards of public health significance in these products (Sections 3.1.3 and 3.1.4, Appendix 3). In most surveys for *E. coli*, *Listeria* spp, *Salmonella*, and *S. aureus*, no micro-organisms were detected.

There is little data on the presence or absence of pathogens in raw milk and raw milk products in Australia. A small number of surveys however looked at *Aeromonas*, *L. monocytogenes*, and *Y. enterocolitica* in Australian raw milk. *Aeromonas* was detected in 27 – 60% of raw cows milk samples (Table 17, Appendix 3), *L. monocytogenes* was not detected in raw cows milk samples, but was detected in 1.4% of raw goats milk tested (Table 18, Appendix 3). *Y. enterocolitica* was detected in 12.8% of raw goats milk sampled (Table 19, Appendix 3). In addition a number of *Salmonella* isolates have been reported by NEPPS in both raw cows milk and raw goats milk (Table 1, Appendix 3).

A total of 43 dairy products and foods containing a dairy component have been recalled in Australia between 1990 and August 2005 (Section 3.1.2, Appendix 3). The recalls represent 6% of the total number of recalls that have occurred over this time. The products recalled include both domestically produced and imported dairy foods, with most of the recalls attributed to milk, cheese and cream. The majority of recalls were due to the presence of *L. monocytogenes* in product.

2.2 Occurrence of microbiological hazards in dairy products overseas

Surveys conducted overseas showed that pathogens are frequently isolated from raw milk (Section 3.2, Appendix 3). Pathogens were detected in raw milk in 85% of 126 surveys identified in the literature. Pathogens detected in raw cows milk included: *Aeromonas* spp., *B. cereus, Brucella* spp., *Campylobacter* spp., *Coxiella burnetii*, pathogenic *E. coli, L. monocytogenes, Mycobacterium* spp., *Salmonella, S. aureus, Streptococcus* spp., and *Yersinia* spp. Pathogens detected in raw goats milk included: *Brucella* spp., pathogenic *E. coli*, *Mycobacterium* spp., and *S. aureus*. Pathogens detected in raw sheep's milk included: *Aeromonas, Brucella* spp., pathogenic *E. coli, Mycobacterium* spp., and *S. aureus*.

While pathogens are rarely isolated from pasteurised milk they are more frequently found in pasteurised milk products, with affected products being cheese, infant formula and milk powder (Section 3.2, Appendix 3).

In addition, pathogens have been reportedly been detected in raw milk cheeses. However, in the few surveys of cheese documented, it could not be ascertained whether the cheese was manufactured from raw or pasteurised milk. In these few surveys where raw milk cheeses were specifically identified, pathogens were however rarely detected (Section 3.2, Appendix 3).

In analysis undertaken by AQIS from 2002 to 2004, a low percentage (up to 6.4%) of imported cheese samples failed for *E. coli* and *L. monocytogenes* testing (Table 7, Appendix 3).

3. Food-borne illness associated with dairy products

Food-borne illness causes a range of symptoms associated with gastroenteritis, but may also cause a number of other types of illnesses such as meningitis, septicaemia, neurological conditions and hepatitis. In addition, certain illness may have sequelae including reactive arthritis, irritable bowel syndrome and Guillain-Barré syndrome.

The national Gastroenteritis Survey results have estimated 5.4 million cases of gastroenteritis in Australia each year attributable to food. The most common pathogens associated with these being pathogenic *E. coli*, norovirus, Campylobacter and non-typhoidal Salmonella (Australian Government Department of Health and Ageing, 2005).

It has been reported that food prepared in restaurants and catering establishments, along with the mishandling of food by consumers is responsible for the majority of food-borne illness.

Prior to the introduction of pasteurisation, dairy products such as liquid milk were frequently implicated in various forms of food-borne illness. In the 19th century, milk was a common vehicle for communicable diseases such as scarlet fever, diphtheria and tuberculosis.

In Australia, a major outbreak of typhoid fever in the Melbourne suburb of Moorabbin in 1943 was attributed to raw milk. It lead Dr F.V.G. Scholes, Medical Superintendent , Queen's Memorial Infectious Diseases Hospital at Fairfield to state:

'*But the great lesson of the outbreak is that it is not safe to drink raw milk*'.

The result was that pasteurisation was soon made mandatory, and milk-borne diseases became much more uncommon.

3.1 Food-borne illness associated with dairy products in Australia

Prior to 2000, there was no nationally coordinated system of surveillance of food-borne illness. However since this time, Australia has introduced a national mechanism to enhance surveillance of food-borne illness and to provide a means for facilitating the national investigation of, and determining the causes of, food-borne illness (OzFoodNet).

During 1995-2000, a survey conducted by State and Territory health departments identified six outbreaks of food-borne illness associated with dairy products (Appendix 2, Table 1,) (Dalton *et al*., 2004). In addition a literature review of outbreaks prior to this time, also identified an outbreak of salmonellosis in 1977 associated with infant formula (Appendix 2, Table 1) (Forsyth *et al*., 2003).

Between January 2001 and December 2004 a total of 390 outbreaks of food-borne or suspected food-borne disease have been reported (OzFoodNet, 2005). Of these outbreaks, 3.9% (16/405) were potentially associated with the consumption of dairy products (Table 2, Appendix 2). In four of the sixteen outbreaks, a specific dairy product was identified as the food vehicle that caused infection. The remaining twelve outbreaks involved a food vehicle that contained dairy products as an ingredient.

Of the eleven outbreaks that could be attributed to a specific dairy product (excluding infant formula), unpasteurised milk was responsible for eight outbreaks, cheese, gelati, and cheese sauce for the remaining three outbreaks (Appendix 2, Table 2). These 11 outbreaks affected 268 people, with a median number of 13 cases per outbreak and a range of 8 to 111 cases.

In 45% of these outbreaks the organism responsible was identified as *Campylobacter* (5 outbreaks). Single outbreaks were caused by *Cryptosporidium*, *Salmonella* Typhimurium 44, *Salmonella* Oranienberg and *Salmonella* Bredeney. In the outbreak associated with consumption of cheese sauce, the organism responsible was identified as C*lostridium perfringens*, and was likely to have been due to poor food handling. A causative organism could not be identified in one outbreak.

Four of the outbreaks occurred on school camps where unpasteurised milk was consumed and two on farms where unpasteurised milk was consumed. Unpasteurised milk was also consumed and led to single outbreaks in the community and in a school. Single outbreaks occurred from gelati serve at a restaurant, cheese sauce prepared by a caterer and from infant formula consumed in the community. The four outbreaks from specific dairy products identified in the OzFoodNet Outbreak Register were investigated using three point source cohort studies and one case control study. Data from before 2001 does not identify how outbreaks were investigated.

In twelve of the outbreaks identified in during 1995-2004, dairy products were an ingredient in the food vehicle identified as the source of infection (Table 2, Appendix 2). Dairy products are a component of many foods; therefore it is often difficult to determine whether they are the ingredients in the food vehicle identified as the cause of an outbreak.

The twelve outbreaks involved: cream filled cakes (4 outbreaks), custard as a food or part of a food (4 outbreaks), ice cream (3 outbreaks), and cheesecake (1 outbreak). Many of these foods also contain raw eggs, hence it is possible that the eggs and not the dairy component of the food was responsible for the infection. The agent responsible for these dairy related outbreaks was *S.* Typhimurium (11 reports). No organism was identified in one outbreak.

It is important to recognise that these outbreak data represent a small proportion of actual cases of food-borne illness, as many outbreaks go unrecognised. It should also be noted that it can be difficult to identify the key ingredient causing food-borne outbreaks, or critical factors contributing to their occurrence.

3.2 Food-borne illness associated with dairy products overseas

While commercial dairy products have rarely been identified as sources of food-borne illness in Australia, there have been a number of reports of outbreaks of illness associated with consumption of dairy products internationally. Information tabulated from a search of the international literature describes 163 outbreaks associated with dairy products from 1973-2003 (Appendix 2, Tables 3-12).

There have been twenty-two outbreaks attributed to pasteurised milk (13.5%) and seventeen outbreaks to cheese made from pasteurised milk or pasteurisation not stated so assumed pasteurised (10.4%). Faults with the pasteurisation process or a post pasteurisation contamination has been identified or suspected as the source of infection in each case.

Unpasteurised dairy products are the most common cause of dairy associated outbreaks of illness. There have been 30 outbreaks attributed to unpasteurised milk (18.4%), 18 unpasteurised milk cheese (11.0%), and 13 unpasteurised milk from non-bovine species (8.0%). The total number of dairy outbreaks associated with unpasteurised products is 61/163 (37.4%).

Ice cream was responsible for 23 outbreaks (14.0%), with raw eggs identified as an ingredient and a possible source of infection in fourteen of these outbreaks. Butter was associated with 6 outbreaks (3.7%) and yoghurt and fermented products associated with 2 outbreaks (1.2%). Dried milk products were associated with 5 outbreaks (3.0%). Eight outbreaks (5.9%) of illness resulted from foods where a dairy product was a component. Infant formula was associated with 19 outbreaks of illness (11.7%).

The extent to which outbreaks of food-borne illness can be attributed to raw or pasteurised milk or milk products does not enable risk assessors to clearly determine the relative risk that raw milk products pose to consumers. The literature is often unclear about the heat treatments given to milk and the term unpasteurised may apply to raw or thermised or improperly pasteurised milk (De Buyser, et al., 2001). Raw milk is as frequently involved as pasteurised milk in outbreaks, yet only a small proportion of milk and milk products are unpasteurised.

3.3 Attribution of food-borne illness to dairy products

While there is enhanced quantitative data on the incidence of illness due to specific pathogens, there is often not the ability or capacity to identify or distinguish specific food vehicles. The causative agent of an illness is usually determined through epidemiological studies, but confirming the identity of a key ingredient or the original source of product contamination, or critical factors contributing to their occurrence is problematic.

This inability to attribute cases of food-borne illness to causal vehicles is a major issue internationally, and is especially difficult where illness is linked to foods with multiple ingredients. Problems arise because of difficulties with:

- Food recall biases when gathering food consumption histories;
- Long exposure windows with specific pathogens;
- Inability to obtain representative food samples for analysis; and
- A lack of precision in, or suitable methods for, sample analysis.

Critical in this process is the capacity to link epidemiological data to animal and food monitoring data.

The development of public health interventions requires accurate data defining the source from which humans are acquiring pathogens and how specific foods contribute to the total burden of food-borne illness. However, outbreak data represents only a small component of actual cases of food-borne illness, as many outbreaks go unrecognised. People do not always seek medical attention for mild forms of gastroenteritis, and not all food-borne illnesses require notification to health authorities.

Nevertheless, the existing epidemiological data supports the proposition that pasteurised dairy products represent a low risk to public health, and that pasteurisation is an effective means of reducing the risk of human illness from dairy products.

4. Primary production factors impacting on milk safety

Raw milk has a mixed microflora which is derived from several sources including the interior of the udder, exterior surfaces of the animals, environment, milk-handling equipment, and personnel. In general, there are two means by which pathogens contaminate raw milk. Contamination may occur when micro-organisms are shed directly into raw milk from the udder as a result of illness or disease, or through contamination from the external surface of the cow and the milking environment. Primary production factors that impact on these routes of contamination and the microbiological quality of the raw milk include:

- animal-related factors e.g. animal health, herd size, age and production status;
- environment-related factors e.g. housing, faeces, feed, soil, and water; or
- milking and operation of milking equipment factors.

Some of these primary production factors can be managed to reduce the risk of contamination of raw milk by pathogens, while management of others will have limited impact on the final microbiological status of raw milk.

4.1 Animal factors impacting on milk safety

Animal factors that may impact upon the microbiological quality of raw milk include, animal health, herd size, and age and production status of cattle. These factors impact upon the prevalence of micro-organisms in animal herds and subsequent shedding in animal faeces, or directly into the milk itself. Shedding of micro-organisms in the faeces can lead to further contamination in the farm environment. Faecal material can also contaminate raw milk directly from the animal's udder, hide, or hair, thereby introducing pathogens into the milk.

4.1.1 Animal health

The health status of animals has a significant impact on the microbiological quality of raw milk. Major diseases of milking animals include mastitis, an inflammatory disease of the mammary tissue caused by organisms colonising the teat duct and the interior of the udder, where high numbers of micro-organisms and somatic cells[12] are shed directly into milk.

However sick and diseased animals may also shed other agents into their milk and/or their faeces, including *Mycobacterium bovis, Brucella abortus, Br. melitensis, Br. suis, L. monocytogenes*, salmonellae and *Coxiella burnetii*, and viruses such as Foot and Mouth Disease. In addition to clinically infected animals, animals may be asymptomatic carriers of agents also shedding organisms in faecal material and/or directly into milk.

4.1.1.1 Mastitis

There are over 140 different organisms that can cause mastitis in cows, and they may be found on the cow and in the environment (Nickerson, 2002). However, most mammary gland infections are caused by only a few types of bacteria, including streptococci, staphylococci and coliforms (Nickerson, 2002).

The most important contagious (spread from infected to uninfected cows) mastitis-causing micro-organisms are the bacteria *Streptococcus agalactiae, S. aureus* and *Corynebacterium*

[12] Somatic cells refers to white blood cells/body cells from the cow. Somatic cells do not increase after the milk leaves the cow.

bovis. The infected mammary glands are the chief reservoirs of these micro-organisms and transmission from cow to cow occurs during the milking process. The infection is commonly subclinical and of long duration, with the micro-organisms shed into milk from the infected udders in large numbers (Nickerson, 2002). *S. aureus* and *Corynebacterium bovis* can cause illness in humans, whereas there is debate whether *St. agalactiae* is a human pathogen (Appendix 5).

In the absence of good hygiene, *St. agalactiae* can spread rapidly throughout a herd. However it is easily eradicated from dairy herds through strategic antibiotic treatment. Udders infected with *St. agalactiae* typically have a high somatic cell count resulting in elevated levels in the bulk milk, and the milk itself may be slightly discoloured (Nickerson, 2002).

S. aureus are not commonly found on healthy teat skin, but they readily colonize and grow in the teat canal. Teat skin chapping, resulting in lesions or sores, promotes colonisation and infection. Symptoms are most often subclinical with periodic flare-ups into clinical cases of mastitis requiring treatment. Chronic infections are extremely difficult to treat with antibiotics due to development of scar tissue at multiple sites which impede the antibiotic coming into contact with the bacteria (Nickerson, 2002).

Mammary infections with *Corynebacterium bovis* usually result is only a slight elevation in somatic cell counts in the raw milk. Outbreaks are most commonly reported in herds that do not practise post milking teat dipping and dry-cow therapy (Nickerson, 2002).

Current mastitis control programs are based on hygiene, including pre- and post-milking teat disinfection, antibiotic therapy during lactation and at drying-off and culling of chronically infected cows (Oliver and Pighetti, 2002). *St. agalactiae, S. aureus* and *Corynebacterium bovis* can be controlled by: (1) good udder hygiene, (2) correct use of good milking machines, (3) dipping teats after milking; and (4) treatment of all udders at drying-off (Nickerson, 2002).

Environmental pathogens including streptococci, other than *St. agalactiae,* and gram-negative bacteria, also cause mastitis. These micro-organisms gain access to the teat canal and enter the interior of the udder between milkings when teats are exposed to mud, manure and dirty bedding materials (Nickerson, 2002). Most environmental pathogens elicit an elevated somatic cell count in the milk. Control of environmental mastitis pathogens is best achieved by maintaining a clean, dry environment for lactating and non-lactating cows and preventing infection (Oliver and Pighetti, 2002).

Listeria and *Salmonella* mastitis are not common; however, shedding of these organisms in raw milk has been documented via intramammary infections (Wiedmann and Evans, 2002; Poppe, 20052). The incidence of *E. coli* mastitis is low and intramammary *E. coli* O157:H7 infections have not been documented (van Kessel *et al*., 2004).

As mastitis is the cause for elevated cell counts in raw milk, the European Economic Commission has regarded milk or milk products made from raw cow's milk with cell counts above 400,000 cells/mL as unsuitable for human consumption (Directive 92/46). Other importing customers are increasingly using this standard.

Mastitis Control in Australia

The Australian Mastitis Advisory Council comprising dairy farmer and processor peak bodies has initiated a program called Countdown Downunder to help farmers and their advisors achieve mastitis control and reduce cell counts. The industry goal is for more than 90% of all farms to supply milk of less than 250,000 cell/mL and 100% of farms to reach a cell count of less than 400,000 cells/mL. Countdown Downunder encourages farmers to adopt best practice for mastitis control by providing clear, consistent management recommendations to milk harvesting in Australia through guidelines, technotes, seminars, and short courses.

4.1.1.2 Other zoonotic diseases/infections

Sick and diseased animals may also shed other disease agents in the milk including those which cause illnesses. In addition to clinically infected animals, animals may be asymptomatic carriers of agents also shedding organisms in faecal material or directly into milk. Zoonotic micro-organisms that can cause illness and disease in animals: *Mycobacterium bovis, Brucella* spp., and *Coxiella burnetii.* These disease agents may be shed in milk, and subsequently cause illness in humans (brucellosis, Q Fever and tuberculosis) (Appendix 5). Although these have declined with the control or elimination of infection in milking animals, the risk of other zoonoses is a constant concern. For example, *M. avium* subsp. *paratuberculosis* (Appendix 5) is currently topical as debate continues regarding a possible link between consumption of contaminated milk and Crohn's disease.

Listeria can cause disease both in humans and animals. Encephalitis, abortion, septicaemia and mastitis due to *L. monocytogenes* has been documented in cattle. The clinical manifestation of Listeriosis in sheep and goats include encephalitis, septicaemia, and abortion. Sheep are especially susceptible to Listeriosis. In addition to clinically infected animals, a significant number of animals may be asymptomatic carriers of *L. monocytogenes* often shedding the organism in faecal material (Wiedmann and Evans, 2002).

Salmonellosis is a common intestinal illness caused by numerous *Salmonella* serovars and may clinically manifest itself in animals and humans alike as acute or chronic enteritis, an acute septicaemic disease or as subclinical infections. However, ingestion of *Salmonella* by cattle does not necessarily lead to infection or disease (Torrence and Isaacson, 2003). Animals with subclinical infections or animals recovered from clinical salmonellosis may become carriers, shedding the organism in large numbers in the faeces (Poppe, 20052). Infection rates of 10-15% in dairy cattle have been reported (Poppe, 2003). Cattle infected with *Salmonella* may also only shed the organism intermittently (Kabagambe *et al*., 2000) Foot and Mouth disease (FMD) is a virus which is shed in milk. It is of major concern to the dairy industry because contaminated milk can be the vehicle for animal infection and therefore be disseminated countrywide (Desmarchelier 2001). Australia is officially free from (FMD)[13].

Australia is free from bovine brucellosis[14] (*Brucella abortus*), and bovine tuberculosis *(Mycobacterium bovis)*[15] (Australian Quarantine and Inspection Service 1999; Animal Health Australia 2005a; Animal Health Australia 2005b; Animal Health Australia 2005c).

[13] FMD has not occurred in Australia for more than 120 years, the last reported outbreak was in 1872

[14] A national eradication program commenced in 1970, and Australia has been free of bovine brucellosis since 1989

[15] A national eradication program commenced in 1968, with Australia being recognised as bovine tuberculosis free on 31 December 1997

Ovine brucellosis (*Brucella melitensis*) has never been reported in livestock in Australia (Australian Quarantine and Inspection Service 1999).

The zoonotic micro-organism *Cryptosporidium* can infect animals, the predominant species isolated from dairy cattle being *C. parvum* (Becher *et al*., 2004). Infection in milking animals leads to diarrhoea and shedding in the faeces. *Cryptosporidium* can also cause illness in humans (Appendix 5).

Bovine spongiform encephalopathy (BSE) is a non-inflammatory disease of central nervous system in adult cattle, which originated in the United Kingdom in the 1980s. Australia is currently free from BSE. FSANZ has undertaken a comprehensive risk assessment of the available scientific data and information for BSE and has developed a policy/framework (including a Standard) to manage the risk of BSE in food.

4.1.2 Herd size

Herd size in some cases may have an effect on the prevalence of some micro-organisms (*e.g. Salmonella, E. coli* and *Campylobacter*) shed by animals. Large herd sizes in the US (>100 cattle) have been associated with salmonellosis and *Salmonella* shedding in cattle herds (Kabagambe *et al.,* 2000; Huston *et al*., 2002; Fossler *et al*., 2004). Herd size however does not appear to have any affect on shiga-toxin producing *E. coli* (STEC) prevalence(Hussein and Sakuma, 2005).

Higher stocking rates in dairies, compared with other grazing cattle and a large herd size (>100 cattle) have been identified as a possible risk factors for *Campylobacter* prevalence (Wesley *et al*., 2000; Bailey *et al*., 2003; Torrence and Isaacson, 2003). Size of herds in Australia have been increasing, with an average size of 195 cows per herd. However, there are some very large farm operations which may support up to ten individual herds of 500-1,000 dairy cattle.

4.1.3 Age/production status

Age and production status (*e.g.* lactating, dry) may influence the prevalence of some micro-organisms in cattle. A higher prevalence of *E. coli* O157:H7 has been found in lactating cows compared to dry cows (Fitzgerald *et al*., 2003) and higher prevalence rates have also been reported in culled[16] dairy cattle. It appears stress may be a factor in the increase in prevalence of STEC in lactating and culled cows. Dairy calves are also more susceptible to STEC infection than older cattle due to their lower immunity to infection (Garber *et al*., 1995; Cobbold and Desmarchelier, 2000). Prevalence has been found to increase in calves before weaning (Hussein and Sakuma 2005).

Becher et al., (2004) studied the prevalence of dairy cattle infected by *Cryptosporidium* at two farms in Western Australia. The combined prevalence rate was 48.1%, with a significantly higher isolation rate of *Cryptosporidium* from calves ≤3 weeks of age. Young calves are considered more susceptible to *Cryptosporidium* infection due to their lowered immunity, which often leads to diarrhoea and can result in the death of the animal (Duffy and Moriarty, 2003).

[16] Cows were selected to represent the distribution of cows within a herd, *i.e.* Lactating, dry, or sick. Cull cows were defined as cows expected to be culled within the next 7 days.

4.2 Environmental factors impacting on milk safety

Pathogens may originate from the dairy environment, for example, housing, faeces from cattle, manure handling, feed, soil, and water. These environmental factors may therefore influence the microbiological quality of raw milk. Dairy farming practices in the southern hemisphere are different to those observed in the northern hemisphere, particularly in the way animals are fed and housed and in general animal husbandry practices.

4.2.1 Housing

In the northern hemisphere, dairy farms generally consist of intensive production systems, with cattle often housed inside during winter to protect the animals from temperature extremes. This practice is not common in Australia (Bailey *et al.,* 2003).

Intensive housing of cattle may increase the risk of contamination of cow udders due to the closer proximity of cows to each other, concentration of faeces, contact with bedding etc. Intensive housing systems also mean increased supplementary feeding, which may also impact indirectly on the microbiological quality of raw milk. Studies have found relatively high levels of *L. monocytogenes* in cattle under these conditions which, may be a reflection of these more intensive production systems (Bailey *et al.,* 2003). Communal housing also appears to be a factor in the prevalence of *E. coli* and *B. cereus* in dairy cattle (Rahn *et al*., 1997; Christiansson *et al*., 1999).

Farm buildings can be directly contaminated with micro-organisms following outbreaks of disease in the herd or colonisation of animals, or indirectly from other sources such as contaminated water used for cleaning or access by wild animals (Torrence and Isaacson, 2003). *Salmonella* has been isolated from farm buildings (Torrence and Isaacson, 2003).

Similar effects of stocking density on the microbiological quality of sheep's milk have been reported (Sevi *et al*., 1999). Milk from ewes housed in straw-bedded pens with 2 square metres per animal had lower somatic cell counts and lower levels of mesophiles, psychrotrophs and faecal coliforms than milk from animals housed with only 1 or 1.5 m^2/animal. Mastitis was observed in animals at the higher stocking densities, but not at the lower density.

Australian farming is mainly year round pasture based (*i.e.* animals are not housed indoors), therefore Australia does not tend to experience the same problems seen in intensive farming systems. The level of *Bacillus* spores in raw milk in Australia are slightly lower than that reported overseas, due to this fact (Cook and Sandeman, 2000). The majority of isolates in a Victorian survey closely resembled *B. licheniformis*, which on a worldwide scale has been consistently amongst the most frequently isolated mesophile from raw milk. The next most common isolates recovered in this Australian survey included *B. subtilis, B. pumilus* and *B. cereus* (Cook and Sandeman 2000).

4.2.2 Faeces

The faeces of milking animals may contain a variety of enteric pathogens. Pathogens present in the faeces may result from infection of the milking animal, or through ingestion of the organisms from either feed or water.

Faecal soiling of the hide, hooves and the udder is often unavoidable. Faecal material is widely disseminated in the farm environment and potentially contributes to the farm cycle of food-borne pathogens via faecally contaminated water, feed, soil and inadequately treated

faecal waste used to fertilize pastures. Faecal material can also contaminate raw milk directly from the animal's udder, hide, or hair, thereby introducing pathogens into the raw milk. In addition, faecal material can contaminate raw milk during the milking process if the milking suction cups are kicked off by the cow onto the floor, which may be covered with a significant amount of faecal material. Some of this faecal material can then be subsequently be sucked up by the fallen cups directly into the milk line.

4.2.2.1 Prevalence of pathogens in cattle faeces

The reported prevalence of microbiological pathogens in faeces from dairy cattle, both in Australia and overseas, varies significantly. This reflects wide variations in geographic and climatic conditions, animal health, and management practices followed by individual dairy farms.

In general, dairy cattle infected with enteric pathogens will excrete the organisms in large numbers in their faeces. In particular *Listeria*, *Salmonella*, and *E. coli* are shed in the faeces of infected animals (van Kessel *et al.,* 2004). Infection of dairy cattle with STEC (see Appendix 5, Section 5.11 for definitions of pathogenic *E. coli*) can occur from grazing or consuming forages that were fertilised by contaminated manure. Faecal material can also contain very high numbers of *Bacillus* spores (Cook and Sandeman 2000).

Salmonella serotypes have been frequently isolated from cattle showing symptoms of diarrhoea (Torrence and Isaacson, 2003). Calves are generally more susceptible to *Salmonella* infection, with the mortality rate among calves with salmonellosis reported to be between 19 and 24 percent. The prevalence of *Salmonella* in US dairy cattle faeces is also highly variable, ranging from 2.1-27.5% (Losinger *et al*., 1995; Kabagambe *et al.,* 2000). Cattle that recover from *Salmonella* infection may not become permanent carriers, depending upon the *Salmonella* serotype (*e.g.* excretion of *S*. Typhimurium usually limited to a few weeks or months, whereas animals infected with *S*. Dublin may become permanent carriers (Torrence and Isaacson, 2003).

Prevalence of faecal shedding of *Listeria* spp. in European cattle has been found to vary substantially, with reported prevalence from 2%-52% (Husu, 1990). Faecal excretion of *L. monocytogenes* is very common after clinical Listeriosis. The number of *Listeria* isolations from faeces has been suggested to be associated with the prevalence of *Listeria* in feeds (Husu 1990).

Table 3: Prevalence of *L. monocytogenes* in faecal samples from dairy cattle

Country	Prevalence (%)	Reference
Finland	6.7	(Husu 1990)
Netherlands	6.0-15.3%	(Kampelmacher and Noorle Jansen, 1969)
Netherlands	2.0%	(Dijkstra, 1965)
Denmark	52%	(Skovgaard and Morgen, 1988)
Germany	33%	(Weber *et al*., 1995)
Canada	14.5%	(Fedio and Jackson, 1992)
Yugoslavia	19%)	(Buncic, 1991)
Scandinavia	3.1% - spring to autumn on pasture to 9.2% - winter indoors	(Husu 1990)

The prevalence of *C. jejuni* and *C. coli* in dairy cattle ranges from 5-53% depending on methods of isolation, age of animal (calf or adult), season, and sample analysed (faeces or intestinal contents) (Stanley *et al*., 1998). In a US survey, (Wesley *et al.,* 2000) found 37.7% of faecal samples from dairy herds to be contaminated with *C. jejuni,* and 1.8% of faecal samples contaminated with *C. coli*.

Studies on the prevalence of pathogenic *E. coli* in overseas herds concentrate on *E. coli* O157:H7 as this has historically been the STEC serovar of most human clinical significance.

Cattle appear to be a major reservoir of STEC. In a review of reported prevalence of STEC in dairy cattle faeces worldwide, Hussein and Sakuma (2005) demonstrated contamination varied between 0.2-48.8% for O157 and 0.4-47.0% for non-O157 STEC. Faecal excretion of *E. coli* O157 by cattle is considered transient, typically lasting 3-4 weeks (Lejeune *et al*., 2001a) and appears to be seasonal with the highest prevalence seen in cattle in late summer to early autumn (Herriott *et al*., 1998).

Table 4: Prevalence of pathogenic *E. coli* in faecal samples from US and UK dairy cattle

Country	Prevalence (%)	E coli type	Reference
US *cattle* *herds*	0.28% (10/3570) 8.3%	O157:H7	(Hancock *et al*., 1994)
UK *lactating cows* *non lactating cows* *calves*	0.9% 6.3% 9.3%	O157:H7	(Mechie *et al*., 1997)
US	1.2%	Verotoxin-producing O157	(Garber *et al*., 1999)
US	0.3-6.1%	O157:H7	(Kudva *et al*., 1998)

In Australia, the prevalence of STEC in dairy cattle faeces have been found to be similar to those derived in surveys from the Northern hemisphere (Cobbold and Desmarchelier 2000). A study of *E. coli* in faecal samples (n=588) found the prevalence of STEC in Australian dairy cattle was 16.7%. *E. coli* O157:H7 represented 11.2% of the total STEC isolates and *E. coli* O26:H11 represented 10.2% (or 1.9% and 1.7% of total samples respectively) (Cobbold and Desmarchelier 2000). The rate of STEC faecal shedding by Australian cattle in South-east Queensland was higher during weaning. The cattle in this study grazed on native pastures in summer and rye grass in winter with various forms of supplemental feed provided. A wide range of environmental samples were analysed in the study, including a variety of soils, tank water, dam water and sediment, river water and sediment, creek water and sediment, trough water and sediment, slurry and irrigation water samples, brewers' grain, molasses, flies (from the surface of the molasses) and feeds for milkers and weanlings. Evidence of STEC presence was demonstrated in a wide range of water samples on each of the farms. The occurrence of environmental contamination was generally low (Cobbold and Desmarchelier 2000).

In a survey of 25 faecal samples taken from six dairy farms in New South Wales and Queensland (total of 150 samples) undertaken by (Bailey *et al.,* 2003) *Campylobacter* was isolated from all farms, with a median prevalence of 6% (range 0-24%) of faecal samples being positive for *Campylobacter*. *C. coli* was isolated in 4% of samples whereas all other *Campylobacter* isolates were *C. jejuni*. *Listeria ivanovii* was isolated from one sample. No *Yersinia enterocolitica* was isolated from any of the dairy farms studied.

4.2.2.2 Survival of pathogens in faecal material

Food-borne pathogens such as *Salmonella* and enteropathogenic *E. coli* can survive for months in faecal material. *L. monocytogenes* have been found to survive in faeces stored at 5°C for several years (Husu 1990). In the environment, C*ampylobacter* has been found to remain viable at 4°C for up to three weeks in faeces and five weeks in urine (Blaser *et al*., 1980).

Wang *et al*. (1996) studied the survival of *E. coli* O157:H7 in bovine faeces and found that it could survive for up to 70 days when stored at 5°C (initial concentration of 10^5 cfu/g). Survival in faeces stored at 37°C was determined to be 49 days. At these higher temperatures, the faecal samples had low moisture contents (about 10%) and water activities of <0.5.

4.2.2.3 Manure handling

Effluents from dairy farming operations include raw manure, untreated slurry (a mixture of manure, urine, spilt feed, and water that is held without aeration), and treated slurry (aerated) that is filtered to separate the solid fraction from the liquid fraction. Dairy effluent contains many bacteria, viruses and parasite eggs and cysts.

Most large farms wash animal faeces, urine, and spilt feed from milking areas creating a slurry mixture. The slurry is held in settling tanks or ponds away from the milking operation and undergoes anaerobic degradation (untreated slurry) for more than 1 month before disposal. Some farms reduce the bulk of untreated slurry by using a mechanical aeration technique that separates the solid and liquid portions of the slurry. Appropriately treated liquids are released into the environment, while the solids, which occupy less space, are degraded by anaerobiosis before being used as fertiliser.

Where cows are housed, the practice of flushing alleys with water to remove manure appears to distribute faecal flora throughout the cow-housing environment, thus exposing large numbers of animals to faecal material. Herds maintained on farms on which alleyways were flushed with water to remove manure were eight times more likely to have samples test positive for *E. coli* O157 than herds maintained on farms cleaned by use of other methods of manure removal (Garber *et al.,* 1999).

The effluent from dairy farms undergoes primary treatment (generally in anaerobic or aerobic lagoons) before application as fertilizer to land used for silage, grazing, or cultivation. Unless appropriately processed, this effluent is a potential hazard capable of transmitting biological agents. Studies have shown that a variety of conditions in the manure can influence the survival of pathogenic bacteria that subsequently infect livestock. These conditions include temperature, solid content, pH, bacterial concentration, aeration, and the length of time that manure or slurry is held before it is applied to pasture land (Kudva *et al.,* 1998). *E. coli* O157:H7 has been shown to survive for more than 1 year in a non-aerated ovine manure pile exposed to environmental conditions by (Kudva *et al.,* 1998). In similar aerated ovine manure and bovine manure piles, the organism can survive for 4 months and 47 days, respectively (Kudva *et al.,* 1998). Proper aeration for appropriate lengths of time (1-3 months) is required before being used as fertilizers to ensure slurry is not a vehicle for environmental spread and propagation of pathogens (Kudva *et al.,* 1998).

In the past, animal waste and bedding were composted for several days, and the compost reached temperatures of 70ºC or more before being used as fertilizer. Composting and drying of manure is known to reduce the number of viable pathogens. While composting is ideal, it

is generally not a practical approach for processing cattle manure as advancements in mechanised farming have led to large numbers of animals per farm, and faster and more efficient methods for disposal of wastes are required (Kudva *et al.,* 1998).

In Australia, dairy effluent is either spayed direct onto pastures or transferred to a pond system for storage and later irrigation. Storing of effluent in ponds helps to kill pathogenic bacteria and viruses.

4.2.3 Feed

Feed plays an important role as a primary vehicle for contamination of cattle at the farm level and as an indirect source of contamination of raw milk. Major types of feeds fed to cattle are grasses (pasture), silage, grains and concentrate. Contamination of feed may originate from storage on farm or from the source of the feed (including contamination during feed manufacture). Animal feed can be contaminated with pathogens of faecal, plant and soil origin (Desmarchelier 2001).

The potential for faecal contamination in feed exists on the farm. For example, equipment used to clean manure from pens is often used for feed handling, and, on many dairies cattle are fed on concrete slabs that receive vehicle and foot traffic (Lynn *et al*., 1998). Regular cleaning of feed troughs may reduce the potential for on-farm contamination.

4.2.3.1 Grasses (pasture)

Irrigation of dairy effluent onto pastures and crops is widely practiced to increase pasture growth. Therefore poorly treated effluent may present a risk of microbial contamination to pastures and crops. Manure fertilizers may also contaminate grasses.

Use of recycled water or reclaimed water (*i.e.* water derived from sewerage systems) must be suitable treated to a standard that is suitable for its intended use.

Listeria contamination in grasses was found to be, to a large extent dependent on their moisture content. Hay, with very low moisture content, was found to be free of Listeria. While in a study by (Fenlon *et al*., 1996), no *L. monocytogenes* was found in pasture at time of harvest, it was detected within 24 hours of cutting (9/10 samples). *C. jejuni* was more frequently recovered in herds where alfalfa or whole cottonseed or hulls were fed (Wesley *et al.,* 2000).

4.2.3.2 Silage

Silage is a moist conserved fodder produced as a foodstuff for cattle. The fodder in silage is naturally preserved by lactic acid fermentation of sugars by bacteria after the fodder has been wilted and stored to exclude air. Silage has a high moisture content and is preserved by the combined effects of a rapid pH reduction to pH 4.2 and storage under anaerobic conditions. Silage is used during seasons when fresh forage is unavailable.

Traditionally, silage was prepared in bunkers, pits, or large concrete or steel silos on farm. The recent trend has been to produce silage in large bales, which are sealed by wrapping in plastic. Silage has higher moisture content than hay and is only microbiologically stable when anaerobic conditions are maintained. Such conditions prevent the growth of aerobic spoilage organisms, while the lactic acid fermentation provides acidity that inhibits the growth of any anaerobic spoilage organisms or pathogens such as *Cl. botulinum*.

Undesirable micro-organisms can enter the silage storage by soil and livestock waste during harvest or increase in numbers when the silage storage environment is suitable. Hazards include *Enterobacteria, Listeria, and Clostridia* (Mickan 2002). Both *Bacillus* spp. and *Listeria* spp. have also been isolated from silage (Husu 1990; Sutherland and Murdoch, 1994).

Micro-organisms from contaminated silage can spread to the alimentary tract of the animal being fed, resulting in the same organisms being present in the faeces (Fenlon *et al.,* 1996). In addition large numbers of spores in silage may lead to infection of dairy cattle and subsequent excretion of high numbers in faeces (Cook and Sandeman 2000).

E. coli and other *Enterobacteriaceae* and *Bacillus* and *Clostridia* species are a risk when effluent is applied to paddocks closed for silage production. Effluent sprayed onto these paddocks must be well washed in by rain before harvesting (Mickan 2002).

A slow fermentation favours the growth of enterobacteria in the early phase of fermentation as they compete more strongly with the desirable lactic acid bacteria for water-soluble carbohydrates (Mickan 2002).

L. monocytogenes is found in soil, faeces and rotting vegetation and can reproduce at low temperatures as well as in heating silage. *L. monocytogenes* is a ubiquitous organism and is likely to naturally occur in plant materials used for silage preparation and/or in contaminating soil. It is more commonly found in the outer layers of baled silage although it may occur in the layer just below the plastic sheet in chopped stack silage. Damage to the plastic wrapping or ineffective sealing of the silage may result in aerobic spoilage, and this is known to create conditions highly selective for the growth of *L. monocytogenes* and very high numbers of the organism can be present (Fenlon, 1986). Under these circumstances, a combination of rising pH, moisture, and slow air ingress favour the growth of *Listeria*.

Faecal contamination from birds and other animals, especially rodents, which can be asymptomatic carries of *Listeria,* can also contribute to *Listeria* contamination of silage (Wiedmann and Evans, 2002). The occurrence of *L. monocytogenes* in poor quality silage is well documented (Husu 1990). Correctly fermented silage from contaminated grass has been shown to contain little *Listeria* contamination, whereas spoiled silage and residues of silage remaining in feeding troughs had higher levels, up to 1.5×10^5 cfu/g^{-1} (Fenlon *et al.,* 1996).

Silage appears to be the most likely source of infection of Listeriosis in cattle and sheep. Goats that are grazed and not exposed to any silage have been known to develop Listeriosis, most likely through abrasion to the mouth by rough grazing materials providing an entry port for the organism (Wiedmann and Evans, 2002).

Spores of *Clostridia* can survive the passage through the alimentary tract of the dairy cow, and are subsequently transferred to milk via faeces, mainly though faecal contamination of the udder (Driehuis and Oude Elferink, 2000). The occurrence of clostridial spores in milk however mainly impairs its quality; the species most relevant for the dairy industry is *Cl. tyrobutyricum*. Poor silage quality can also lead to high clostridial spore levels in raw milk (Vaerewijck *et al*., 2001). *Cl. botulinum*, is the cause of botulism and may present a health hazard to animals fed silage. However, *Cl. botulinum* has a limited acid tolerance and does not grow in well fermented silage (Driehuis and Oude Elferink 2000). However risk of *Cl. botulinum* is increased substantially if the crop is contaminated with animal remains. *Cl.*

botulinum has been isolated from silage to which poultry manure has been applied (Mickan 2002).

Proliferation of *Bacillus* spp. (*B. cereus, B. lentus, B. firmus, B. sphaericus, B. licheniformis,* and *B. polymyxa)* usually occurs during the later stages of aerobic spoilage of silage. High numbers of *Bacillus* spores have been detected in the surface layers of grass and maize silage (Driehuis and Oude Elferink 2000).

In Australia, silage is now a major component on many farms as a feed resource and pasture management tool. Considerable areas of Australia are devoted to forage crops and pastures, which are either used for grazing or harvested and conserved as hay or silage. (Kaiser and Piltz 2 A.D.). Australian silage has been found to contain very high numbers of *Bacillus* spores (Cook and Sandeman 2000).

4.2.3.3 Concentrates and grains

Feed concentrate is a dried, pelleted supplementary feed ingredient for cattle. It can be composed of different ingredients such as grains or cereals (corn and barley), maize gluten feed, citrus pulp, soy bean meal and manioc or coconut cake meal (Vaerewijck *et al.,* 2001). Concentrates, particularly grain mixes which are commonly based on cereal grains such as barely, and may include other ingredients such as lupins, canola, cottonseed, maize, oats, sorghum, soybean, sunflower, rice and wheat; carrot and citrus pulps; potato by-product; and whey, are fed as supplements to pasture in Australia (Department of Primary Industries 2005).

In Australia, grain is fed to supplement dairy cattle either as "straight", "cracked" or "rolled", or as a mix of cracked or rolled. Grain is more often fed to dairy cattle rather than as a component of pelletised concentrates. The amount of supplementary feed provided to Australian dairy cattle varies throughout the year; 10 kg or more per cow per day of feed concentrates are fed in summer when no green pasture is available, with little or no supplementary feeding in spring when there is sufficient green pasture.

Faecal contamination of concentrates and grain may occur prior to delivery of feeds to the farm and can be due to faecal contamination preharvest (through manure application to crops) or post harvest by contamination from bird or rodent faeces during storage or shipment. Concentrated feed can be contaminated with *Salmonella, E. coli,* and *Campylobacter* (Weis and Seeliger, 1975; Kabagambe *et al.,* 2000; Torrence and Isaacson, 2003).

B. cereus has been isolated from feed concentrates (te Giffel *et al*., 1995; Slaghuis *et al*., 1997; Christiansson *et al.,* 1999; Vaerewijck *et al.,* 2001), including grains and pellets in Australia (Cook and Sandeman 2000). However, feed concentrates have not been shown to be a source of *Listeria* contamination as most are subjected to heat treatment during manufacture, particularly the pelleted variety, and contain moisture levels which are too low to sustain *Listeria* growth (Fenlon *et al.,* 1996). Table 5 lists the prevalence of some pathogens in feeds, and Table 6 lists the incidence and level of *L. monocytogenes* contamination in various habitats.

Table 5: Prevalence of pathogens in feed

Country	Feed type	Organism	Prevalence/levels	Reference
Netherlands	Feed concentrate	*B. cereus spores*	10^1-10^2 spores g^{-1}	(te Giffel *et al.,* 1995)
US	Feed concentrate	*B. cereus spores*	<10-10^3 spores g^{-1}	(Slaghuis *et al.,* 1997)
Sweden	Feed concentrate	*B. cereus spores*	<150-850 spores g^{-1}	(Christiansson *et al.,* 1999)
Belgium	Feed concentrate	aerobic sporeformers	4.0×10^3 - 1.1×10^6 g^{-1}	(Vaerewijck *et al.,* 2001)
UK	Silage	*B. cereus*	10^5 cfu g^{-1}	(Crielly *et al*., 1994)
Finland	Grass Silage	*L. monocytogenes*	15.6%	(Husu 1990)
Finland	Pasture grass	*L. monocytogenes*	38.2%	(Husu 1990)
US	Feed concentrate	*E. coli* *E. coli* 0157	30.1% 0%	(Lynn *et al.,* 1998)
Australia	Feed concentrate	*B. cereus spores*	1.1×10^2-6.3×10^2 g^{-1}	(Cook and Sandeman 2000)
Australia	Silage	*B. cereus spores*	7.2×10^4-3.4×10^5 g^{-1}	(Cook and Sandeman 2000)

Table 6: Incidence and level of *L. monocytogenes* contamination in various habitats (Fenlon *et al.,* 1996)

Date	Sample/site	Positive samples (Total no. samples)	Level (g^{-1})
March 1993	Soil field 1	1 (3)	Present
	Soil field 2	2 (3)	0.9 Present
	Soil field 3	1 (2)	Present
	Silage (field 1) (spoiled)	2 (2)	4.6×10^4 >2.3-<20
	Silage (field 2) (spoiled)	1 (2)	1.5×10^3
	Cattle faeces (silage field 1)	1 (1)	>2.3-<20
	Cattle faeces (silage field 2)	4 (4)	0.4 3.6×10^2 5.0×10^2 20
July 1993	Cattle faeces (grazing)	3 (10)	0.4 0.4 Present
	Stored 1 year-old used silage bags	1 (6)	Present
February 1994	Following season silage	4 (6)	8.1×10^4 1.0×10^3 2.0×10^3 Present

4.2.4 Soil

Soil represents an important source of pathogens for grazing animals. A wide variety of organisms, including pathogenic bacteria, may be found as typical soil microflora, plus faeces and urine from grazing animals may contaminate soil by the application of fertilisers and effluent.

Bacillus and Clostridium species are commonly found in soil. The levels of *B. cereus* in soil has been found to vary from <50-380,000/g (Christiansson *et al.,* 1999). In Australia, levels of *Bacillus* spores have also been found at levels between 5.6×10^2-1.8×10^3 cfu/g (Cook and Sandeman 2000).

L. monocytogenes is a ubiquitous organism that is naturally found in the soil and thereby finds its way into grasses and silage made from pastures. Soil contaminated with *L. monocytogenes* (and other micro-organisms) can be inadvertently transferred into the milking parlour, from farms to factories via milk tankers, and on the feet of employees.

Food-borne pathogens such as *Salmonella* and enteropathogenic *E. coli* can survive for months in contaminated soil (Desmarchelier 2001).

4.2.5 Water – stock drinking

Water is used extensively on dairy farms for cleaning, cooling, stock drinking and irrigation. Dairy cattle consume large amounts of water daily. Running water and water from wells, bores and large fenced dams are generally less contaminated than stagnant water supplies, particularly those to which cattle have free access (Agriculture Western Australia 2005). Sediment in water can support bacterial growth and may be a reservoir for pathogenic micro-organisms. Depending upon the water source, water may contain high levels of bacterial contamination even before it enters water troughs. High spore counts have been observed in Australian dam water, probably due to run off from paddocks containing a high level of suspended soil and organic material (Cook and Sandeman 2000).

E. coli O157:H7 has been reported to survive in pond water at 13ºC and in river water at 18ºC for 20 and 13 days respectively (Wallace, 1999) and can survive in trough sediments for 4 months and in water for 8 days at 5ºC (Rice and Johnson, 2000).

Water may be a significant reservoir of *Campylobacter*, where it has been shown to be able to remain viable for up to 4 weeks at 4ºC (Blaser *et al.,* 1980). Transmission of *C. jejuni* has been observed from groundwater to dairy cattle (Stanley *et al.,* 1998).

Salmonella and *E. coli* O157 were detected in water troughs with a prevalence of 0.8% and 1.3% respectively (Lejeune *et al*., 2001b). However it has been reported that *E. coli* O157 could be repeatedly isolated from environmental sources on farms and be present in as many as 10% of water troughs (Lejeune *et al.,* 2001b). Water troughs can become contaminated with cud and/or faecal material. Extraneous matter including dust, feed, or bedding may also enter the trough.

The position of water troughs on farm may also affect prevalence of *E. coli*. It has been demonstrated that bacterial contamination is higher in troughs that are closest to the feed troughs (Lejeune *et al.,* 2001b). Water troughs close to feed bunks (<7.62 m) have been shown to have higher numbers of *E. coli* O157 than those placed further away (Lejeune *et al.,* 2001b). Metal troughs also have shown lower *E. coli* O157 counts compared with troughs that were manufactured from concrete or plastic (Lejeune *et al.,* 2001b). Water trough sediments contaminated with faeces from cattle excreting *E. coli* O157 therefore may serve as a long-term reservoir of this organism on farms and a source of infection for cattle (Lejeune *et al.,* 2001a).

In addition *E. coli* contamination of cattle has been found to be positively associated with the protection of the water trough from direct sunlight, lower concentrations of protozoa in the trough water, and warmer weather (Lejeune *et al.,* 2001b). Frequent cleaning of water troughs and/or treatment of drinking water with chlorine, UV light, or ozone to kill or inactivate pathogens will also reduce the potential for replication and/or survival of pathogens.

Wet and muddy conditions around drinking points may result in contamination of the legs, udder and tail of cattle with mud and faecal material. This increases the risk of bacterial infections of the teat and udder, leading to environmental mastitis, and high bacterial counts on the udder and teats leading to possible contamination of raw milk during milking. It is important, therefore, to maintain the surroundings of watering points in a dry, solid and stable condition to prevent them becoming boggy and muddy.

4.3 Microbiological monitoring and status of Australian milk

The composition and hygienic status of raw milk is determined by a number of tests on arrival at the dairy processing plant. The outcome of these tests has a direct bearing on the money paid to the farmer under performance payment schemes. Monitoring of cell counts may also occur on farm, and this provides an indirect way of estimating the level of subclinical mastitis in the herd and can trigger action to improve or better manage herd health and to improve milk yields. Early detection and treatment of sick animals reduces the risk of severe and intractable cases developing and reduces the likelihood of infection being passed to other cows.

Milk processors, industry associations and programs in Australia, such as Countdown Downunder, provide guidance, assistance and technical support to dairy farmers to ensure consistent milk quality and safety.

Bulk milk cell counts and herd milk cell counts[17] are monitored by *Countdown Downunder* (a program initialled by Australian Mastitis Advisory Council to help farmers and their advisors achieve mastitis control and reduce cell counts). The industry goal is for more than 90% of all farms to supply milk of less than 250,000 cell/mL and 100% of farms to reach a cell count of less than 400,000 cells/mL (Table 7).

Table 7: Australian milk herd cell counts (Dairy Australia 2005)

Year	Herd Milk Cell Counts below 250,000 cells/mL Goal 90%	Herd Milk Cell Counts below 400,000 cells/mL Goal 100%
2000	64%	91%
2004	71%	95%

[17] Herd milk cell count refers to somatic cell count of individual cows in every herd aggregated into a volume-weighted average for the herd.

During the past three years, the inclusion of cell counts in the buying standards and payment schemes of dairy companies has become universal in Australia. Payment schemes are typically based on milk volume and composition, with bonuses or penalties for conformance with specific milk quality indicators (e.g. Bactoscan, Bulk milk cell count (BMCC)[18], total plate counts, thermoduric count, and sediment). Dairy companies differ in the thresholds they use to define premium milk payments depending on the types of products being manufactured (Tables 8-11). Premium payments are typically about one cent per litre extra. In some states there is a ceiling BMCC above which milk processors will not collect the milk. Milk processors report the results of bulk milk cell counts to farmers.

The majority of Australian milk meets the premium milk quality grade 'Band 1' (Figure 1).

Table 8: Milk quality standards for Bactoscan for Australian processors (Dairy Australia, 2005)

Processor	Band 1 (Premium)		Band 2	Band 3	Band 4
A	<80		81-200	201-600	>600
B	<200		201-500	501-2000	>2000
C	<160		160-218	219-493	>493
D	<80		81-200	>200	
E	<51	51-80	81-200	201-1000	>1000

Table 9: Milk quality standards for Bulk Milk Cell Count for Australian processors (Dairy Australia, 2005)

Processor	Band 1 (Premium)		Band 2	Band 3	Band 4
A	<250		251-400	401-800	>800
B	<250		251-600	601-800	>800
C	<250		251-400	401-800	>800
D	<300		301-600	>600	
E	<250	251-350	351-600	501-700	>700

Table 10: Milk quality standards for thermodurics for Australian processors (Dairy Australia, 2005)

Processor	Band 1 (Premium)	Band 2	Band 3	Band 4
A	<1500	1501-3000	3001-6000	>6000
D	<5000	5001-20000	>20000	
E	<2000	2001-5000	5001-10000	>10000

Table 11: Milk quality standards for sediment for Australian processors (Dairy Australia, 2005)

Processor	Band 1 (Premium)	Band 2	Band 3	Band 4
A	Absent			Present
B	<0.5		0.6-2.0	>2.0
C	Advisory			
D	Disc 1-2		Disc 3-4	

[18] Bulk milk cell count refers to the concentration of somatic cells in the total volume of milk in the milk vat.

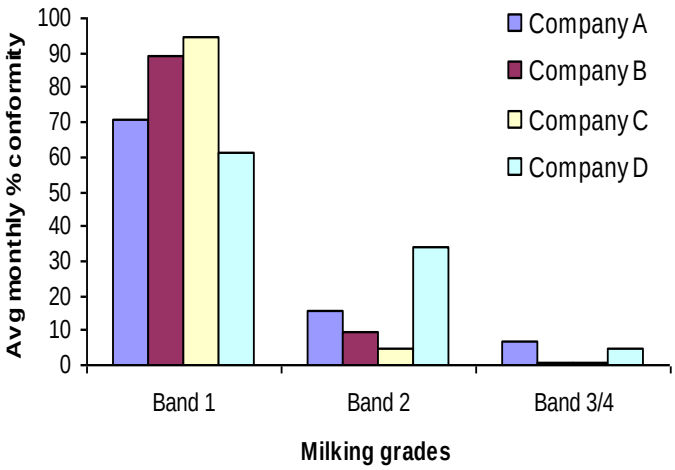


Figure 1: Average monthly percentage of suppliers for each Milk Quality Grade 2004-2005

4.4 Effect of milking on milk safety

4.4.1 Milking practice

4.4.1.1 Teat washing

Poor milking practices may lead to contamination of raw milk. The teat surface is the major avenue of entry of micro-organisms into raw milk. It is well recognised that there is significant opportunity for teats to become contaminated by faeces and soil (as dust or mud) (Cook and Sandeman 2000; Vaerewijck *et al.,* 2001). If not removed before milking, this dirt, together with the large number of micro-organisms associated with it, may be washed into the milk during milking. Contamination of teats is less when cows are pasture-based rather than intensively housed (Slaghuis *et al.,* 1997).

Significant relationships have been observed between the number of *Bacillus* spores isolated from the surface of the teat, as well as from the surface of cup liners, with the number of spores found in bulk raw milk. These observations were related to the cleanliness of the teat at milking, and may represent accumulative build-up of dirt and bacteria from the teats in milking equipment (Cook and Sandeman 2000). The contribution of *B. cereus* from the exterior of the udder has been shown to decrease following cleaning and disinfecting the animal surface before milking (Christiansson *et al.,* 1999).

In Australia, washing cow teats is only recommended if they are dirty. If teats are washed, it is important that they are dried to minimise the risk of the animal developing mastitis (Brightling *et al*., 2003). If teats are left wet, more bacteria may be found on the teats (Slaghuis *et al.,* 1997). Hence, minimising the use of water on udders and teats is beneficial for teat skin health and also generally leads to better milk quality (reduced coliform counts, sediment, etc) unless very careful drying techniques are used. Research has shown that there is no reduction in milk quality when teat cups are applied to visibly clean, dry teats (Hubble and Mein, 1986).

4.4.1.2 Teat disinfection

Post milking teat disinfection is an effective procedure for reducing the rate of subclinical and clinical mastitis during lactation (Slaghuis *et al.,* 1997). Teat disinfection helps keep teat skin healthy and heal skin lesions, and these actions may be its most important contribution to mastitis control (Hillerton, 1997). The majority of Australian dairy farmers rely on post-milking teat disinfection, applied by spray techniques, as an integral part of their mastitis control programs (Lee, 1994).

One of the biggest variables in successful use of teat disinfection is the quality and consistency of application. Failure to cover the whole teat of every cow at every milking is the most common error in teat disinfection. Disinfectant is applied by dipping each teat separately in a cup or spraying disinfectant on to the teats from below. Dipping has the advantage that complete coverage of the teat barrel is fairy easy to achieve. Spraying disinfectant often coats one side of each teat only. Spraying is the method of teat disinfectant application in most Australian herds as it is quicker and easier to incorporate into milking routines.

The effectiveness of teat sprays is dependent on the quality of water used to make up the disinfectant. Water quality characteristics that alter the bacterial killing power of teat disinfectants include alkalinity, water hardness, organic matter and chlorine concentration. Countdown Downunder recommends using cooled hot water to minimise the bacterial load (Countdown Downunder, 2000).

The Rural Industries Research and Development Corporation recommends that mastitis in sheep be minimised by disinfecting the teats after each milking, which prevents the entry of mastitis-causing bacteria into the teat canal, which is naturally enlarged after milking (Bencini and Dawe 2005).

4.4.1.3 Milk segregation

In Australia, colostrum milk must be withheld from the vat for at least eight milkings after calving. Colostrum has a high number of somatic cells (not due to mastitis) and its changed composition has a significant effect on the processing efficiency of dairy products.

Similarly, milk derived from cows treated with antibiotics must be segregated from the bulk milk collection system. The approval of veterinary medicines is strictly controlled in Australia, and their use must be under the supervision of a veterinarian. Withholding periods for antibiotics must be strictly adhered to, resulting in a minimum period of time that must elapse between the last treatment of an animal with a veterinary medicine and the supply of milk from that animal for food consumption.

4.4.1.4 Cooling and filtration of milk

The composition of milk makes it an excellent growth medium for many micro-organisms unless it is frozen or further processed to kill or prevent their growth. As the temperature of raw milk as it leaves the udder is around 37ºC pathogenic bacteria, if present, will grow rapidly. At temperatures between 0 and 5ºC, growth of pathogenic and spoilage mesophilic bacteria are slowed. Therefore, after milking raw milk should be cooled to below 5ºC and stored refrigerated until collection to minimise the potential growth of micro-organisms.

As the milk leaves the udder, it is initially cooled by passing through a heat exchanger (plate cooler) prior to it entering the refrigerated milk vat (bulk milk storage tank). It is further cooled in the vat. Any breakdown in the refrigeration system, or failure to properly cool milk prior to collection may impact on the quality and bacterial load in raw milk.

Filtration of milk before it enters the bulk milk tank provides a safeguard to ensure sediment or other extraneous matter is removed from the milk prior to storage. Although filtration removes most of the soil and other particles, it does not remove all the bacteria adhering to these particles, nor does it remove any dissolved matter from the milk. Sediment that has been trapped by the filter continues to be washed by the milk flowing through. This dislodges bacteria adhering to the particles and thus contributes to an increased bacterial count in the bulk milk. If filters are not adequately cleaned, this process may be a source of cross-contamination of the milk.

Forcing milk through an in-line filter by pump is the most common method of filtering milk in modern milking plants. The filters may be made of various elements including paper, fibre or cloth and they fit over a perforated metal support or cage within a cylindrical tube.

4.4.1.5 Cleaning and sanitation

There are various methods for cleaning milking parlours/yards. Walls and floors are typically cleaned using a combination of manual scraping or brushing and then rinsed using hoses with water under medium or high pressure. Some areas are also fitted with automated cleaning systems that clean the floor of the milking parlour and/or holding areas. This may be accomplished by periodic application of water under pressure or by a flush system in which a large volume of water is released to create a wave of water on floor surfaces that carries manure and urine to a collection pit. However care must be taken in automated systems that flush water does not contaminate milking equipment.

4.4.2 Water use in milking

Water is used extensively during the milking process and may be a possible source of contamination if it is of unacceptable quality. Water is used for teat cup washing, washing of cows teats, milking plant flushing and rinsing, milk vat flushing and rinsing, milk pre-cooling, and teat disinfection.

The greatest risk of milk contamination from water is from water used to flush the milking plant following milking.

A qualitative assessment undertaken by Dairy Australia, estimates the risk of microbiological contamination of milk via water in Australian dairy farms to be negligible. However, the use of untreated water to flush the milking plant following milking was found to be of a slightly higher risk (low/medium) (Dairy Australia, 2004).

4.4.3 Milking equipment

Milking equipment and methods have an important effect on both animal health and the microbiological status of raw milk.

Cows with mastitis are responsible for elevated levels of somatic cells and bacterial cells in raw milk. The use of milking equipment is estimated to lead to about 20-25% of mastitis infections in Australia (Countdown Downunder 2003). The main milking-related mechanisms of spread of mastitis infections include:

- spreading of organisms via contaminated liner surfaces, milker's hands and teat lesions;
- impacts and possible reverse pressure gradients may assist the passage of organisms into the teat canal;
- teat damage and loss of keratin lining of the teat canal can lead to a decrease in the natural effectiveness of the teat canal as a barrier; and
- less frequent or less complete emptying of the udder.

Proper sanitation and prevention of faecal contamination of equipment during milking is critically important. Contamination of raw milk can also occur from equipment used for milking, filtering, cooling, storing, and distribution of milk. Milk handling equipment contributes a large proportion of raw milk microflora (ICMSF, 1998). Poor cleaning and hygiene standards in the waiting area and at milking; wet udder preparation using one towel for many cows; failure to apply fore-milking; and poor maintenance of milking equipment can lead to contamination of raw milk with pathogens (Sanaa *et al*., 1993).

Milk residues left on equipment surfaces after inadequate cleaning provide nutrients and high ambient temperatures favour the growth of microbial contaminants. Surfaces often remain wet for long periods, permitting build-up of micro-organisms that adhere to equipment surfaces. During subsequent use of equipment, these micro-organisms can contaminate milk. The type and number of organisms introduced from milking equipment is largely dependent on the efficiency of cleaning and disinfecting. Bacteria will proliferate in milk residues left on equipment and increase rapidly if milk is cooled slowly, or inadequately (National Milk Harvesting Centre 2000).

Cleaning of milk handling equipment involves a combination of chemical, thermal and physical processes. The key principles of a good cleaning system involves sufficient hot water (temperature and volume), correct wash solutions (detergent), adequate contact time and sufficient turbulence to prevent build up of milk residues and bacteria in the equipment.

Table 12: Mean number of *Bacillus* spores present in environmental and bulk milk samples from Cobden and Stanhope, Victoria (Cook and Sandeman 2000).

Sample	Cobden	Stanhope		
	Mesophilic spores	Mesophilic spores	Thermophilic spores	Anaerobic spores
Bulk milk (cfu/mL)	73	7	2	7
Teat skin (cfu/mL)	7.8×10^3	2.2×10^3	7.0×10^2	1.9×10^3
Cupliners (cfu/mL)	1.3×10^3	82	25	48
Foremilk (cfu/mL)	1.2×10^2	13	4	8
Hot water (cfu/mL)	25	1.0×10^2	1.1×10^2	3.8×10^2
Cold water (cfu/mL)	23.5×10^2	3	1	3
Faeces (cfu/g)		6.8×10^2	1.3×10^3	8.6×10^2

4.4.4 Hygiene of milking personnel

Personnel in direct contact with cows and milking equipment are also a potential source of contamination. Milk handling personnel may contribute various organisms, including pathogens, directly to milk. Micrococci and staphylococci from skin and upper respiratory tissues may gain entrance, especially during hand milking. Workers with illness and/or infections who come into direct contact with dairy equipment can also introduce contamination.

4.5 Raw milk collection and transport to processors

Raw milk must be adequately protected during transport to prevent any further contamination. It is also important to ensure milk is kept cool to prevent the growth of organisms. Cleaning of milk tankers is a critical process to minimise contamination and to maintain a high quality supply of raw milk.

In Australia raw milk is collected from the farm every 12-48 hours in heavily insulated stainless steel tankers. Collection interval depends on herd size, size of the milk vat, and season. In Australia, there is a trend to collect milk less frequently due to financial incentives offered to farmers by milk processors.

Milk is unloaded into insulated silos at the processing facility before results of microbiological testing and somatic cells counts are known. Typically, these silos contain milk from a series of tankers, and this results in significant dilution effects where milk of high bacterial count is mixed with high quality milk. As these silos are often not refrigerated, the temperature of the milk will be reflect the temperatures achieved on farm.

Trucks are cleaned and sanitised at the dairy plant or at an intermediate wash station.

4.6 Summary of major primary production risk factors for milk production in Australia

There are two means by which pathogens contaminate raw milk. Contamination may occur when micro-organisms are shed directly into raw milk from the udder through illness or infection of the animal, or through contamination from the external surface of the cow and the milking environment. However there are many factors that impact on these routes of contamination. Table 13 summarises the major risk factors in the production of milk.

Table 13: Major risk factors in production of milk

Risk factor	Effect	Control
Animal health	Disease in, sickness of, and carriers in milking animals can increase shedding of pathogens directly into raw milk, or in animal faeces.	Animal health and mastitis programs
Herd size	Herd size may have some effect on the prevalence of some pathogens (*e.g. Salmonella, E. coli* and *Campylobacter*)	Biosecurity and animal husbandry
Age/ production	Calves have an increased susceptibility to infection, and have been reported to have higher prevalence rates of some pathogens (*e.g. E. coli*)	Calves kept separate from milking herd
Housing	Intensive housing practices may increase risk of contamination of udders due to close proximity of animals, concentration of faeces, bedding etc. This has been shown to be a factor in the prevalence of *Bacillus spp., E. coli,* and *L. monocytogenes*	Australian dairy farming is mainly pasture based
Faeces	Faeces may contain various pathogens – reflecting either illness/infection, or through ingestion of contaminated feed and/or water with faeces. Faeces may contaminate the exterior of the udder and introduce pathogens into raw milk.	Udder hygiene at milking
Effluent	Effluent (containing manure) can also contaminate pasture.	Appropriate treatment and disposal of effluent
Feed	Contamination of feed can lead to shedding of pathogens into faeces. Poorly made silage can be a source of pathogens (*e.g. E. coli, Bacillus spp., Listeria,* and *Clostridia*).	Control over preparation and storage of feed, especially silage
Water – stock drinking	Water is a potential source of contamination. Sediment in water can support bacterial growth and be a reservoir for pathogens. Water sources can become contaminated with cud and/or faecal material, feed, etc.	Ensuring water is of suitable quality
Milking	Poor milking practices, including dirty teats, inadequate cleaning and maintenance of milking equipment, and poor personnel hygiene can lead to contamination of raw milk.	Maintenance, sanitation and cleaning of equipment, appropriate animal and good personal hygiene
Water use - milking	Water is a potential source of contamination during washing of teats and cleaning of milking equipment.	Ensuring water used is of suitable quality
Storage	Inappropriate temperature control of milk after milking can lead to growth of pathogens	Rapid cooling of milk and regular collection.
Transport	Inappropriate temperature control of milk during transportation can lead to out-growth of pathogens. Contamination can occur if tankers do not adequately protect milk, and/or are inadequately cleaned.	Temperature control, tanker maintenance, and cleaning and sanitation

5. Processing and the impact of processing on milk and milk products

5.1 Processing of milk and milk products

After transport from the dairy farms, raw milk is pumped from the bulk milk tanker into insulated silos at the processing plant. After initial screening and testing, the raw milk is processed into a diverse range of dairy products using a range of processes and technologies.

Processing of dairy products is intended to:

- produce microbiologically safe products of acceptable shelf-life;
- develop or maintain desired sensory qualities (appearance, flavour, texture); and
- isolate particular constituents of milk which are used directly or as part of other foods or for non-food purposes.

In order to produce microbiologically safe dairy products, processing of raw milk requires a microbiocidal processing step to eliminate, remove or destroy any vegetative pathogens present. Heat treatment (i.e. pasteurisation) applied by an appropriate time/temperature combination is traditionally used as the key microbiocidal step in the manufacture of dairy products. However this processing step reduces the number of micro-organisms only at the point in the manufacturing process where it is applied, and its effectiveness in terms of end product safety depends on the initial microbial load of the raw milk, the effect of any post-treatment contamination and/or growth, and the implementation of further control measures.

Pasteurisation of raw milk is not normally applied as the sole control measure but is used in combination with a number of preventive measures (hurdles[19]). For example pasteurisation is followed by packaging and refrigeration of liquid milk while heat treatment, fermentation, salting and aging are applied in the manufacture of cheese.

Microbial growth is dependent upon many conditions such as nutrients, water activity, pH, temperature, presence of preservatives, competitive micro-organisms, and atmospheric conditions. Control of these conditions can therefore be used to limit, retard or prevent microbial growth.

Therefore depending on the type of dairy product being manufactured, the processing methods and hurdles employed may include:

- heat treatment (thermisation, pasteurisation, ultra-high temperature (UHT) sterilisation;
- cold treatment (chilling/refrigeration, freezing);
- mechanical treatment (separation, centrifugation, homogenization, filtration, agitation);
- removal of water (concentration, dehydration, curing/ageing);
- microbiological or biochemical fermentation (acid production, lipolysis, proteolysis); and/or
- combinations of these methods.

Detailed descriptions of the equipment and processes employed to convert liquid milk into processed dairy products may be found in the ***Dairy Processing Handbook*** (published by

[19] The ***hurdle concept*** (Leistner and Rodel, 1976) describes the effect of multiple factors (e.g. temperature, pH, water activity) on microorganisms. Several different hurdles at sub-optimal levels can be used to control the growth of microorganisms in food products, rather than a single, severe hurdle.

Tetra Pak processing Systems, Sweden). The effect of specific processes on microbiological hazards in various dairy commodities is discussed in more detail in Appendix 1. A brief summary of the findings in Appendix 1 is presented in Table 14.

Table 14 Impact of processing on microbiological safety in various dairy commodities

Dairy product	Processing and impact on microbiological safety
Milk and cream	Milk is derived from mammalian animals e.g. cows, sheep, goats, etc. Cream is produced from whole milk by separation. The main steps in milk and cream processing include filtration; homogenisation; and heat treatments e.g. pasteurisation, sterilisation and UHT processing. Pasteurisation is sufficient to destroy the most significant milk-borne vegetative bacteria.
Cheese	Cheese making normally begins with heat treatment of milk, followed by addition of starter culture and rennet, resulting in production of a cheese curd through coagulation and acidification. Mild heating separates the whey, which is drained away. Curds are salted, pressed into moulds and ripened under controlled conditions. A number of processing factors influence the growth and survival of pathogens in cheese, including the severity and duration of heat treatment (including curd cooking); pH; salt concentration; water activity; and maturation/ripening.
Dried milk powders	Liquid milk is initially concentrated and spray-dried to form a powder. Micro-organisms associated with dried milk powders will not grow, however, they may survive for long periods of time and resume growth when the powder is reconstituted and stored under favourable conditions. The presence of micro-organisms depends on factors such as the bacterial load in the raw milk, preheating temperatures, operating conditions of the evaporator and dryer, and plant hygiene. Post-process contamination is a major factor impacting on the contamination of milk powders. The major factors affecting the survival of pathogenic micro-organisms are evaporation and drying.
Infant formulae	Infant formulae are a sub-set of dried milk powders. Formulae may contain milk, soy protein or protein hydrolysates, fat, carbohydrate, vitamins and minerals. These products are either manufactured in powdered form through evaporation and spray-drying, or in liquid form, followed by a high temperature treatment. Microbial pathogens in powdered formulae are the same as those for dried milk powders and as such are not able to grow due to the absence of water.
Concentrated milk products	Evaporated and sweetened condensed milks are both manufactured from milk and sugar that are heated, evaporated and homogenised. Evaporated milk receives a UHT or sterilisation treatment before cooling and packaging, while condensed milk is seeded with lactose, cooled and packaged. Pathogens are generally not associated with these milks due to their low water activity.
Butter	Butter is produced from pasteurised cream. Churning the cream produces butter. Growth of micro-organisms in salted butter is unlikely, due to it's moisture distribution and salt content.
Ice-cream	Ice-cream is a frozen aerated emulsion made from cream and/or milk products, and other ingredients. The ice–cream mix is pasteurised, homogenised, aged and whipped to incorporate air while being frozen. The heat treatment applied to ice cream mix destroys pathogenic micro-organisms. However, pathogens may be introduced with the addition of ingredients. Pathogens will not grow in ice-cream, but may survive freezing.
Cultured and fermented milk products	Cultured and fermented milk products are prepared by fermentation of milk using specific micro-organisms which reduce the pH and coagulate milk proteins. In the production of yoghurt, milk is homogenised and heat treated. After cooling a starter culture is added and allowed to ferment for several hours. Flavours and other ingredients can be added before the product is packaged. The heat treatment of milk is sufficient to destroy vegetative micro-organisms and rapid growth of starter cultures inhibits the outgrowth of spore-formers. Pathogenic micro-organisms are prevented from growth by the low pH; the presence of lactic acid, and by refrigerated storage.
Dairy desserts	Dairy desserts can be based on fresh milk, milk powder or milk protein concentrates to which flavours, colours and sweeteners may be added. Dairy desserts mixes typically undergo a heat treatment; and further processed (e.g. whipping and freezing). Heat treatment by pasteurisation or UHT results in the destruction of vegetative cells. Contamination may occur after heat treatment with the addition of further ingredients, or through survival of spores of *B. cereus*.
Dairy-based dips	Dairy-based dips range from processed cheese-type products to sour cream-based dips to which herbs/spices, dehydrated vegetables and flavouring agents are added. Where pasteurisation or other heat treatments are employed, vegetative cells will be destroyed. However, spore-formers can survive heat treatments and other hazards can be introduced with the addition of heat labile ingredients after heating. The low pH of these products assists in their microbial stability
Casein, whey and other functional milk derivatives	These products are derived from milk by concentrating components from whey, skim milk, etc. Normally, heat treatment is followed by steps such as ultrafiltration, acid precipitation or proteolysis. These products are derived from milk and cream that have received at least a pasteurisation heat treatment and so will be free from vegetative cells. Most of these products are dried, thus the low water activity ensures that outgrowth of pathogens is very unlikely.

Dairy product	Processing and impact on microbiological safety
Colostrum	Colostrum is the initial mammary secretion after the birth of the calf. Colostrum obtained by milking is pasteurised, concentrated by evaporation, and either spray-dried or freeze-dried. Pathogens may be protected by the elevated fat and total milk solids content compared to standard bovine milk. Contamination after processing is a concern, although the low water activity of colostrum powder will prevent growth and vegetative cells will eventually die off.

Given the efficacy of the pasteurisation process, post-pasteurisation contamination remains a major concern for the safety of dairy products. Rigorous controls over hygiene, cleaning and sanitation, and product handling are necessary to ensure the final product is not contaminated with pathogenic micro-organisms and opportunities for growth are limited. Contamination may result from the environment, including equipment, personnel or contamination of finished product with raw materials.

Of particular concern is *L. monocytogenes,* particularly in moist and chilled products. Its psychrotrophic nature enables the organism to colonise and grow in wet and cold environments including condensation on walls and ceilings, equipment surfaces, drains, floor puddles, condensate collected in refrigeration units and condensation in compressed air lines. Likewise, *Salmonella* has presented problems particularly with dried milk products. Dust and powder residues from ledges, filterhoods, wall ceilings, floors and ancillary equipment are common sources of contamination. Powder, dust and water supply the nutrients and the warmth in some processing environments provides ideal growth conditions for *Salmonella*.

5.1.1 Cleaning and sanitation of processing equipment

The safety and quality of dairy products also depends on proper cleaning and disinfection of processing equipment. The soil encountered in dairy processing plants consists mainly of adhering products and product particles such as milkfat, protein and milk minerals. Residues left on equipment surfaces after inadequate cleaning provide nutrients for microbial growth and will permit build-up and adherence of bacteria films on equipment surfaces.

Micro-organisms commonly found on food contact surfaces include enterobacteria, lactic acid bacteria, micrococci, streptococci, pseudomonas, and bacilli (Wirtanen et al, 200?). Inadequate cleaning may result in large numbers of lactococci, coliforms, and other Gram-negative organisms such as *Pseudomonas*, *Alcaligenes*, *Flavobacterium* and *Chromobacterium* (ICMSF, 1998). These organisms are heat sensitive and are readily destroyed by chlorine disinfectants (ICMSF, 1998).

Inadequate cleaning allows micro-organisms to adhere and grow on equipment surfaces and form protective extracellular matrices – biofilms. Once formed, elimination of biofilm is very difficult. Equipment design and choice of surface materials are crucial to combating biofilm formation, as is the cleaning regime. Dead ends, corners, cracks, crevices, gaskets, valves and joints are vulnerable points for biofilm accumulation. Biofilms in the dairy industry are characterised by the predominance of a single species of bacteria e.g. *Streptococcus thermopiles* or *Bacillus* spp. (Flint et al, 1997).

Effective cleaning, disinfection and post-rinsing are all important in eliminating micro-organisms. Automated cleaning (or CIP – cleaning in place) systems are frequently used in the dairy industry, and can provide high and reproducible standards of cleanliness (ICMSF, 1998). Automated cleaning systems allow rinsing water and detergent solutions to be circuited through tanks, pipes and process lines without being dismantled. The passage of high velocity flow of liquids over equipment surfaces generates a mechanical scouring effect which dislodges dirt deposits.

In addition to automated cleaning systems, it may also be necessary to dismantle and manually clean equipment. Steam, hot water or chemical sanitisers may be used to sanitise the plant and equipment. Water used in cleaning should be of potable quality.

The efficiency of cleaning and sanitation should be subject to regular environmental monitoring and verification. In addition to verifying the effectiveness of cleaning procedures to ensure residual material is removed, regular monitoring of the environment in and around the processing plant can be an effective early warning system for identifying potential sources of contamination of dairy products.

The Australian Dairy Authorities' Standards Committee (ADASC) is responsible for developing and administering legislation and inspection procedures to ensure Australian dairy products are hygienically manufactured and do not present a risk to public health. ADASC has worked with dairy companies to develop manuals to assist the dairy industry to control *Listeria* spp and *Salmonella* spp in the dairy processing environment. State Dairy Authorities have also developed Codes of Practice for Dairy Food Safety. Standards Australia also has standards which set out accepted practices for cleaning and sanitising dairy factory equipment (AS1162 – 1991, *Cleaning and Sanitising Dairy Factory Equipment*).

5.2 Pasteurisation

The *Australia New Zealand Food Standards Code* (the Code) requires that milk and liquid milk products must be pasteurised (or undergo an equivalent heat treatment)[20]. In the case of cheese manufacture, the Code specifies pasteurisation or thermisation (in combination with a minimum storage time). Standard 1.6.2 of the Code specifies that, for the pasteurisation of milk in Australia, the minimum heat treatment is no less than 72ºC for no less than 15 seconds, or any other time and temperature combination of equal or greater lethal effect. These processing measures have been in place historically as an important public health measure to manage the microbiological hazards that may be present in raw milk. There is currently no mechanism in the Code, by which non-thermal processes (*e.g.* ultra high pressure treatments) may be considered as valid, alternative processes to pasteurisation.

This means raw milk and raw milk products are not permitted to be sold in Australia, unless expressly permitted by a State or Territory or if a specific exemption has been given as a result of an assessment process. The sale of raw goat milk is permitted in South Australia, Queensland, New South Wales and Western Australia. In addition, some specific raw milk cheeses are permitted in the Code where an assessment has shown that they can be produced to an equivalent level of safety as cheeses made from heat-treated milk.

[20] Milk and liquid milk products includes those used in the production of cream and cream products, fermented milks, yoghurt, dried, condensed and evaporated milks, butter and icecream.

5.2.1 History of pasteurisation[21]

In 1911, the National Milk Standards Committee in the United States was the first professional body to recommend a minimum time-temperature combination for the pasteurisation of milk: 62.8ºC (145ºF) for 30 minutes (now known as the batch or holder method). This heat treatment was slightly above what many people at the time considered to be adequate for the destruction of *M. tuberculosis*, one of the main milk-borne pathogens of concern in that era.

However, it was not until further research and investigation of commercial equipment that the 'holding method' of milk pasteurisation was officially and legally recognised as an adequate method of pasteurisation in the United States where, in 1924, the first Pasteurised Milk Ordinance was published. In the Ordinance, pasteurisation was defined as '*a heating process of not less than 142ºF (61.1ºC) for 30 minutes in approved equipment*'. However, it is noteworthy that a temperature 3ºF lower than that which had been recommend earlier, in 1911, was officially adopted.

Following further studies on the thermal destruction of *M. tuberculosis* and other pathogens, a High Temperature Short Time (HTST) pasteurisation standard - 161ºF (71.7ºC) for 15 seconds - was included in the 1933 edition of the U.S. Public Health Service Milk Ordinance and Code.

In the late 1930s, it became apparent that *Coxiella burnetii*, the causal agent of Q Fever, was more heat resistant than *M. tuberculosis*/*M. bovis*. Studies reported in 1956 showed that if *C. burnetii* cells were present in raw milk in large numbers, some might survive 143ºF (61.7ºC) for 30 minutes. These studies resulted in a recommendation by the US Public Health Service to increase the standard for the 'holding method' of pasteurisation to 145ºF (62.8ºC) for 30 minutes. It was also suggested that at least an additional 5ºF (2.8ºC) be added to the holding temperature for products with a fat content higher than whole milk or with added sugar.

Apart from some rounding of numbers to take account of Fahrenheit-Celsius conversions, the above standards for pasteurisation have remained unchanged to the present day. According to the International Dairy Federation, the minimum time-temperature combinations now recognised world-wide are 63ºC for 30 minutes or 72ºC for 15 seconds.

5.3 Impact of pasteurisation on pathogens in raw milk

The impact of pasteurisation on pathogens in raw milk has been discussed widely in recent years. While there is extensive literature on the subject, there has been no definitive study of the impact of pasteurisation on raw milk in Australia. In February 2005, FSANZ commissioned a study titled 'Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products'. In addition, a separate food safety quantitative risk assessment model on the pasteurisation efficacy of the Australian Dairy Industry was developed in collaboration between the University of Tasmania and the Dairy Research and Development Corporation (now Dairy Australia) (Section 10.5).

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[21] Text in this section is from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

The objectives of the 'Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products' study were to:

- Define the effect of pasteurisation on levels of pathogenic micro-organisms in milk;
- Determine how current industry pasteurisation practices compare with regulatory requirements; and
- Identify possible alternative methods and processes for the destruction of pathogenic micro-organisms in milk and milk products, including:
  - the current state of knowledge on their effects on micro-organisms; and
  - methods for validating their effectiveness, as a basis for any future equivalence comparisons with pasteurisation.

The study involved:

- a desk-top review of the available scientific literature and epidemiological data, from Australian and overseas sources, on the effect of milk pasteurisation and thermisation on the levels of pathogenic micro-organisms in milk intended either for human consumption as a liquid milk product or for further processing into other dairy products; and
- a survey of the commercial dairy industry in Australia, with the objective of determining current industry practices for the pasteurisation of milk including the methods employed and time/temperature combinations and their relationship to minimum regulatory requirements.

5.3.1 Methods for determining heat resistance of pathogens and interpretation of the data

Many different techniques and types of equipment have been used to measure heat resistance of milk-borne pathogens, ranging from the very simple to the very sophisticated and from micro- to commercial-scale. However, there is ample evidence to indicate that the method used to determine heat resistance is a major factor in determining:

- the reliability of the heat resistance data generated; and
- its relevance to commercial pasteurisation practice.

Hence, methodology should always be considered when assessing the veracity of any conclusions about the ability of an organism to survive/not survive commercial heat treatments.

From a commercial perspective, it is the overall impact of the integrated heating profile, plus any other relevant system inputs, on the survival/destruction of any pathogens that may be present in the raw milk. Other system inputs during commercial processing include turbulent flow and, in some cases, homogenization. Thus, greatest weight should be given to the results of heat resistance studies carried out using actual HTST pasteurisation equipment, either pilot plant or commercial-scale. Such equipment should, however, comply with recognised design and operational standards.

5.4 Ability of bacterial pathogens to survive pasteurisation[22]

Heat resistance studies conducted using either pilot plant- and/or or commercial-scale HTST pasteurisation equipment, together with additional data from studies using various laboratory techniques, have confirmed that the vegetative forms of 11 of 18 pathogenic species considered in this review are destroyed by both batch (63ºC for 30 minutes) and HTST (72ºC for 15 seconds) pasteurisation, with a reasonable margin of safety. These species are:

- *Brucella abortus*
- *Campylobacter jejuni*
- *Campylobacter coli*
- *Coxiella burnetii*
- Pathogenic *Escherichia coli* (0157:H7)
- *Listeria monocytogenes*
- *Mycobacterium tuberculosis*
- *Mycobacterium bovis*
- *Salmonella enterica serotypes*
- *Streptococcus pyogenes*
- *Yersinia enterocolitica*

The effect of pasteurisation on selected organisms are presented in Table 15.

Table 15: Effect of pasteurisation on selected micro-organisms [23]

Mycobacterium avium subsp. paratuberculosis (MAP)	The heat resistance of this organism has been subject to extensive study during the past decade using various laboratory techniques and pilot scale HTST equipment. Obtaining definitive heat resistance data for this organism has proved to be difficult. While there appears to be ample evidence that this organism is destroyed by batch pasteurisation, studies on the ability of MAP to survive heating at 72ºC for 15 seconds, even with pilot scale HTST equipment, have given conflicting results. However, more recent, well-controlled studies have shown that a minimum 4-$\log_{10}$ reduction is obtained during HTST pasteurisation. In view of the numbers MAP likely to be present in raw milk, this level of kill in fact provides a reasonable margin of safety for the consumer. However, population reductions in the order of 6-7- $\log_{10}$ have been reported. The fact that it is necessary for operational reasons to operate HTST equipment at temperatures slightly higher than 72ºC - apart from any decision to use higher temperatures for other reasons - provides an additional margin of safety. A fundamental unanswered question with respect to MAP, is whether it is a human pathogen, or whether its postulated association with Crohn's disease is just serendipitous, rather than causal. If studies eventually establish that there is no causal connection between MAP and Crohn's disease, any concerns that this organism might be able to survive HTST pasteurisation will prove to be unfounded.
Bacillus cereus	Although there is limited data available specifically on the heat resistance of the vegetative form of this organism, and none using commercial HTST equipment, it is generally accepted that the vegetative cells are readily destroyed by both batch and HTST pasteurisation. However, there is more than ample evidence to indicate that the spores of *B. cereus* are very heat resistant and readily survive any heat treatments in the normal pasteurisation range. The pasteurisation heat treatment is sufficient to heat activate the fast-germinating spores of *B. cereus*, but not the slow-germinating spores. Similarly, pasteurisation inactivates diarrhoeagenic toxins produced by *B. cereus*, but not the emetic toxin.
Brucella melitensis	No definitive data on the heat resistance of the organism (which is not present in Australia) were located. However general statements from authoritative sources indicate that the organism is destroyed by pasteurisation.
Enterobacter sakazakii	Although the data is somewhat variable, and data using commercial HTST equipment is lacking, the consensus view is that the heat resistance of this organism falls within the safety margins of commercial pasteurisation. Its presence in pasteurised milk products has been found to be due to re-contamination of the pasteurised product after the pasteurisation step.

[22] Text in this section is from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

[23] Text in this section is from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

Staphylococcus aureus	Although this organism has relatively high heat resistance for a mesophilic non-sporing bacterium, and despite the fact that data using commercial HTST equipment is lacking, there is ample evidence from laboratory studies that it is destroyed by both batch and HTST pasteurisation heat treatments with a wide margin of safety. However, the thermal stability of the enterotoxins produced by *S. aureus* greatly exceeds that of its vegetative cells, and they readily survive pasteurisation by a wide margin.
Streptococcus agalactiae	Only one report on the heat resistance of *S. agalactiae* was located. This indicated - under relatively crude experimental conditions - that the organism was inactivated at unspecified population levels in cream by batch pasteurisation.
Streptococcus zooepidemicus	Not a single report on the heat resistance of *S. zooepidemicus* was located. However, human infection with this organism can usually be traced to an animal source, including ingestion of unpasteurised milk and cheese.

5.4.1 Data gaps on heat resistance of pathogens

The main gaps in data and knowledge in respect of pasteurisation identified were:

- definitive evidence on whether or not MAP can be classified as a human pathogen; and
- quantitative heat resistance data for *Brucella melitensis, Streptococcus agalactiae* and *Streptococcus zooepidemicus* in milk.

In addition, it must be noted that:

- heat resistance data obtained using commercial HTST pasteurisation equipment appears to be lacking for the vegetative cells of several of the pathogenic species covered in this review, *e.g. Bacillus cereus, Enterobacter sakazakii* and *Staphylococcus aureus*; and
- the most recent heat resistance data obtained by any method that is available for some of the pathogens is quite dated, *e.g. M. tuberculosis* (1927), *Coxiella burnetii* (1956 and 1961), *M. bovis* (1960), and *St. agalactiae* (1974).

Standardised protocols and methodologies for the determination of heat resistance appear to be lacking. Studies using methodologies known to give unreliable results, *e.g.* open tubes, are still being reported in the literature.

5.5 Quantitative modelling of the effect of pasteurisation on pathogens

The food safety risk assessment model on the pasteurisation efficacy of the Australian Dairy Industry[24] developed in collaboration between the University of Tasmania and Dairy Australia model is a stochastic simulation model and is designed and built in Microsoft Excel with Palisade's @Risk as the simulation engine. The model includes variables on the farm that are used to describe conditions during storage on farm, transport to the processing plant, and following pasteurisation.

The pathogens modelled were selected by a group of dairy industry technical managers and included: *E. coli; Salmonella; L. monocytogenes; Campylobacter* spp.*; Yersinia enterocolitica; B. cereus; and S. aureus.* These pathogens were identified as representing the priority food safety hazards relevant to the Australian dairy industry, recognising that not all hazards could be modelled given the resources available to the project.

[24] Text in this section is from the food safety risk assessment model on the pasteurisation efficacy of the Australian Dairy Industry developed in collaboration between the University of Tasmania and Dairy Australia (Ross *et al*., 2005)

The model uses a variety of biological and physical parameters as inputs, including:

- State (factors such as herd size, production per cow and seasonality effects);
- herd size;
- probability that the herd is contaminated (whether with a pathogen or with a residue);
- for contaminated herds, the number of animals within the herd that is contaminated;
- for each 'contaminated' cow, amount of hazard transferred into raw milk;
- the volume of milk produced per cow;
- time and temperature in the farm milk vat (used to predict growth of pathogens);
- dilution upon mixing into tankers;
- time and temperature of the tanker (used to predict growth of pathogens);
- processing factory silo size (used to estimate effect on hazard concentrations); and
- time and temperature of pasteurisation (used to model thermal inactivation).

The model also includes several steps involving time and temperature combinations to model the progress of pasteurised milk from the factory into storage and distribution, through the retail chain and, finally, to consumer transport, storage and eventual consumption.

The concentration of bacteria remaining in milk after pasteurisation is a function of the initial concentration in raw milk and the combination of the time and temperature conditions of pasteurisation. Under pasteurisation conditions employed in the Australian dairy industry the results for each pathogen are listed in Table 16.

Table 16: Modelled effect of pasteurisation on pathogens (Ross *et al*., 2005)

Pathogen	Effect of pasteurisation
E. coli	*E. coli* is predicted to be effectively eliminated during pasteurisation. The maximum estimated concentration of *E. coli* in raw milk, based on >1 million iterations of the model, was 1.8×10^{-3}/ml. The model estimates the minimum effect of pasteurisation is a 13-log reduction in EHEC numbers. A huge contamination on farm would not overwhelm the effect of pasteurisation against this organism. In 95% of cases pasteurisation achieves a 26-log reduction in concentration, a median value being a 112-log reduction.
Salmonella	*Salmonella* are moderately destroyed by pasteurisation. The highest concentration of *Salmonella* estimated in raw milk is 1.6×10^{6}/ml. To achieve these high levels of the organism in raw milk, high on farm contamination and temperature abuse during transport and storage needs to occur. The model estimates that the minimum thermal inactivation of *Salmonella* during pasteurisation will achieve a 5-log reduction. However, in 95% of cases the pasteurisation process will result in a 7-log reduction in numbers and a mean reduction of 9-log. These estimates were based on the most thermotolerant strains, more 'typical' strains would experience reductions greater than that predicted for EHEC.
L. monocytogenes	*L. monocytogenes* is effectively eliminated by pasteurisation. The highest concentration of this organism was estimated by the model to be 3.9×10^{-1}/ml. With the exception of the large-scale contamination of raw milk on farm, the concentration of *L. monocytogenes* in Australia's raw milk is likely to be low. The model estimates that the minimum reduction of this organism during pasteurisation is 7-log. In 95% of cases an 11-log reduction was estimated. The mean reduction is 59-log.
B. cereus	Spores of *B. cereus* are not inactivated by pasteurisation. The model assumes that any *B. cereus* cells that become contaminants of raw milk enter the factory processing stage of the model as spores. The mean concentration of *B. cereus* spores in milk is estimated at 72 spores L^{-1}. The expected log reduction in the concentration of *B. cereus* spores in milk is 0.02-log, effectively no change in concentration. Growth of *B. cereus*, was estimated using a separate stochastic model during simulated distribution, retail storage and home storage conditions. The results included variability in initial *B. cereus* loads, minimum growth temperatures for *B. cereus*, temperature and storage life (due to temperature variation). The results from modelling suggest that 1 in 100,000 litres of milk could contain in excess of 100,000 cells per 100 milk at the time of consumption.

Pathogen	Effect of pasteurisation
C. jejuni	*C. jejuni* has relatively low thermal tolerance and is effectively eliminated by pasteurisation processes used by the Australian dairy industry. The highest level estimated to be present in raw milk entering the factory is 6.1×10^{-1}/L. Even with high contamination rates and high numbers entering milk at the farm, the effect of pasteurisation will negate risk from this organism. The model estimates that the minimum reduction in numbers of *Campylobacter* spp during the pasteurisation of milk is 61-log. In 95% of cases pasteurisation achieves approximately a 113- log reduction, and the mean reduction is 2000-log.
Y. enterocolitica	*Y. enterocolitica* has a relatively low thermal tolerance and is effectively eliminated by pasteurisation. The highest estimated level of *Y. enterocolitica* in raw milk entering the factory is 7×10^{7}/L. Minimum reduction during pasteurisation is 41-logs. In 95% of cases a 72-log reduction will result from pasteurisation - mean estimate is a 1500-log reduction.
S. aureus	The predicted reduction in *S. aureus* levels is less than that predicted for Gram negative, food-borne, pathogens but a large reduction in hazard levels is still predicted to occur. The organism is a common contaminant of raw milk, with some reports as high as 100% of raw milk samples being positive for the bacterium. The model predicts that the maximum concentration that *S. aureus* will reach just prior to pasteurisation is 7.7×10^{4}/L, considerably less than the levels required to produce toxin levels that would lead to emetic reactions. The minimum predicted effect of pasteurisation on *S. aureus* is a reduction of 7-log. In 95% of cases a 10-log reduction can be expected.

5.6 Overall summary of effectiveness of pasteurisation

The study 'Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products' concluded that consumers of pasteurised milk and dairy products in Australia can be assured that pasteurisation continues to be a very effective public health measure. Three complementary observations allow this conclusion to be drawn:

i) Ample heat resistance data to indicate that the vegetative cells of the most significant milk-borne pathogens are destroyed by pasteurisation, with a reasonable margin of safety [though it is recognised that there are still some gaps in the data for some organisms and that there are other forms (*e.g.* spores) or products (*e.g.* toxins) of some species that can withstand pasteurisation];
ii) With a small number of exceptions (which are related more to process control issues or the interpretation of what constitutes an equivalent treatment, rather than significant deficiencies in the actual times and temperatures used), pasteurisation of milk and cream in Australia meets the minimum time and temperature standards prescribed in the Code, or recognised equivalents; in many cases, the product is heated to a temperature and/or a time often in excess of the prescribed minimums; and
iii) Lack of evidence in epidemiological data indicating that pasteurised milk products have been implicated in any outbreaks of food-borne gastrointestinal illness in Australia in recent years whereas, in contrast, such outbreaks continue to be associated with consumption of raw milk, both in Australia and in other countries.

The modelling undertaken by University of Tasmania concluded that the likelihood of survival of pasteurisation by vegetative pathogens is very remote. The modelling estimates range from 1 *Salmonella* survivor among the entire Australian liquid milk production every 2.5 years to 1 *Campylobacter* spp. survivor in approximately 10^{2000} years. With regard to the *Salmonella* data, the estimate is based on the most heat resistant strains known. Data for enterohaemorrhagic *Escherichia coli*, which may be more representative, lead to estimates of 1 survivor in 10^{100} years of Australian production at current levels. A summary of the effect of pasteurisation on bacterial contaminants in milk is presented in Table 17.

Table 17: Summary of the effect of pasteurisation on bacterial contaminants in milk (Ross *et al*., 2005)

Organism	Pasteurisation effect (Log reduction)			Time to encounter a single cell in Australia's milk production (years)
	Min.	95th percentile	Median	Mean
E. coli	13	26	112	10^{100}
Salmonella	5	7	9	2.5
L. monocytogenes	7	11	59	10^{45}
B. cereus	0.02	0.04	0.10	<1
C. jejuni	61	113	2000	10^{2000}
Y. enterocolitica	41	72	1500	10^{1500}
S. aureus	7	10	25	10^{13}

Such estimates highlight the huge margin of safety afforded by pasteurisation of liquid milk products when such equipment is operated reliably. This quantitative risk assessment indicated that the vast majority of liquid milk processors have sufficient controls in place to prevent milk that has not received adequate pasteurisation from reaching the market. Conversely, the results indicate that Australian pasteurisation processes have virtually no effect on spores of *B. cereus*, some strains of which are psychrotrophic. *B. cereus* is expected to be commonly found in raw milk at low levels (~100 spores/litre).

While there are no data to indicate harm to public health, simulations of the potential for germination and outgrowth of cells of *B. cereus* in pasteurised milk during normal distribution and storage suggest that levels of 100,000 cells per 100 ml of liquid milk could occur in 1 in 100,000 servings.

While these studies concluded that pasteurisation of raw milk destroys the vegetative cells of the most significant milk-borne pathogens, it should be noted that dairy products containing elevated levels of fat or solids such as ice-cream mixes, cream and yoghurt warrant higher time/temperature combinations than those currently specified in the Code to compensate for the protective effect of fat and solids on micro-organisms (Appendix 1).

5.7 Times and temperatures used for the pasteurisation of milk in Australia[25]

From the Australia-wide industry survey conducted during this study and additional data from a survey of the Victorian dairy industry by Dairy Food Safety Victoria in 2004, it is clear that batch pasteurisation is used in Australia by small-scale processors.

However the batch method would account for only a very small percentage of all milk pasteurised in Australia. Temperatures and times of heat treatment for batch pasteurisation covered a range, from 62-90ºC and from 15 seconds to 30 minutes. The type of product being manufactured was a major influence on the temperature-time combination used.

Several processors reported using what is essentially a HTST treatment, *e.g.* 72ºC for 15 seconds or similar, under batch conditions.

[25] Text in this section is from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

All of the temperatures and times for pasteurisation of milk by the HTST method reported during the industry surveys showed that the minimum heat treatment for HTST pasteurisation as specified in the Code, *i.e.* 72ºC for 15 seconds, was being achieved by all respondents. Beyond that, however, temperatures ranged from 72-86ºC and times from 15-50 seconds, with many different combinations within those ranges. As with batch pasteurisation, type of product was again a major influence on the heating regime used, with time and temperatures reported generally being within the expected range for the type of product.

Of particular note is that HTST treatment of milk for liquid milk products, at least by most of the large processors and some of the smaller ones, was mostly in the range 74-78ºC for 15-30 seconds. This reflects a recommendation by the peak Australian dairy industry organisation in 2000 that the times and temperatures for HTST pasteurisation of milk for the liquid milk trade be increased as a precaution against the presence in the raw milk of any MAP organisms that might be resistant to the minimum pasteurisation treatment of 72ºC for 15 seconds. Whether use of this enhanced heat treatment is still warranted in the light of more recent studies on the heat resistance of this organism that have been conducted using commercial HTST equipment, particularly in areas of Australia where Johne's disease in cattle is reported to be not endemic, is a matter for conjecture.

Only one processor reported that they were using the 62ºC for 15 seconds heat treatment (thermisation) option for cheese milk permitted in the Code.

Some processors, particularly those in the small and medium size categories, reported that design of their pasteurisers and operational considerations largely dictated the limits on the times and temperatures of heating that they could use in practice.

5.8 Alternative technologies to pasteurisation of milk and milk products[26]

Several alternatives to the traditional thermal processes for the pasteurisation of milk have been under investigation by various research groups around the world. A major driver of this research has been the demand by consumers for 'natural' foods which, they believe, have the colour, flavour and nutritive value of the raw material. Many of the alternative technologies investigated so far have the potential to achieve this aim. To date, however, no single alternative technology has been shown to be capable of replacing heat - applied via the traditional thermal pasteurisation processes - as an effective and reliable means of destroying all of the pathogenic vegetative bacteria that can be found in raw milk.

Overall, the following observations can be made about various alternative technologies:

- There is a lack of information on the effect of most of the alternative technologies on many of the pathogens considered in this review.
- The most researched technologies are high pressure and pulsed electric field. A considerable amount of data is also available for hydrogen peroxide treatment.
- For each technology and each pathogen, a range of bactericidal effects have been reported. While high log reductions and 'complete inactivation' have been reported in many cases, the reported log reductions usually cover a wide range. This may be due to different experimental conditions but the possibility of different resistances amongst

[26] Text in this section is from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

the strains of bacteria cannot be ignored.

- A wide range of treatment conditions can be used for each technology and therefore it is difficult to compare data from different reports.

In summary, no one alternative technology can be used with confidence at this time as an alternative to thermal pasteurisation. Considerable research still needs to be performed on the technologies with the pathogenic bacteria of interest before properly informed risk assessments can be performed. A summary of the applicability of the major alternative technologies to the dairy industry are outlined in Table 18.

From an Australia-wide industry survey conducted during this study, it was established that, on average, 22% of respondents had 'some knowledge' of the various alternative processes that have potential application for the destruction of pathogenic organisms in milk. There is industry interest in the application of alternative technologies, for a range of reasons. Some interest is purely economic (*e.g.* reduced costs), some is technological (*e.g.* making a better cheese) and some is philosophical (*e.g.* keeping milk in its natural state). Conversely, some respondents also had concerns about the alternative technologies, *e.g.* technical feasibility, effects on manufacturing process and product quality, capital and operating costs, food safety and operator safety.

Table 18: Applicability of major alternative technologies to the dairy industry

Technology	Overall conclusion
High pressure and pulsed electric field	Capable, under certain conditions, of inactivating pathogenic micro-organisms that may occur in milk and milk products. However, neither is effective against bacterial spores, although very high pressure at elevated temperature has been shown to be sporicidal. To date, neither technology has been used commercially in the dairy industry. This is partly because the processes have not been adequately validated for food safety and partly because the technologies have not been scaled up to commercial capacities. Both technologies can be expected to be applied in selected areas of the dairy industry in the medium term, provided regulatory issues can be resolved.
Hydrogen peroxide	Capable of reducing the load of bacteria, including pathogens in liquid foods. It is effective against a range of organisms but may not completely destroy some pathogenic organisms.
Microfiltration	Already being used in the processing of market milk and milk for cheese making in some countries. The extent of removal of bacteria is usually only of the order of 3 logs, although there have been reports of higher reductions. The risk of bacteria entering the final product through faulty membranes or equipment without detection is a constant concern. At present, microfiltration is used in conjunction with normal pasteurisation. A major drawback of microfiltration is that whole milk cannot be treated.
Bactofugation	Used commercially, although largely restricted to cheese milk for removal of *Clostridium tyrobutyricum* spores. The maximum level of removal is ~2 logs so this technology cannot be used alone for 'pasteurising' milk.
Ultrasonication	Shows promise but insufficient research on its effect on pathogens has been reported to enable a proper assessment for treating milk at this time.
Irradiation	Effective against most if not all pathogens. However, public attitude together with the risk of off-flavour production will prevent its use for milk and milk products in the foreseeable future.

6. Distribution, retail display, and the consumer-end of the dairy supply chain

After manufacture, dairy products remain vulnerable to contamination (particularly unpackaged products) and susceptible to temperature abuse at all stages up until consumption. As many dairy products do not undergo a further pathogen reduction step prior to consumption (*e.g.* cooking), avoidance of contamination and attention to storage time and temperature are of particular importance in minimising the potential exposure to pathogens.

6.1 Post-processing contamination

Cross-contamination is potentially the most important means by which dairy products are contaminated after processing. The potential for microbiological hazards to be introduced during transport and distribution; retail; food service; and the consumer-end of the supply chain may occur through environmental contamination and via cross contamination with other products.

Microbiological hazards can be introduced into dairy products through environmental contamination including soil and dust, air, birds, rodents and insects. Most dairy products however, are packaged for distribution, thus the integrity of the packaging must be maintained to prevent environmental contamination.

Cross-contamination of dairy products with microbiological hazards can occur through inadequate food handling practices at retail and in the home. Unpackaged cheeses in delicatessens are particularly vulnerable to cross contamination with other foods, food utensils, and from display cabinet surfaces. For example unpackaged cheeses may become contaminated with *L. monocytogenes* from surrounding foods on display, or through contaminated utensils, etc. *L. monocytogenes* is a concern for dairy products in particular as most dairy products require refrigeration, and growth of this organism can occur at refrigeration temperatures.

6.2 Storage time and temperature

Storage time and temperature during retail display, food service and/or consumer household, including transportation, will impact on the number of micro-organisms present in dairy products. Improper storage of dairy products may allow growth of pathogenic micro-organisms to levels likely to cause illness. Spores, which have survived processing, may grow if storage temperature and times are not controlled. Furthermore, low levels of pathogens, which may have been introduced through environmental contamination during processing, may also grow if storage temperatures and time are not controlled (*e.g. Salmonella, Listeria* and bacteria such as *E. sakazakii*). Correct storage (refrigeration) of dairy products throughout the transportation and retail supply chain and through to the consumer is important to maintain safety, shelf-life, and quality.

Published and unpublished data obtained from surveys in Australia and overseas consistently show the refrigerated retail cabinet as a weak link in the cold chain. Data on retail storage temperatures during a Meat and Livestock Australia study (Figure 2) show that while the majority of temperatures recorded were below 5°C, some temperatures recorded were as high as 15°C (Meat & Livestock Australia, personnel communication).

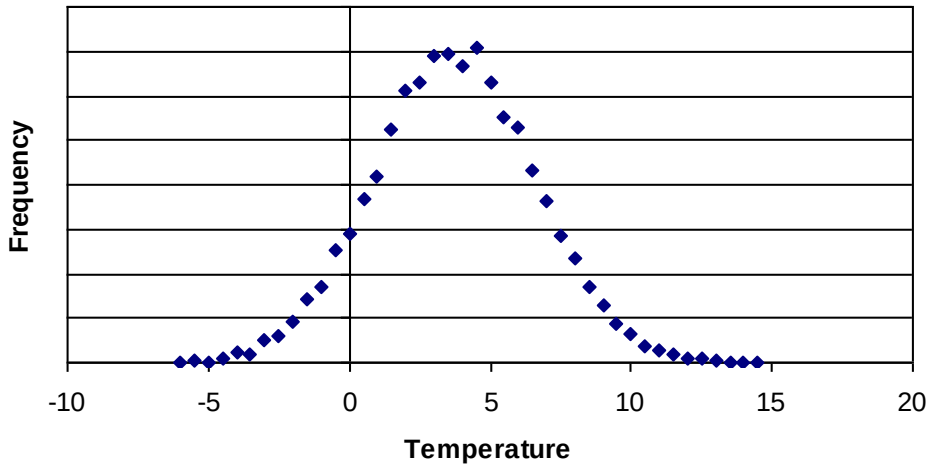


Figure 2: Frequency distribution for retail temperatures in Australia

There is a lack of available data for storage conditions in the food service sector, however temperature control of food stored in domestic refrigerators in Australia is generally poor. In a 1998 survey, 36% of Australian domestic refrigerators (n=171) had their fresh-food compartments above 5°C for greater than 50% of the time (Jay et al 1998). Data on temperatures in domestic refrigerators during the Meat and Livestock Australia study (Figure 3) confirm this finding (Meat & Livestock Australia, unpublished data).

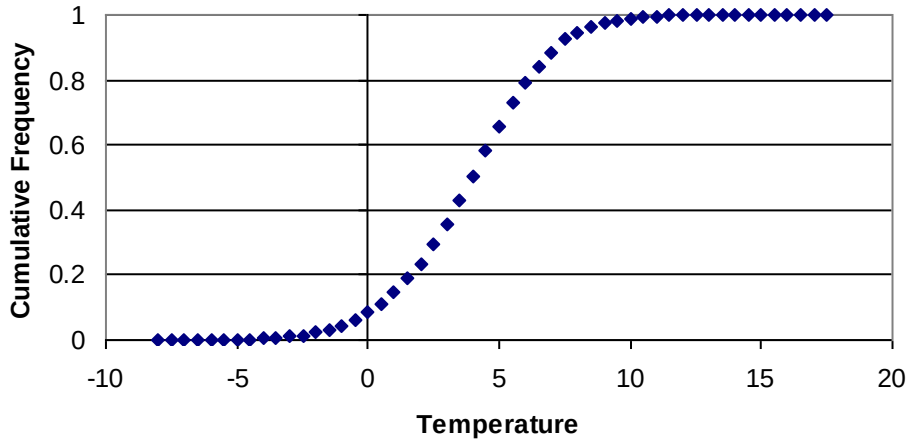


Figure 3: Cumulative frequency distribution for domestic refrigerator temperatures in Australia

As the growth and levels of micro-organisms in dairy products are influenced by temperatures during storage, the better the cold chain is maintained the less growth will occur. This is particularly important for *L. monocytogenes* as it can grow at refrigeration temperatures.

6.3 Food handling practices

Poor hygiene and inadequate food handling practices can also lead to contamination of dairy products post manufacture, especially in unpackaged products.

Infected food handlers can also be a source of contamination. The microflora on the hands and outer garments of food handlers generally reflects the environment and habits of the individuals. This flora would normally consist of organisms found on any object handled by the individual as well as those picked up from dust, water, soil etc. Some pathogens are specifically associated with the hands, nasal cavities and mouth of personnel. For example, micrococci and staphylococci from skin (particularly cuts and wounds) and upper respiratory tissues may contaminant dairy products during handling.

Other pathogens that may be transferred to dairy products include intestinal pathogens such as *Salmonella* and *Shigella* which can be deposited onto equipment and surfaces if good sanitary practices are not followed either at retail, during food service operations, or in the home. Viruses such as hepatitis A and noroviruses may also be transmitted to dairy products through infected food handlers. Hepatitis A in particular is excreted in high numbers in the faeces and is spread from person to person by the faecal oral route. In addition, asymptomatic persons may transfer the virus to food during the incubation period for the disease. Viral shedding may begin several days before onset of symptoms and continue after symptoms have ceased. Hepatitis A is also able to survive on environmental surfaces.

Product stored in the home, once opened is also vulnerable to contamination and temperature abuse. Most dairy products have a relatively short shelf-life, especially milk (10-16 days under optimum storage conditions) thus storing dairy products according to manufacturer instructions and following good hygiene and handling practices in the home is also important.

Pathogens such as *B. cereus*, *Salmonella* and *E. sakazakii* can grow in reconstituted milk powders and infant formulae if stored above 5°C for a sufficient time and multiply very readily at room temperatures. Good hygiene practices in the home during reconstitution, storage and feeding are essential to avoid recontamination and/or multiplication of pathogens in these products. The National Health and Medical Research Council guidelines recommend that infant formula be reconstituted with cooled boiled water then stored in the refrigerator for a maximum of 24 hours (NHMRC, 2003). Studies both in Australia and overseas have indicated that some consumers prepare infant formula incorrectly with warm tap water, leave bottles at room temperature for more than 2 hours and store prepared warm infant formula in insulated carriers when travelling (Lilburne *et al*., 1988; Beck-Fein and Falci, 1999).

7. Discussion and summary

The purpose of the microbiological risk profile was to bring together scientific and technical information on microbiological hazards that may be associated with dairy products in order to identify the public health and safety risks associated with microbiological hazards in dairy products. The profile identifies and examines hazards along the dairy supply chain from milk production through to consumption of dairy.

Raw milk has a mixed microflora, which is derived from several sources including the interior of the udder, exterior surfaces of the animals, environment, milk-handling equipment, and personnel. In general, there are two means by which pathogens contaminate raw milk. Contamination may occur when micro-organisms are shed directly into raw milk from the udder as a result of illness or disease, or through contamination from the external surface of the cow and the milking environment. Primary production factors that impact on these routes of contamination and the microbiological quality of the raw milk include:

- animal-related factors e.g. animal health, herd size, age and production status;
- environment-related factors e.g. housing, faeces, feed, soil, and water; or
- milking and operation of milking equipment factors.

There is relatively little data on the presence or absence of pathogens in raw milk in Australia although it is well established that raw milk can be contaminated with pathogenic micro-organisms, including *Salmonellae, S. aureus, L. monocytogenes. E. coli* (0157:H7), *Campylobacter* spp. and *Yersinia enterocolitica*. Overseas data demonstrate that pathogens are frequently isolated from raw milk. Pathogens were detected in raw milk in 85% of 126 surveys identified in the literature.

The safety of processed dairy products relies on:

- the quality of raw materials;
- correct formulation;
- effective processing;
- the prevention of recontamination of product; and
- maintenance of temperature control during distribution and retail sale of the product.

The most important risk factors affecting raw milk microbiological quality on-farm can be summarised as follows:

Risk factor	Effect	Control
Animal health	Disease in, sickness of, and carriers in milking animals can increase shedding of pathogens directly into raw milk, or in animal faeces.	Animal health and mastitis programs
Herd size	Herd size may have some effect on the prevalence of some pathogens (*e.g. Salmonella, E. coli* and *Campylobacter*)	Biosecurity and animal husbandry
Age/ production	Calves have an increased susceptibility to infection, and have been reported to have higher prevalence rates of some pathogens (*e.g. E. coli*)	Calves kept separate from milking herd
Housing	Intensive housing practices may increase risk of contamination of udders due to close proximity of animals, concentration of faeces, bedding etc. This has been shown to be a factor in the prevalence of *Bacillus spp., E. coli,* and *L. monocytogenes*	Australian dairy farming is mainly pasture based
Faeces	Faeces may contain various pathogens – reflecting either illness/infection, or through ingestion of contaminated feed and/or water with faeces. Faeces may contaminate the exterior of the udder and introduce pathogens into raw milk.	Udder hygiene at milking
Effluent	Effluent (containing manure) can also contaminate pasture.	Appropriate treatment and disposal of effluent
Feed	Contamination of feed can lead to shedding of pathogens into faeces. Poorly made silage can be a source of pathogens (*e.g. E. coli, Bacillus spp., Listeria,* and *Clostridia*).	Control over preparation and storage of feed, especially silage
Water – stock drinking	Water is a potential source of contamination. Sediment in water can support bacterial growth and be a reservoir for pathogens. Water sources can become contaminated with cud and/or faecal material, feed, etc.	Ensuring water is of suitable quality
Milking	Poor milking practices, including dirty teats, inadequate cleaning and maintenance of milking equipment, and poor personnel hygiene can lead to contamination of raw milk.	Maintenance, sanitation and cleaning of equipment, appropriate animal and good personal hygiene
Water use - milking	Water is a potential source of contamination during washing of teats and cleaning of milking equipment.	Ensuring water used is of suitable quality
Storage	Inappropriate temperature control of milk after milking can lead to growth of pathogens	Rapid cooling of milk and regular collection.
Transport	Inappropriate temperature control of milk during transportation can lead to out-growth of pathogens. Contamination can occur if tankers do not adequately protect milk, and/or are inadequately cleaned.	Temperature control, tanker maintenance, and cleaning and sanitation

Pasteurisation represents the principal process for rendering dairy products safe for consumption. Pasteurisation will eliminate most significant milk-borne vegetative micro-organisms of concern. This is confirmed by microbiological survey data for pasteurised dairy products in Australia that shows a very low incidence of hazards of public health significance in these products. Pathogens such as *L. monocytogenes, Salmonella* and *S. aureus* are rarely isolated.

However, the effectiveness of pasteurisation is dependent upon the microbiological quality of the incoming raw milk. Control of risk factors on-farm will minimise the opportunity for microbiological hazards to contaminate raw milk and reduce the likelihood and concentration of these hazards.

A survey of Australian dairy manufacturers determined that the vast majority met the minimum time and temperature standards prescribed in the Code for the pasteurisation of milk and cream. In many cases, milk was heated to a temperature and/or a time in excess of the prescribed minimums. For the majority of dairy products, pasteurisation also represents

an initial treatment before specific processes are used to transform raw milk into various manufactured products.

Dairy products containing elevated levels of fat or solids such as ice-cream mixes, cream and yoghurt, necessitate higher time/temperature combinations than those currently specified in the Code in order to compensate for the protective effect of fat and solids on pathogenic micro-organisms.

The effect of pasteurisation on dairy processes on the major microbiological hazards that have been associated with food-borne illness in various dairy commodities can be summarised as follows:

Pathogens	Significance in dairy products
Salmonella	*Salmonella* is destroyed by pasteurisation, however it can be present in the environment and can gain access to product after heat treatment. Initial source is often birds and rodents, although occasionally present in the raw milk. Non-dairy ingredients can be an important source of contamination.
Listeria monocytogenes	*L. monocytogenes* is destroyed by pasteurisation. Its presence in heat-treated products is due to post-pasteurisation contamination. *L. monocytogenes* is a concern to the dairy industry as it can grow down to 0ºC (refrigeration temperatures).
Staphylococcus. aureus	*S. aureus* is destroyed by heat-treatment, however its toxins are heat stable, thus control of growth of this organism prior to heat treatment is essential. However, *S. aureus* does not grow well at low temperatures (i.e. refrigeration).
Bacillus cereus	Vegetative cells of *B. cereus* do not survive pasteurisation, however spores will survive heat treatments. *B. cereus* is rapidly outgrown by gram-negative psychrotrophs at refrigeration temperatures, but in their absence, *B. cereus*, if present may then be able to grow to high levels. This is a concern with extended shelf-life chilled products such as desserts.
Escherichia coli	*E. coli* is found in cattle and may enter milk through faecal contamination, however *E. coli* is heat-sensitive and does not survive pasteurisation.
***Campylobacter* spp.**	*Campylobacter* spp. is destroyed by pasteurisation and its presence in milk products is due to environmental contamination after heat treatment. *Campylobacter* spp. are fragile organisms unable to grow in foods.
Yersinia enterocolitica	*Y. enterocolitica* is destroyed by pasteurisation and its presence in heat-treated milk products is due to environmental contamination after heat treatment. *Y. enterocolitica* is able to grow in dairy products held at refrigeration temperatures and therefore may be considered as a hazard in prolonged shelf-life products.
Enterobacter sakazakii	*E. sakazakii* will not survive pasteurisation. Recontamination of powdered infant formulae during manufacture is a risk. *E. sakazakii* cannot grow in a dry substrate, but it can survive a long period of time and is potential hazard when the powder is reconstituted and held for long periods of time at favourable temperatures. Contamination and subsequent growth may occur during reconstitution and preparation.

Post-pasteurisation contamination is a major risk factor for the safety of dairy products. Contamination may result from the environment, including equipment, personnel or contamination of finished product with raw materials. Rigorous control over hygiene, cleaning and sanitation, and product handling is therefore critical to safety of dairy products.

The major processing factors affecting the safety of specific dairy products are summarised overleaf:

Dairy product	Processing and impact on microbiological safety
Milk and cream	Pasteurisation is sufficient to destroy pathogenic milk-borne vegetative bacteria. Illness resulting from consumption of pasteurised milk is rare. However, where outbreaks have occurred, these were attributed to inadequate pasteurisation, post-pasteurisation contamination and/or temperature abuse.
Cheese	A number of processing factors influence the growth and survival of pathogens in cheese, including the severity and duration of heat treatment (including curd cooking); pH; salt concentration; water activity; and maturation/ripening. A number of outbreaks of food-borne illness have been linked with the consumption of cheese (Appendix 2). These outbreaks have resulted from faulty controls in cheese production; use of contaminated starter cultures or contaminated ingredients; post-pasteurisation contamination; or mishandling during transport and/or distribution.
Dried milk powders	Micro-organisms in dried milk powders will not grow due to low water activity, however, they may survive for long periods and resume growth when the powder is reconstituted and stored under favourable conditions. Heat-treatments given prior to spray-drying are severe enough to destroy all vegetative pathogens in raw material. However, there is opportunity for environmental contamination during spray-drying and subsequent storage. Dried milk powders have been implicated in a number of food-borne illness outbreaks (Appendix 2). The outbreaks were caused by preformed staphylococcal enterotoxin; poor plant hygiene; contamination and abuse of reconstituted products; and outgrowth of bacterial spores.
Infant formulae	Microbial pathogens of concern are the same as those for dried milk powders, however control over these hazards is essential because of the vulnerable status of infants. The microbial quality of dry-blended products depends on the quality of ingredients as there is no heat treatment to destroy bacteria in the final product. Several outbreaks have been associated with infant formulae (Appendix 2), many of which have been caused by improper preparation and handling of infant formulae by consumers.
Concentrated milk products	Microbial pathogens are generally not associated with concentrated milks due to the low water activity of these products.
Butter and butter products	Pasteurisation of cream used in butter manufacture results in the destruction of vegetative micro-organisms, although preformed toxins and spores may carry over to butter. The preservative properties of butter are based on moisture distribution. In addition salt in moisture droplets also have a preservative effect. Several outbreaks of food-borne illness have been linked to the consumption of butter (Appendix 2).
Ice-cream	The heat treatment applied to ice cream mix destroys pathogenic micro-organisms. However, pathogens may be introduced with the addition of ingredients. Pathogens will not grow in ice-cream, but may survive freezing. There have been documented outbreaks of food-borne illness due to consumption of ice-cream (Appendix 2). The outbreaks have been linked to the use of raw ingredients or improper heat treatment during preparation of ice-cream in the home, and contamination during commercial ice-cream manufacture.
Cultured and fermented milk products	The heat treatment of milk is sufficient to destroy vegetative micro-organisms and rapid growth of starter cultures inhibits the outgrowth of spore-formers. Pathogenic micro-organisms are prevented from growth by the low pH; the presence of lactic acid, and by refrigerated storage. Cultured and fermented milks have been associated with only limited outbreaks of food-borne illness (Appendix 2).
Dairy desserts	Heat treatment by pasteurisation or UHT results in the destruction of vegetative cells. Contamination may occur after heat treatment with the addition of further ingredients, or through survival of spores of *B. cereus*.
Dairy-based dips	Where pasteurisation or other heat treatments are employed, vegetative cells will be destroyed. However, spore-formers can survive heat treatments and other hazards can be introduced with the addition of heat labile ingredients after heating. The low pH of these products assists in their microbial stability.
Casein, whey and other functional milk derivatives	Milk fats, casein and whey protein components are derived from milk and cream that have received at least a pasteurisation heat treatment and so will be free from vegetative pathogens. In some products, vacuum drying leads to the destruction of vegetative cells, and low water activity of many products ensures that the outgrowth of pathogens is very unlikely and would lead to eventual die off.
Colostrum	Bovine colostrum is pasteurised before drying, however, pathogens may be protected by the elevated fat and total milk solids content compared to standard bovine milk. Contamination after processing is a concern, although the low water activity of colostrum powder will prevent growth and vegetative cells will eventually die off.

As dairy products rarely undergo a further pathogen reduction step prior to consumption (*e.g.* cooking), prevention of contamination and control over bacterial growth, storage time and temperature is of particular importance in minimising potential exposure to pathogens. Most dairy products have a relatively short shelf-life, especially milk (10-16 days under optimum storage conditions) thus storing dairy products according to manufacturer instructions and following good hygiene and handling practices in the home is also important.

In addition, good hygiene practices in the home during reconstitution, storage and feeding of reconstituted products such as dried milks and infant formulae are essential to avoid recontamination and/or multiplication of pathogens in these products.

In Australia, illness from dairy products is rare. Between 1994-2004, there were only eleven reported outbreaks directly attributed to dairy products and eight were associated with consumption of unpasteurised milk. The majority of outbreaks associated with unpasteurised milk were a result of consumption of raw milk on a farm or camp setting. In the other outbreaks in Australia where a dairy product was one component of the food vehicle identified, the affected foods typically included cream filled cakes and custards. In these cases it is possible that eggs or other ingredients in these products, and not the dairy component, may have been responsible for the illness.

While commercial dairy products have rarely been identified as sources of food-borne illness by health departments in Australia, there have been a number of reports of outbreaks of illness associated with consumption of dairy products internationally. Of a total of 135 outbreaks associated with dairy products reported during the period 1973-2003, 16.2% were attributed to pasteurised milk and 12.5% were attributed to cheese produced from pasteurised milk. However, in these outbreaks, a fault with the pasteurisation process or post-pasteurisation contamination has been identified or suspected as the source of infection. While ice-cream was responsible for a number of these outbreaks (16.2%) but in the majority raw egg ingredients were identified as the source of infection rather than the dairy component.

Unpasteurised dairy products are the most common cause of internationally reported dairy-associated outbreaks of illness (43.4%). Over 22.8% of outbreaks were attributed to unpasteurised cows milk and 11.8% of outbreaks were attributed to unpasteurised cheese produced from raw cows milk. Clearly both internationally and domestically, unpasteurised dairy products are the most common cause of dairy-associated outbreaks of illness.

The lack of epidemiological data linking pasteurised dairy milk products in Australia to outbreaks of food-borne illness attests to the safety of these products. In contrast, outbreaks continue to be associated with consumption of raw milk, both in Australia and overseas.

Dairy products likely to support the growth of pathogens and prone to contamination after final heat treatment may be categorised as higher risk than other dairy products. While dairy products that are inherently stable with respect to pathogens, if correctly formulated, can be classified as low risk. The degree of risk is based on:

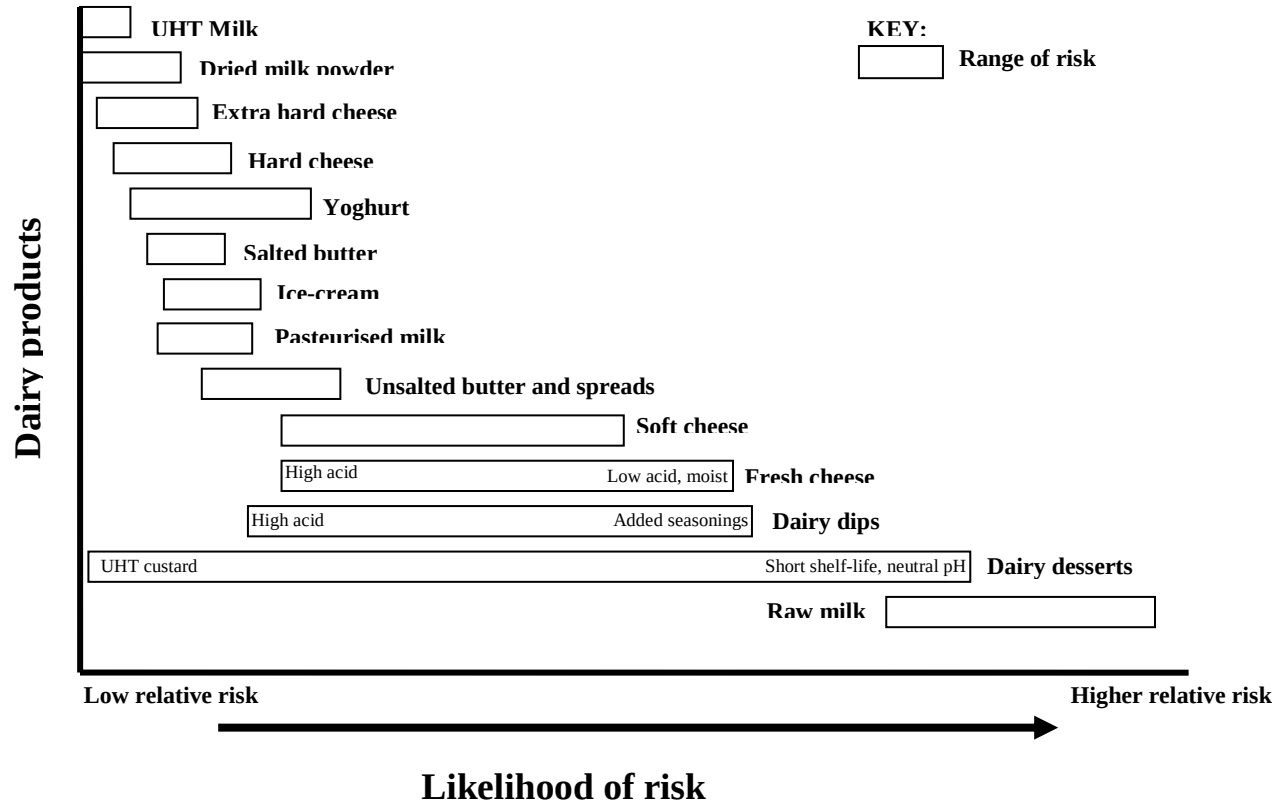
- intrinsic properties of the product (*i.e*. the impact of water activity, pH, salt concentration, etc and their effect on the growth of contaminating micro-organisms);
- extent to which food is exposed to factory environment or handling after heat treatment;

- hygiene and control during distribution and retail sale; and
- degree of reheating or cooking before consumption (many dairy products are ready-to-eat, so this is rarely a factor).

Qualitative objective methods of describing the relative risk to public health associated with dairy products is extremely difficult. The following table attempts to provide a relative rating for selected dairy products, and to convey a perception of some level of risk to consumers.

Risk	Dairy product	Context
Higher risk	Soft cheeses Dairy desserts	Mild pH, long shelf-life Mild pH, fermentable carbohydrate, long shelf-life
Intermediate risk	Unsalted butters/low fat spreads Pasteurised milk and cream	Absence of salt, high moisture content Mishandling and poor hygiene, minimal post-pasteurisation hurdles
Low risk	Yoghurts Salted butters Hard cheeses Extra hard cheeses	Low pH High salt concentration Low a_w, low pH Low a_w, low pH

The relative risk from dairy products may also be expressed graphically as a continuum:



The actual ranking of the dairy products is quite variable. Once a shelf-stable UHT product is opened, it may become contaminated and when subjected to temperature abuse it could become a high-risk food. In contrast, the low pH and low water activity of extra hard cheese means its will be very robust and unlikely to support the growth of any pathogen that adventitiously contaminates the surface. Dried milk powders and infant formulae are inherently stable products due to their low water activity, however these products may be prone to contamination, and upon reconstitution become higher risk, especially if improperly reconstituted and stored.

8. Conclusions

Australian dairy products enjoy a reputation for high standards of quality and safety. There have been few reported failures i.e. incidents of food-borne illness attributed to dairy products in the market place in recent years. While dairy products have been the vehicle in some outbreaks, the cause is often multifactorial involving contaminated non-dairy ingredients, post-processing (post-pasteurisation) contamination and/or poor hygiene.

The safety of dairy products is due to the use of heat treatment and a combination of control measures up and down the supply chain. Control of animal health, adherence to good milking practices, cooling of milk, and control over milking parlour hygiene have been important in reducing the microbial load in raw milk entering Australian dairy processing facilities.

The almost universal use of pasteurisation in milk processing in Australia has resulted in the marketing of dairy products with an excellent reputation for safety and product quality. The processing industry has introduced significant measures to ensure product safety, including the adoption of codes of practice, adherence to Listeria control protocols, and the extensive use of HACCP-based food safety programs supported by laboratory verification.

Notwithstanding the above, there is need for ongoing vigilance and further development of safety control measures. Over the past twenty years we have seen the emergence of new pathogens and the re-emergence of traditional pathogens in various foods. These organisms often occupy specific environmental niches and may arise through changing technologies, methods of food handling and preparation, dietary habits and population. Post-processing contamination in-plant and the maintenance of control over contamination and storage conditions during transport, retail display and home use remain major factors impacting on the safety of dairy products.

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PART B: CHEMICAL RISK PROFILE

1. INTRODUCTION

As part of the development of a Primary Production and Processing (PPP) Standard for dairy products, an evaluation has been undertaken of 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 that any public health and safety concerns are addressed, and also to identify any gaps in the current regulation which should be addressed through a PPP Standard.

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

There are many regulations currently in place that control the use or presence of chemicals in dairy products. Agricultural and veterinary chemicals, which are used extensively in primary production, are assessed as part of a pre-market evaluation and approval process and generally have maximum residue levels identified in the Code (the Code). Similarly, food additives and processing aids undergo pre-market evaluation and approval and have maximum use levels identified in the Code. Sanitisers are assessed before use and their use controlled through HACCP-based food safety plans. 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 so-called '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.

On-farm QA and legislated food safety programs require farmers to use all agricultural and veterinary chemicals according to label instructions, accurately identify treated animals, keep records of all chemical use and separate milk from treated animals for the duration of the prescribed WHPs. Most dairy companies also carry out screening for antimicrobials in milk at the farm level and from bulk milk tankers.

Sources of potential chemical risks

A paddock-to-plate flowchart identifying potential chemical inputs into dairy products is presented in Figure 1. This perspective helps to define the nature of the chemical inputs at specific stages through the dairy supply chain.

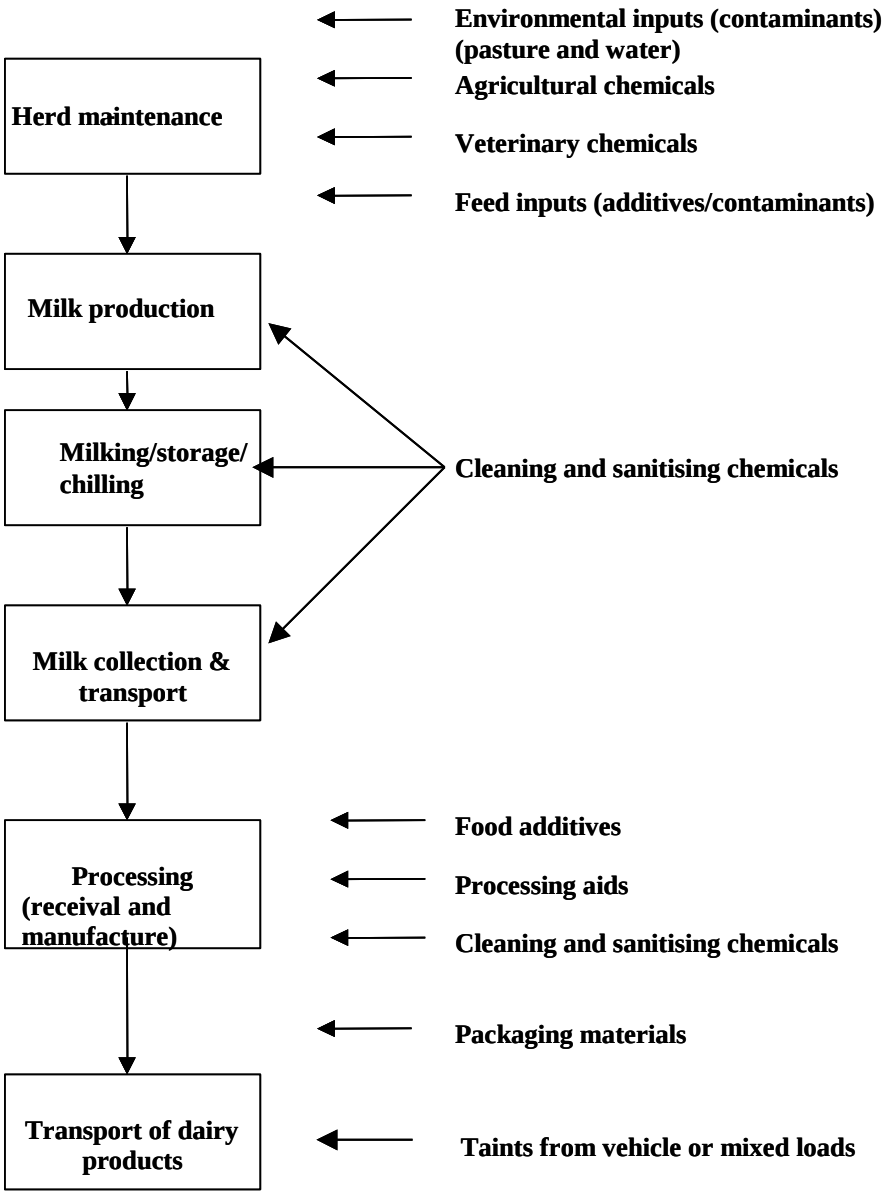

Figure 1 Potential gateways for the introduction of chemicals into the dairy primary production and processing chain

In this assessment the potential sources for chemicals to be introduced into milk and milk products that have been considered include, biological sources, agricultural practices and food processing. Contaminating chemicals, such as heavy metals, endogenous plant toxicants, mycotoxins, or anthropogenically-produced chemicals, such as dioxins, may be ingested by dairy cattle as a result of their presence in the soil or feed. Agricultural chemicals such as herbicides and pesticides are used in association with dairy production. Veterinary applications also include the administration of antimicrobials and antihelmintics, which can be carried-over into the milk. Direct contamination, for example from sanitisers has also been considered. In addition, the potential for undesirable endogenous chemicals to form within dairy products due to processing (*e.g.*, polycyclic aromatic hydrocarbons) or microbiological activity (*e.g.*, biogenic amine or fungal toxin production) has been assessed. Food additives and processing aids may be used during production of dairy products.

Packaging of dairy products may also lead to the unintentional migration of chemicals from the packaging material into dairy produce. Finally, the transport of milk and milk products may lead to potential chemical contamination from containers or from other food commodities.

Physiology & pharmacokinetics of the blood/milk barrier in dairy animals

Milk is synthesised in the alveolar gland. For milk synthesis, milk components and their precursors have to pass the blood milk barrier, a membrane that separates the blood flowing in capillaries from the alveolar epithelial cell of the udder. However, during this process certain environmental chemicals present in the blood can also potentially pass through the membrane and be incorporated into the milk at concentrations comparable to chemical levels in other fatty compartments in the body.

Agricultural and veterinary chemicals, and environmental contaminants, can enter the bloodstream by the two main routes: ingestion and dermal contact (Burnam and Palmiter, 1987; Hale and Ilett, 2002). These chemicals circulate in the bloodstream, either bound to carrier proteins such as albumin and lipoproteins or in their free form, and distribute among tissue compartment throughout the body (Figure 2). The individual carry-over rates of chemicals into milk depend upon the physiology of the animal, the bioavailability of the compound, the general stability, the amount taken up and the time and frequency of exposure in addition to the chemico-physical properties of the chemical molecule (e.g. lipo-or hydrophilicity, molecular weight and preference to carrier proteins). These factors may all influence and limit the transfer of molecules across the gut, into the blood. Furthermore, ingested and absorbed chemicals may be re-distributed, detoxified and excreted by other body organs. The specific detoxification of metal contaminants has been attributed to glutathione metabolism and metallothioneins. These low molecular weight metal-binding proteins actively scavenge sulphydryl-reactive metals (i.e. cadmium, arsenic, mercury and lead) (Burnam and Palmiter 1987).

Indirect contamination from pesticides and herbicides may occur via oral or dermal exposure. In all cases, the chemical may be absorbed, subsequently metabolised and eventually excreted into the milk of the lactating animal.

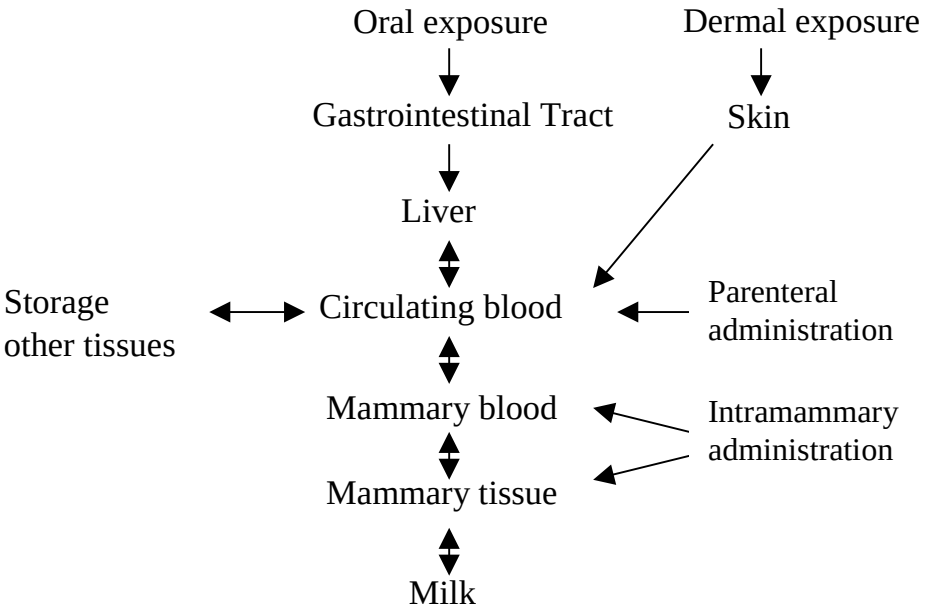

Figure 2: Schematic model of pharmacokinetic pathways in dairy animals

Following administration and absorption, the rate of distribution of chemicals within the body is a function of tissue perfusion, which is the rate of blood flow through the various tissues. The chemicals accumulate first in highly vascular organs. Then, as equilibrium states are reached and metabolic pathways are activated, the chemicals redistribute. Chemicals with high lipid solubility concentrate in tissues with higher fat content, such as adipose tissue, brain, liver, kidney, and may in the case of lactating animals include the milk (Landrigan *et al*., 2002).

There are two pathways by which chemical compounds can cross the blood-milk barrier: 1) paracellular diffusion, which relies on open junctions between cells, and is relevant in colostrums composition and 2) transcellular diffusion, which involves chemicals passing through cells. The latter occurs when the open junctions have 'closed' (about 72 - 96 hours after the mother gives birth). From a physiological perspective, open junctions are important in the transfer of immunoglobulins to colostrum from the mother to the suckling new born. Contaminants and therapeutic compounds can also potentially transfer from blood into milk via paracellular diffusion immediately after birth (Hale and Ilett, 2002). Although this is of limited significance for milk produced in Australia, as routine industry practice excludes the first eight milkings from the supply chain, it may be relevant to the complementary medicine industry producing colostrum-based products.

Transcellular diffusion, occurs mainly via passive transport, which in general allows the passage of lipophilic components of molecular weight <800 Da; thus, lipid solubility of a chemical is a primary factor for its incorporation into milk. Factors that affect the lipophilic character of a chemical include its chemical structure and its degree of ionisation in the body compartments. Low molecular weight water-soluble chemicals (<200 Da) can also cross cell membranes with the bulk transfer of water. Chemicals of high molecular weight (>800 Da) tend not to pass through the membrane and are unlikely to carry-over into milk.

Also, chemicals (e.g. heavy metals) that are highly bound to either plasma proteins or erythrocytes are unlikely to passively diffuse into milk (Clewell and Gearhart, 2002b).

Other factors that affect the presence of chemicals in milk are its degree of biotransformation and its elimination rate. Some weak bases can preferentially enter the milk as a result of the pH gradient that exists between the blood (pH = 7.4) and milk (pH = 6.5-6.8), whereas other ionic compounds are transported into the milk via active uptake mechanisms (Clewell and Gearhart, 2002a).

2. Existing framework for through-chain management of chemicals for dairy products

As mentioned in the General Introduction, the dairy industry (farm and manufacturing sectors) is subject to a number of food safety regulations, both at the Commonwealth and State levels.

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. The APVMA undertakes its responsibilities under the authority of the Agricultural and Veterinary Chemicals Code Act 1994. 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. When an agricultural or veterinary chemical is registered for use or a permit for use granted, following a risk assessment, the APVMA publishes maximum residue limits (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 (see Appendix 8). When the APVMA registers a new product it also approves the associated product label, which provides the approved directions for use, including withholding periods.

State government agencies have responsibility for administering controls regarding the use of Agvet 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 Agvet 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 vet chemicals), and control of off-target movement, e.g. spraydrift; and
- audit, compliance and enforcement activities

State/Territory governments and statutory bodies, including SDAs, have legislative responsibility for administering controls regarding the use of agricultural and veterinary chemicals from the point of retail sale.

Australian dairy farms have hazard analysis critical control point (HACCP)-based on-farm food safety programs as required by State government legislation. These food safety programs require record keeping and are subject to audit by State/Territory authorities or company auditors.

The dairy industries' traceability systems are extensive and include QA programs and food safety plan requirements, vendor declarations, record keeping, livestock identification, inventory controls and product recall plans. A summary of the monitoring and auditing of chemical residues in milk and other dairy products are conducted through the Australian Milk Residue Analysis (AMRA) survey) and the Australian Total Diet Survey (ATDS). Although not strictly relevant to the dairy industry, the National Residue Survey (NRS) serves as an additional source of data and reflects the general standards of animal husbandry in Australia, providing verification of control measures for chemical residues. Dairy cattle comprise approximately 12 – 16% of the "cattle" meat commodity monitored in the NRS and therefore chemical residue data from this survey is also included in this report.

2.1 Australian Milk Residue Analysis (AMRA) Survey

The Australian Milk Residue Analysis (AMRA) survey is a national program that audits potential chemical inputs into Australian dairy production, including on-farm administration, prior to harvest, through to the final dairy product. The farm and manufacturing sectors of the dairy industry is subject to a number of food safety regulations at both the Commonwealth and State levels (see Appendix 8 for additional information).

Additional data is collected from targeted testing and from testing conducted by dairy companies.

The Australian Quarantine and Inspection Service (AQIS) uses the AMRA Survey when certifying the residue and contaminant status of milk and milk products for export and is the competent authority that approves the core annual AMRA Survey.

A national dairy auditing program was established in 1998 that is summarised as the AMRA survey. Prior to this, industry and States conducted similar monitoring and auditing programs. The AMRA Survey is conducted by ANZDASC on behalf of the Australian dairy industry, with Dairy Food Safety Victoria (DFSV), a statutory authority, coordinating the Survey. The Survey is funded by the Australian dairy industry through the industry-owned service company, Dairy Australia. Funding for Dairy Australia is derived through an industry milk levy, and for research purposes, contributions from the federal government.

In the risk assessment to determine which chemicals will be included in the AMRA survey, the following matters are considered:

- importing country requirements, including those of the European Union;
- availability of the chemicals for use in Australia;
- approved use patterns of the chemicals for dairy cattle in Australia;
- previous conclusions of AMRA Survey reviews;
- other possible sources of chemical contaminants
- test results from:
  - previous AMRA Surveys;
  - company testing programs conducted either for regulatory or customer requirements;
  - results of the National Organochlorine Residue Management (NORM) Program;

- results of the National Antibacterial Residue Minimisation (NARM) Program;
- results of the NRS Cattle Meat Survey; and
- results of the NRS Grain Products Survey

The core AMRA survey is designed to monitor bulk milk for the presence of chemical residues. The experimental design of the survey is risk-based; samples are collected and analyses carried out on a statistical basis. It provides a mechanism to monitor compliance with required conditions of use, including withholding periods (WHPs[27]) after treatment with antibiotics

For the period 2001-2004, 11,000 analyses have been conducted in the core survey to investigate the potential presence of AgVet chemicals in bovine milk and dairy products. Seven detections above the relevant MRL/ML/ERL for all the agricultural and veterinary chemicals, environmental pollutants, natural toxicants or heavy metals tested, have been found during this period, equating to a compliance of level of 99.94%. There have been a few instances of detection in milk of the antihelmintic, Triclabendazole, which is not approved for use in lactating dairy cattle in Australia.

There have also been single breaches of the MRLs for the antibiotics, penicillin G and Cloxacillin.

Gentamicin was also detected on one occasion in milk although it is not approved for use in lactating dairy cattle in Australia.

A summary of the formal AMRA Survey test results from 1 January 1998 to 30 June 2004 is shown in Tables 2 to 5. Results are shown as the total number of analyses and the number detected with residues above the maximum residue limit (MRL), ML or extraneous residue limit (ERL) (ADASC, 2003).

2.2 Australian Total Diet Study (ATDS)

FSANZ monitors the food supply to ensure that existing food regulatory measures provide adequate protection to consumer health and safety. The Australian Total Diet Study (ATDS) is part of that monitoring and is conducted approximately every two years (Appendix 7; (FSANZ, 2005d).

The ATDS, formerly known as the Australian Market Basket Survey, is Australia's most comprehensive assessment of consumers' dietary exposure (intake) to a range of food chemicals including pesticide residues, contaminants, nutrients, additives and other substances. Populations are assessed as a whole as well as for different age and gender groups in Australia. Due to the uncertainties in some of the data used for the assessment, certain assumptions needed to be made. These assumptions are likely to lead overall, to a conservative (*i.e.* over-estimate) for dietary exposures (*e.g.* all foods within a food group are assumed to contain the additive, nutrients or contaminant being assessed.)

[27] The withholding period (WHP) s a stipulated time which must elapse between treatment with a particular chemical and slaughter or harvest. It allows time for the chemical to be metabolised or breakdown or dissipate. Withholding periods are given on the label instructions of any agricultural or veterinary chemical sold and must be obeyed by law.

Exposures are estimated by combining usual patterns of food consumption, as derived from the national nutrition survey (NNS) data, with both current and proposed levels of use of the food chemicals in the food. Food consumption data from the 1995 Australian NNS and the 1997 New Zealand NNS are used for dietary modelling, along with concentration data for the food additives, nutrients or contaminants from a variety of sources (including the Code, manufacturers' use data and analytical data from surveys) (Baines J, 1998). Details on consumption data for dairy products from the NNS are given in Section 2.2 and Appendix 5 of the Microbiological Risk Profile of Dairy Products.

The survey estimates the level of dietary exposure of the Australian population through the testing of food representative of the total diet. In order to achieve more accurate dietary exposure, the foods examined in the ATDS are prepared to a 'table ready' state before they are analysed. As a consequence, both raw and, processed and cooked foods are examined. FSANZ coordinate the survey while the States and the Northern Territory purchase and prepare the food samples.

The Australian Market Basket Survey (ANZFA, 1996b) detected levels of Permethrin residues in cheese of 0.08 mg/kg (the MRL for Permethrin in milk products, in the fat, is 0.05 mg/kg. There have been no subsequent detections of this pesticide.

2.3 National Residue Survey (NRS)

Australia has an active national residue-monitoring program. The National Residue Survey (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.

Industry participation in the NRS is generally required to meet requirements for market access, export certification or national standards. AQIS uses NRS data when certifying the residue and contaminant status of certain commodities for export. The meat from other minor dairy producers, sheep, goat, buffalo and camel, is also included in the NRS. The NRS also covers some commodities that are used in stockfeed. Each commodity is reviewed in consultation with the relevant industry and where necessary, residue monitoring is modified in accordance with emerging needs and changing circumstances.

Residues are classified as being 'present' if their concentration is greater than the limit of reporting (LOR) established for NRS purposes. The NRS typically sets the LOR at 10-20% of the Australian Standard MRL, ERL or ML.

The 2000 – 2001 cattle meat survey showed that of the 60 222 analyses that were conducted there was a 99.99% compliance to the MRLs, with only 5 samples having residue detections exceeding the MRL. There was a single detection of the antimicrobial neomycin that was above the MRL, but this was in a beef cow. The traceback investigation revealed the residue most likely resulted from failure to observe the WHP and inadequate record keeping of individual animal treatments.

3. Potential carry-over of residues from agricultural production systems

3.1 Introduction

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 (AgVet) products. AgVet chemicals are regulated through State authority 'control of use' legislation and the States administer the Food legislation.(see Appendix 8 for further details).

The agricultural and veterinary chemicals used in the dairy production chain undergo rigorous assessment processes by the Office of Chemical Safety, the Environmental Protection Agency, and the National Industrial Chemicals Notification and Assessment Scheme and by State agencies prior to registration by the APVMA. This report provides information on the agricultural chemicals which may have relevance to the dairy industry and the auditing results over the past few years.

In contrast to many of the animal husbandry systems in place internationally, the Australian dairy industry is predominantly pasture-based, *i.e.*, dairy cattle are not confined to dairy sheds for prolonged periods of time. This system, together with the different climatic conditions in Australia, results in different agricultural and veterinary chemicals usage which reflects specific needs. Australia does not experience the same problems as seen in intensive farming systems, which results in a different disease profile and hence different veterinary product usage. APVMA registration requirements in Australia allow for the application of whole herd treatments and a policy of nil milk WHP for antihelmintics.

Agricultural and veterinary residues in milk can arise from a number of potential sources, including indirect exposure through feeds, or direct treatment of cattle.

The same groups of chemicals, for example insecticides, may be used for both agricultural and veterinary purposes, however they are used for different purposes, at different doses and are applied differently.

The AMRA survey is designed such that a particular AgVet chemical can be traced back to its source use. Generally, agricultural pesticides involve indirect exposure, whereas veterinary parasiticides involve direct application, such as drenching or pour-on antihelmintic products.

The MRLs for agricultural and veterinary chemicals for milk, are listed in Appendix 9 (extracted from section 1.4.2 of the Code).

Colostrum

Colostrum is considered within the Dairy Primary Production and Processing Standard and therefore is included in the chemical risk profile. Standard dairy industry practice excludes colostrum from milk entering the production stream, however colostrum is marketed as a complementary medicine, and is regulated by the Therapeutic Goods Agency.

Colostrum is derived from the first four milkings of cows after calving. It is a lactose- and fat-reduced, high-protein product, which is manufactured without the addition of additives or artificial ingredients. However, chemicals may be potentially transferred to colostrum from the cow by paracellular diffusion (see Section 2).

The QA program for colostrum manufacture as a therapeutic is separate from that of the dairy industry. The levels of chemical residues potentially present in colostrum are regulated by MRLs in the Australia New Zealand Code. Although individual manufacturers have strict compliance specifications and immunoglobulin concentrations, bacteria counts, somatic cell counts and antimicrobials are measured, there is no monitoring of colostrum by the State Dairy Authorities.

3.2 Pesticides and Herbicides

Organophosphates and synthetic pyrethroids
Insecticides, such as the organophosphates and synthetic pyrethroids are common agricultural chemicals in use in dairy production in Australia, and are used as grain protectants and pest control in pastures and feeds.

Organochlorines
The broad-spectrum insecticide/acaricide Endosulphan is an organochlorine which is currently used in agricultural production, for example in the cotton industry. Unlike other organochlorines (see Environmental Contaminants section 6.4.3), it shows low persistence in the soil.

Herbicides
Herbicides are used in plant management and in the dairy industry are used to reduce weeds. Selective spot-spraying is common in pastures and raceways, in addition to the use of herbicides, such as glyphosate, in pre-pasture establishment. As for other AgVet chemicals, MRLs for herbicides in milk are included in Appendix 9. A stock WHP applies to most of these herbicides, varying from anything from 1-2 days, up to several weeks.

Fungicides are included in NRS monitoring, however residue levels have been found to be negligible, if present at all. Due to the very low levels of herbicide residues associated with grain products (NRS Grain Program), they are infrequently included in the NRS. A recent review of herbicide usage by Dairy Food Safety Victoria (DFSV) found that the potential risk of herbicide residues in milk is low and therefore the AMRA survey does not currently include testing for herbicide residues.

3.3 Survey results for agricultural chemicals

3.3.1 Milk

In the past seven years of the AMRA survey there have been 33, 382 analyses of organochlorines, organophosphates and synthetic pyrethroids with no detections of residues at levels greater than the MRL/ERL (Table 1). The decline in the numbers of analyses since 1998 reflects the progressive success of management practices. In the 2005-6 AMRA survey, thirty samples will be collected nationally and analysed for organochlorine residues.

Table 1: Comparison of Annual Pesticide Test Results in bulk milk (ADASC, 2002 – 2005)

Survey Year	Pesticides					
	Organochlorines		Organophosphates		Synthetic Pyrethroids	
	No. analyses	No. >ERL	No. analyses	No. >MRL	No. analyses	No. >MRL
1998/1999	2616	0	3597	0	2289	0
1999/2000	1512	0	2079	0	1323	0
2000/2001	1632	0	2244	0	1428	0
2001/2002	896	0	1568	0	784	0
2002/2003	872	0	1526	0	763	0
2003/2004	600	0	1078	0	539	0
2004/2005	240		3864		1932	

As a result of a locust plague, which occurred in South Australia and New South Wales during spring 2000 and summer 2001, approved insecticides were used as control measures by landholders, the Departments of Agriculture and the Australian Plague Locust Commission. During this period, the APVMA issued permits to allow the use of certain synthetic pyrethroid chemicals to control the Australian plague locust; the permits covered the use (*e.g.* WHPs for domestic and export markets) of the active ingredients: Lambda-Cyhalothrin, Gamma-Cyhalothrin, Betacyfluthrin, Alpha-Cypermethrin and Cypermethrin.

Additional monitoring of pesticide residues in milk was carried out as a result of this and the results are shown in Table 2. Results from targeted sampling undertaken during locust plague activity in the vicinity of dairy regions in northern Victoria and southern NSW in summer 2004, are shown in Table 3; no residues were detected.

Table 2: Australian Targeted Milk Testing for Locust Plague Chemical Residues (spring 2001 – summer 2001) (ADASC, 2002)

State	Residue Type	No. analyses	No. samples >MRL
SA	Organochlorines	3	0
	Organophosphates	3	0
	Fipronil	3	0
NSW	Organochlorines	41	0
	Organophosphates	41	0
	Fipronil	41	0

Table 3: Australian Targeted Milk Testing for Locust Plague Chemical Residues (summer 2004) (Dairy Australia personal communication)

State	Residue Type	No analyses tested	No. samples >MRL
Vic (northern)	Chlorpyrifos	60	0
	Cypermethrin	60	0
	Diazinon	60	0
	Fenitrothion	60	0
	Fipronil	60	0
	Malathion	60	0
NSW (southern)	Chlorpyrifos	25	0
	Cypermethrin	25	0
	Diazinon	25	0
	Fenitrothion	25	0
	Fipronil	25	0
	Malathion	25	0

3.3.2 Animal tissue

Table 4 shows a summary of the NRS monitoring results for agricultural chemical residues in cattle tissue (urine, fat, kidney, liver, muscle and faeces), in dairy and beef cattle, from 2000 to 2004 (DAFF 2005a). No pesticide residues were found apart from in the 2000 – 2001 season when Bioresmethrin residues were detected, this chemical is now de-registered and is no longer permitted for use.

Table 4: NRS Cattle Meat Survey Results for Pesticides 1 July 2000 – 30 June 2004 (DAFF 2005a)

	No. of analyses	**Residues Detected > MRL**	**No. of analyses**	**Residues Detected > MRL**	**No. of analyses**	**Residues Detected > MRL**	**No. of analyses**	**Residues Detected > MRL**
	2000 - 2001		**2001 - 2002**		**2002 - 2003**		**2003 - 2004**	
Pesticide *	28 227	4 (Bioresmethrin)	30 986	0	23 211	0	22 028	0

* organochlorines, organophosphates, synthetic pyrethroids and benzoyl ureas.

The 2002 – 2003 NRS survey was also used as a source of information regarding dairy animals other than cattle; no pesticide residues were detected in sheep, goat, buffalo or camel tissue (see Table 10).

3.4 Stockfeed

Although FSANZ does not regulate stockfeed per se, the paddock-to-plate approach necessitates the consideration of safety aspects of stockfeed for different dairy animals. The Australian dairy industry is mainly pasture-based and approximately 60% of cattle feed comes from grazing, the remaining 40% is provided by supplementary feed. Feedlot-based dairy farming is not common, although feedpads for providing supplementary feed are used.

The types of stockfeed consumed by dairy animals are listed in Table 5 and a comprehensive list of stockfeed assumed by the APVMA in their registration assessments is listed in Appendix 13 (APVMA, 2002). The main stockfeed supply chain includes field peas, lupins, cotton, canola, maize, wheat, barley, sorghum, oats, soybean, grass, grains, hay and silage. These crops are used to produce a wide range of stockfeed products including grain, hay, silage and seed meals. Supplementary feed is used to increase energy conversion and performance. There are three aspects to this feed, nutritional, mineral additions and therapeutic. Dairy cattle are fed nutritionally enhanced supplementary feed on a routine basis. In addition to paddock grasses, the cattle also are fed high protein food such as milled or pelleted products which are administered under strict quality assurance (QA) programs. Depending on environmental conditions, mineral additions may be required, this is supplied as salt licks containing minerals such as magnesium, molybdenum, selenium, copper, boron and cobalt. Grain-fed animals are also fed charcoal to prevent bloat. Supplementary feed may also contain the veterinary therapeutics Virginiamycin and Monensin for specific short periods of time (see 4.1.1).

In Australia, supplementary feed used in the dairy industry is generally purchased from the stockfeed milling industry or directly from the grain grower and there are established QA systems which manage chemical risks.

A National Commodity Vendor Declaration form accompanies purchased feed or grain stating which chemicals have been used and that they have been used according to label directions, observing the required WHP. In general, for low input production systems, the majority (90%) of the feed (pasture and silage) is grown on farm as compared to purchased feed (10%), whereas for high production systems, up to 55% of the feed is grown on-farm and 45% is bought in (Dairy Australia, personal communication).

The use of agricultural chemicals can potentially result in the presence of chemical residues in stockfeed that may be subsequently fed to livestock. Measurable residues of the chemical may occur in the milk of livestock consuming the previously treated feed commodity as a normal part of the diet. The APVMA establishes guideline MRLs to cover the residues that may arise in milk (as well as animal tissues and eggs) as a result of livestock dietary exposure (APVMA 2002), however the MRLs for stockfeed are not uniformly legislated; stockfeed legislation in States, other than NSW, is under review or development. These commodities are subject to MRLs based on animal transfer studies and livestock dietary exposure. The anticipated chemical residue levels in animal feed commodities are determined from crop residue trials conducted according to good agricultural practice (GAP). Currently, with the exception of NSW, there is no harmonised legislation for stockfeeds in Australia. Products registered by APVMA will include if necessary label restrictions for grazing and cutting for stockfeed (WHPs) (APVMA 2002). Thus, regulation of stockfeed is currently carried out by each State under separate legislation. Regulation of the NSW Stock Foods Act 1940 Act has most recently been updated by NSW (NSW 2005), and adopts a table of MRLs from the APVMA MRL Standard (APVMA 2005a).

Foreign ingredients in stockfeed are prescribed under the NSW legislation, for example prohibited substances, weed seeds and plants and toxic compounds are listed and the allowed proportion (if any) specified.

Stems and leaves of cotton crops are not used in dairy production due to the risk of the uptake of chemical residues (Blackwood *et al.*, 2000). The advent of bovine spongiform encephalitis overseas precipitated the ruminant feed ban in Australia, which was legislated in 1997 by the Department of Primary Industries, including the abolition of feeding materials such as bone meal, feather meal and fishmeal, with the exception of stockfeed containing tallow, gelatine and/or milk products (Bennet 2002). In 2001, the feed bans were broadened to include chicken litter and chicken faeces (QDPI&F 2005).

Care with stockfeed production is required to ensure that there is no cross-contamination of grain between different species' stockfeed and to prevent the inadvertent delivery of Restricted Animal Materials. QA programs that prevent exposure of ruminants to Restricted Animal Materials by the feed millers supplying pre-mixed feed, to ensure that stockfeed produced for chicken feed which may contain antibiotics, is not fed to ruminants.

Table 5: Stockfeed used in dairy production

Animal	Stockfeed
Buffalo	Can survive on poorer pasture areas that are too wet or of marginal quality for cows, can forage in swampy conditions (e.g. reed beds), can survive on crop stubble and grain/legume by-products
Camel	Trees, grass, shrubs, hay, fresh cut lucerne, pelleted foods, cottonseed meal, barley grass
Cow	Pastures of white clover, perennial ryegrasses, paspalum and kikuyu supplemented with silage, hay, molasses, grain, pelleted foods, green crops
Goat	Pastures, green crops (barley, oats, wheat etc), hay, straw, silage, seeds and grains, protein supplements (coconut, cottonseed, linseed, rapeseed, soybean, peanut meals), cereal bran, sugar cane molasses.
Sheep	Pasture, clover (available in plentiful supply only in high rainfall or irrigated areas), otherwise hay, pelleted feeds

Genetically modified stockfeeds

Australian livestock industries use a wide range of stockfeed components that can potentially be sourced from genetically modified (GM) crops (Lamb and Cunningham, 2003). GM stockfeed may be derived from imported GM grains or feed supplements (and are labelled accordingly) or from Australian-grown GM cotton. Currently cotton is the only GM crop that is included in stockfeed although the Office of the Gene Technology Regulator (OGTR) has also approved GM canola for commercial production, but use is restricted due to State moratoria. Both FSANZ and the OGTR have approved these GM crops as safe for human (and animal) consumption) and that there are no adverse effects resulting from using approved GM crops for stockfeed.

In a recent dairy feeding experiment, GM cottonseed was fed as part of the mixed ration to lactating Holstein cows.

The nutritional value of whole cottonseeds from genetically modified cotton was equivalent to cottonseed from non-transgenic cotton varieties, as indicated by the similar performance of the cows' dry matter intake, milk yield, milk composition, body weight and body condition (Castillo *et al*., 2004).

Many animal feeds are derived from the same GM food crops that are used for human consumption. Concerns are occasionally expressed that this practice may pose an indirect risk to humans, through consumption of the meat, milk and eggs derived from such animals. Scientific evidence published so far (OECD 2003) indicates that feeding GM plant material to livestock and poultry does not affect the nutritional value or safety of the meat, milk and eggs derived from those animals.

Genetically modified stockfeed has been found to have no adverse effects on animal health or commodity production (OECD 2003), and the risk for consumers is considered to be nil or negligible. Novel DNA may not necessarily be present (or detectable) when a GM commodity is used as part of a stockfeed mix (Castillo *et al.,* 2004). Conversely, fragments of plant DNA (both transgenic and non-transgenic) have been detected in animal tissues, including milk, but there is no basis to suppose that novel DNA poses a hazard (OECD 2003). Furthermore, it would be exceptionally unlikely for an expressed protein of any plant gene to be found intact in milk.

Monitoring of chemicals in stockfeed

The Australian dairy industry is largely based on all-year-round pasture feeding, however, as mentioned above, pasture feed is supplemented with additional feed material, predominantly grain. As grains are susceptible to the production of mycotoxins, the AMRA sampling program includes an audit of contaminants in milk originating from cattle feed inputs. In 2005/6 this will include 30 samples to be analysed for residues of Aflatoxin M1, taken from areas that may source hay and straw from crops that are susceptible to aflatoxin e.g. peanuts. A further 120 milk samples will be collected nationally and analysed for organophosphates and synthetic pyrethroids that are contained in registered agricultural products for insect control on harvested and stored grains.

The annual NRS survey is another source of information on chemical residues in agricultural commodities, which may be used in stockfeed. For example, the crops detailed in Table 6, (barley, field peas, lupin, oats, wheat and wheat bran) are all used in stockfeed. The overall compliance levels are high for these crops. Several samples were non-compliant for the organophosphate Fenitrothion.

This chemical is registered for use on cereal grains and for disinfestation of grain storage structures and grain handling equipment, but not registered for use on pulses (lupin and field pea) and no Australian Standard (MRL) is set. One barley sample also had Dichlorvos and Fipronil residues above the Australian Standard (DAFF 2005a). Those samples found with residues greater than the MRL are unlikely to be found at detectable levels in milk. On the whole, the biotransfer of AgVet chemicals from stockfeed to dairy products from stockfeed and via the blood-milk barrier, is considered to be negligible.

Table 6: Agricultural residues measured in crops used for stockfeed, 2003 – 2004 season (collated from NRS survey, 2003-4) (DAFF 2005a)

	Barley		**Field pea**		**Lupin**	
	No. analyses	**Residues detected> MRL**	**No. analyses**	**Residues detected> MRL**	**No. analyses**	**Residues detected> MRL**
Insect-growth regulators	293	0	42	0	51	0
Fumigants	14	0	5	0	5	0
Fungicides	14293	0	42	0	51	0
Organo-chlorines	2932520	0	378	0	459	0
Organo-phosphates	7819	1 (Dichlorvos)	938	1 (Fenitrothion)	1211	1(Fenitrothion)
Synthetic Pyrethroids	1465	0	214	0	255	0
Physiological Modifiers	293	0	42	0	51	0
Other insecticides	586	1 (Fipronil)	84	0	102	0
Environmental contaminants	492	0	24	0	87	0

Table 6 (contd.): Agricultural residues measured in crops used for stockfeed, 2003 – 2004 season (collated from NRS survey, 2003-4) (DAFF 2005a)

	Oat		Wheat		Wheat Bran	
	No. analyses	Residues detected> MRL	No. analyses	Residues detected> MRL	No. analyses	Residues detected> MRL
Insect-growth regulators	32	0	770	0	33	1 (Methoprene)
Fumigants	1	0	34	1 (Phosphine)	-	-
Fungicides	32	0	770	0	33	0
Organo-chlorines	288	0	6561	0	297	0
Organo-phosphates	707	0	21 329	0	693	2 (Fenitrothion)
Synthetic Pyrethroids	96	0	3850	0	165	0
Physiological Modifiers	32	0	770	0	33	0
Other insecticides	64	0	1540	0	66	0
Environmental contaminants	30	0	1755	0	-	-

4. Potential residues from veterinary and animal husbandry systems

4.1 Dairy cattle

4.1.1 Antimicrobials

Antibiotics represent the largest group of veterinary chemicals administered to dairy cattle. These compounds are listed under the S4 schedule and are only prescribed for animals under veterinary control. The different groups of antimicrobials include β-lactams, tetracyclines, sulphonamides, macrolides and aminoglycosides. Some of these groups of antimicrobial agents are also used in human medicine. Further information is provided in Appendix 11.

Antibiotics are used therapeutically for a wide range of infectious conditions in cattle. However, with the exception of occasional outbreaks of disease in a herd, the therapeutic use of antibiotics is on an individual animal basis (JETACAR, 1999).

Therapeutic herd treatment in dairy (and beef) cattle with antibiotics typically include:

- Mastitis in dairy herds (e.g. beta-lactams, tetracyclines, Lincomycin)
- Respiratory infections in cattle (e.g. tetracyclines, Tylosin, Tilmicosin, Ceftiofur, Erythromycin, Neomycin, Trimethoprim – sulphonamide combinations);
- Dry cow therapy for mastitis and sub-clinical mastitis control in dairy herds (e.g. beta-lactams, neomycin, tetracyclines);
- Control of lactic acidosis and bloat (Virginiamycin[28] and polyethers[29])

[28] The approved uses of virginiamycin in sheep and cattle are to reduce the risk of rumen acidosis from grain consumption. Virginiamycin is not used for humans, but is closely related to an antibiotic which is used for humans as an 'antibiotic of last resort' to treat infections which are resistant to other antibiotics. The APVMA published its Review Findings in November 2004 and in February 2005 the APVMA Board made the regulatory decisions to cancel the registration of products whose sole purpose is growth promotion, and to vary the labels of products whose purpose is prevention of lactic acidosis in cattle and sheep so that

- Coccidiosis in young animals (e.g. polyethers);
- Enteric infections (e.g. tetracyclines, neomycin);
- Hoof infections such as footrot (e.g. penicillin, tetracyclines, Ceftiofur, Trimethoprim-sulfonamide combinations).

Antimicrobials are used in dairy animals as chemotherapeutic agents and are not used as growth promotants. However, some antibiotics may be administered in feed for short periods of time, for example to treat lactic acidosis. If antibiotics are introduced via feed, as large molecules they are not readily absorbed by the gut and are unlikely to be carried-over into the milk. In such cases, a nil WHP is assigned.

Antimicrobial drugs are generally used in dairy animals in one of three ways (Agricultural and Veterinary Chemicals Code Act 1994):

1. Whole herd treatments, where there is "blanket" treatment of the majority of animals *e.g*. intramammary dry cow treatments (in some cows);
2. Partial herd treatments, where a minority of the herd is treated, *e.g.* intramammary dry cow treatments; and
3. Individual cow treatments, where only individual or a few animals are treated at any time, *e.g.* lactating cow intramammary treatments and injections for bacterial disease control.

Problems associated with antimicrobial residues

Antibiotic residues in milk can affect the microbial activity required for cheese and yoghurt production, as starter cultures are sensitive to inhibitors in milk including residues of antimicrobial drugs. Furthermore, starter cultures may be inhibited to varying extents influencing the relative proportion of strains in cheese-milk during manufacture. This can impact on cheese flavour profiles. The adverse impact on dairy starter cultures is avoided by setting milk MRLs less than the minimum concentration of antibiotic which inhibits the most sensitive strain of starter culture (APVMA 2003a). The strict compliance of current dairy regulations ensures that antibiotic levels are kept within their regulatory limits so that there are no potentially costly processing problems downstream. Thus, every tanker of milk is tested through on-site facilities and milk is strictly monitored to check that it is "fit for purpose".

In Australia, dairy producers are required to conform with the WHP on the antibiotic label; this is reinforced by the inclusion of a visual marker (a blue dye) in intramammary preparations. Intramammary antibiotics that do not contain blue dye are not registered in Australia, which restricts their availability for use.

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usage is restrained to a maximum of 28 days in cattle and sheep. An additional restraint is that the product may be used only once in a 12-month period and may not be used for any other animal species. The major registrant of virginiamycin products has sought review of the APVMA's decisions in the Administrative Appeals Tribunal. The review was undertaken to assess concerns relating to human health and toxicity, environmental contamination and efficacy (APVMA, 2005).

[29] Monensin is an example of a commonly used polyether ionophore, which may be used for increasing milk yields or to prevent bloat (JETACAR, 1999). It has a nil WHP based on its residue depletion profile and is therefore not tested for. There is no human usage of this antimicrobial.

Antibiotic residues in food have anecdotally been associated with causing allergic reactions and other toxicities in susceptible people. However, the formative risk assessments carried out on antibiotics determine microbiological end points, which are used to set the microbiological Acceptable Daily Intake (ADI). This takes into account the colonisation barrier in the gut, changes in microflora metabolism, and selection for antibiotic resistant organisms (Munro and Reeves, 2005). The ADIs and MRLs for antimicrobial agents are extremely conservative with large uncertainly factors built-in; antimicrobials exceeding the ADI are not eligible for registration, and no MRL is recommended by the APVMA. It is therefore highly unlikely that antibiotics, if carried over in milk, would induce a toxic response.

The potential development for antimicrobial resistance is discussed in Section 5.7.2.

4.1.2 Ectoparasiticides

Some active insecticidal compounds are the same as those used in agricultural practice, and may be used in the control of external parasites in livestock farming practice in Australia; under veterinary use, these are referred to as ectoparasiticides. Ectoparasiticides include botanicals, synthetic pyrethroids, organophosphates, carbamates, and macrocyclic lactones. Macrocyclic lactones may be used as both internal and external parasiticides (i.e. endectocides).

Organophosphate pesticides, such as Chlorfenvinphos, Fenthion, Malathion and Pirimiphos-methyl, were introduced for controlling crop and livestock pests in the 1950s as an alternative to organochlorines. They are generally much less persistent than organochlorines and are degradable, and therefore do not accumulate in animals to any great extent.

4.1.3 Endoparasiticides

Helminths and liver fluke, which are found predominantly in the southern temperate zones of Australia, are commonly controlled with endoparasiticides. Antihelmintic compounds including the benzimidazoles, Levamisole and the macrocyclic lactones are widely used in Australian dairy farming practices, with the latter being the most predominantly used of these three chemical groups. In Australia, the flukicide agent Triclabendazole is only registered for use in non-lactating cattle.

4.1.4 Other veterinary chemicals used routinely in dairy production

In addition to the use of antibiotics and endo- and ectoparasiticides, other veterinary drugs are used routinely in dairy animal husbandry, these may be either whole-herd veterinary drugs or single animal veterinary medicines, all of which are regulated by the APVMA.

Veterinary medicines include reproductive therapy drugs, such as oestrogen, which is used to synchronise endogenous hormones. These chemicals are listed under Table 5 of the MRL standard (APVMA, 2005). This table includes “situations where residues do not or should not occur in foods or animal feeds; or where the residues are identical to or indistinguishable from natural food components; or are otherwise of no toxicological significance”.

Single animal use medicines include non-steroidal anti-inflammatory drugs or anaesthetics. In addition, there are a range of dermatological preparations which are used routinely following GAP in dairy production (APVMA, 2005).

4.1.5 Bovine somatotropin (BST)

Bovine somatotropin (BST) is a hormone produced naturally by all cows, and is necessary to stimulate milk production. Since the early 1980s, it has been manufactured and this form is known as recombinant bovine somatotropin (rBST).

The Australian Agricultural and Veterinary Chemicals Council (now the APVMA) received an application to register rBST in Australia in 1991. However, although all the data requirements for registration were met, for example, regarding efficacy and safety, the application was not approved due to a number of trade concerns. rBST is not routinely monitored for in imported dairy produce, as it is not detectable above natural levels of BST. Recombinant BST is also not approved for use in New Zealand, Canada or in the European Union. However in the U.S.A., treatment of cows with rBST was approved in February 1994, and has been extensively used over the ensuing period. In addition, 24 other countries have given approval for use of rBST.

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed the safety data in 1998 and found that the available data on the identity and concentration of residues in animal tissues provide a wide margin of safety for consumption of residues in food when the drug is used according to good practice in the use of veterinary drugs. The Committee concluded that the presence of drug residues in animal products does not present any human health concerns.

4.2 Survey results for veterinary chemical residues

4.2.1 Milk

In the past seven years of the AMRA survey 3, 467 milk samples were tested for antimicrobial residues (equating to 89, 121 analyses) with 99.997% compliance with the MRL. Table 7 details the number of analyses for each antimicrobial tested from 1998 - 2005 In addition to the detection of Cloxacillin in this survey, there have previously been two samples with antimicrobial residues that exceeded the MRLs. One of these was for Gentamicin during 2000/2001 and the other for penicillin G in 2001/2002. .

Table 7: Comparison of Annual Antimicrobial Test Results in bulk milk (ADASC, 2002 – 2005)

Survey Year	Antimicrobials	
	No. analyses	No. >MRL
1998/1999	15663	0
1999/2000	10143	0
2000/2001	12852	1
2001/2002	14094	1
2002/2003	13689	0
2003/2004	14580	1
2004/2005	8100	0

The AMRA survey also indicated 100% compliance with the MRLs of a large range of endoparasiticides tested over a seven year period; Triclabendazole showed 99.88% compliance with its MRL (Table 8). Of the four detections above the MRL, three occurred during 2000/2001 and one in 2002/2003. Macrocyclic lactone testing commenced in 2002/2003 and there have been no residue detections above the MRLs to date.

Levamisole and benzimidazoles were included in the 2003/4 Survey for the first time. These chemicals are widely used, however validated methodologies for testing for these compounds in Australia have only recently been developed. No detections have been reported to date.

Table 8: Comparison of Annual Antihelmintic Test Results (ADASC, 2002 – 2005)

Survey Year	Antihelmintic (Triclabendazole)		Antihelmintic (macrocyclic lactones)		Antihelmintic (benzimidazoles)		Antihelmintic (Levamisole)	
	No. analyses	No. >MRL	No. analyses	No. >MRL	No. analyses	No. >MRL	No. analyses	No. >MRL
1998/1999	681	0	0	-	0	-	0	-
1999/2000	441	0	0	-	0	-	0	-
2000/2001	476	3	0	-	0	-	0	-
2001/2002	522	0	0	-	0	-	0	-
2002/2003	507	1	436	0	0	-	0	-
2003/2004	540	0	155	0	195	0	38	0
2004/2005	300	0	295		90	0	18	0

4.2.3 Animal tissue

The NRS cattle meat survey results for veterinary chemicals is shown in Table 9; in the 2001 – 2002 season, 63 272 analyses were conducted for the NRS survey and there was a 99.99% compliance with the MRLs/MLs, with only four samples with residues detected above the MRL.

Table 9: NRS Cattle Meat Survey Results for veterinary chemicals 1 July 2000 – 30 June 2002 (DAFF, 2005a) (DAFF, 2005a)

	Number of Analyses	Residues Detected > MRL	Number of Analyses	Residues Detected > MRL
	2000 - 2001		2001 - 2002	
Hormones	5 426	0	3 489	2 (Zeranol and Boldenone)
NSAID*s	650	0	642	0
Beta- agonists	915	0	921	0
Antimicrobials	20 731	1 (neomycin)	22 813	2 (Dihydrostreptomycin and Neomycin
Antihelmintics	3 373	0	3 431	0

*non-steroidal anti-inflammatory drugs

Table 9 (contd.): NRS Cattle Meat Survey Results for veterinary chemicals 1 July 2002 – 30 June 2004 (DAFF, 2005a)

	Number of Analyses	Residues Detected > MRL	Number of Analyses	Residues Detected > MRL
	2002 - 2003		2003 - 2004	
Hormones	4 492	1 (Zeranol)	5068	0
NSAID*s	996	0	972	0
Beta- agonists	1 625	0	1 715	0
Antimicrobials	18 057	0	18 830	2 (neomycin)
Antihelmintics	1 926	1 (Doramectin)	2 033	1 (Triclabendazole)

Zeranol was detected in one cattle faeces sample above the MRL, this can occur in beef cattle as a result of treatment with a hormonal growth promotant (HGP) (dairy cattle are not administered HGP), or naturally in animals through the metabolism of ingested zearalenone, a mycotoxin associated with stored grains (see section 7.5.5). Boldenone was detected in one cattle urine sample above the MRL.

Two kidney samples from beef cattle had antibiotic residue detections above the MRL, one for neomycin and one for Dihydrostreptomycin. Although no cause for Dihydrostreptomycin detection was found, the trace-back investigation for the neomycin detection revealed that the farmer had observed the 30-day withholding period, prior to consigning the cow from which the sample was collected to slaughter.

In the 2002 – 2003 season, 53, 827 analyses were conducted as part of the NRS Cattle Meat Survey and there was 99.94% compliance for Agricultural and Veterinary (AgVet) chemicals. In the 2003-2004 season, 51,627 analyses were carried out with 99.99% compliance. One cattle liver sample contained Triclabendazole residues exceeding the MRL and trace-back investigations indicated that the most likely cause of this residue was accidental treatment of an animal destined for slaughter.

4.3 Minor species (goat, sheep, buffalo)

There is little monitoring of chemical residues in milk from the "minor" dairy producers (goat, sheep, camel and buffalo). Furthermore, a recent risk-based assessment of unpasteurised goat milk identified that many of the veterinary products used were not registered for use in goats (Appendix 14; (Pointon *et al*., 2004). There is only a limited range of registered antibiotics for the control of mastitis in goats (Appendix 14; a complete list of registered products can be found on the APVMA website (APVMA, 2005)). Similarly, there is only a limited range of antihelmintics that are registered for use in dairy goats. This may lead to off-label use and potential unregulated residues in goat milk.

The benzimidazoles (e.g. Fenbendazole) have a zero WHP in cattle whereas they have a different profile (lower plasma levels) in goats compared with sheep. Therefore, to achieve comparable efficacy it is common practice to double-dose goats. This is also the case for the macrocyclic lactones Ivermectin and Moxidectin. The MRL for these macrocyclic lactones in bovine milk is 0.08 mg/kg, but no MRL is set for goats and thus the residue level must be zero.

Closantel is an Antihelmintic used in sheep against blood feeding worms (such as Barbers Pole and Liver Fluke); there are no MRLs for cow or goat milk. Also, some AgVet chemicals may only be permitted in specified States, for example, there is currently a permit for Trichlorfon (neguvon) and Morantel in NSW; neither product has been identified as being used in dairy goats in SA (Pointon *et al*., 2004).

A preliminary investigation was recently carried out by the Dairy Authority of South Australia, in conjunction with a South Australian Veterinary Clinic, into potential residue problems with Moxidectin and Closantel usage in goats. There are no MRLs for these drugs in goat milk and therefore the level in milk should be zero, however, it was found that residues for both drugs were detectable in the milk eight days after treatment (Pointon *et al*., 2004).

The presence of veterinary residues in sheep, goats, camels and buffalo is monitored in the NRS (albeit in non-dairy matrices) (Table 10); more chemicals are monitored in sheep reflecting the relative agricultural importance of the commodity. The only non-compliance in the 2002 – 2003 survey was for Moxidectin use in goats.

Table 10: Summary of residues found in sheep, goat, buffalo and camel meat; NRS survey results 1 July – 30 June 2003 (DAFF, 2005a)

Analyte	No. Anal.	Res > MRL	No. Anal.	Res > MRL	No. Anal.	Res > MRL	No. Anal.	Res > MRL
	Sheep		Goat		Buffalo		Camel	
Hormones	2045	0	-	-	-	-	-	-
NSAIDs	879	0	-	-	-	-	-	-
Beta-agonists	1830	0	-	-	-	-	-	-
Pesticides	21 406	0	2522	0	180	0	260	0
Antimicrobials	16 452	0	-	-	-	-	-	-
Antihelmintics	1841	0	515	3*	-	-	-	-

No. Anal: number of analyses Res. >MRL: number of residues exceeding the MRL

* non-compliance of Moxidectin use. Moxidectin is not registered for use in goats so there is no established MRL. Moxidectin use under veterinary advice was confirmed in one animal at traceback, with the goat being slaughtered before the WHP recommended by the veterinarian had elapsed. Moxidectin use, without veterinary advice, was confirmed in the other two animals. The WHP for sheep was observed, which is not appropriate for goats (DAFF 2005a).

The overall potential risk with minor dairy producing species is associated with off-label usage of veterinary chemicals. More specifically, there is often no pharmokinetic data to determine WHPs for minor species, so these need to be conservatively applied with advice from a technical expert

5. Potential residues from environmental and anthropogenic chemicals

5.1 Potential contaminants in dairy products

Contaminants in food are regulated, in part, by establishing MLs for various foods in the Code. However MLs are part of a broader risk management framework for food contaminants. 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. The public health risks associated with food contaminants can also be managed through establishing guidelines, codes of practice, or through education of the public on safe food consumption patterns.

In many cases, the potential for contamination of food is limited as a result of these other regulations and specific food regulation may be unnecessary. When a food standard is considered necessary for a particular contaminant as a risk management measure, 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 maximum levels (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).

Although the Australian dairy industry relies upon all-year-round pasture feeding, additional feed material, predominantly grain, also forms part of the feeding program. Other minor feedstuffs such as hay, straw and such as crop trash may also be used, particularly when feed is less plentiful such as during Australia's recent drought conditions. Some minor feedstuffs such as peanut hay and sorghum are susceptible to fungal infection so monitoring of residues of aflatoxin M1 contaminants in milk, originating from hay and straw and from these crops is included in residue testing programs. During times of drought, more novel feedstuffs may also be utilised, such as oranges and bakery products.

The presence of environmental contaminants in forage is subject to wide variations, mainly depending upon the vicinity of the crops to a source of contamination including mould infestation.

As part of a review of potential chemical hazards for dairy products, eleven environmental contaminants, eight groups of stockfeed contaminants, two forms of natural chemical toxins/residues and one chemical formed during food processing, were reviewed in addition to endogenous plant toxins, which may be present in pasture (Table 11).

Regular monitoring of environmental contaminants occurs to some degree through the AMRA survey and NRS, although not all the potential contaminants covered in this paper are considered in these surveys. Inclusion of particular contaminants in these surveys will depend on the results of previous surveys, the likelihood of significant contamination, and the potential public health risk associated with the contaminant.

Heavy metals are included in the AMRA survey every 2-3 years in order to meet trade criteria and were first included in the AMRA Survey in 2001/2002. No residues were found (in 112 samples) at levels greater than the ML, and for this reason, heavy metals were not included in 2002/2003 or 2003/2004 survey. The NRS Cattle Residue Survey also monitors environmental chemical residues such as PCBs, heavy metals and the mycotoxin, Zeranol. Although this survey monitors matrices other than milk, it provides an insight into the levels and types of residues found in all cattle (including ~15% dairy cattle) and residues in other milk-producing animals.

Table 11: Potential chemical contaminants for dairy products and their sources

Contaminant	Source
CHEMICAL CONTAMINANTS	
Arsenic	Environmental contamination.
Cadmium	Environmental contamination
Dioxins and dioxin-like Polychlorinated biphenyls	Environmental contaminant. Contaminated feed (Belgium)
Iodine	Environmental contaminant; milk production and processing
Lead	Environmental contaminant.
Mercury	Environmental contaminant; manometers with mercury used in dairy farms
Polychlorinated biphenyls (PCBs)	Environmental contaminant
Radionuclides	Environmental contaminant
Selenium	Environmental contaminant; AgVet contaminant
Zinc	Environmental contaminant
NATURAL TOXINS	
Biogenic amines	Endogenously formed within dairy products
Fungal toxins	Formed within dairy products
Aflatoxin M1, M2 and M4	*Aspergillus flavus,* and *A. parasiticus* contamination of corn, peanuts and other feed ingredients
Corynetoxin	*Rathayibacter toxicus* contamination of annual rye grass
Cyclopiazonic acid	*Penicillium sp*. and *Aspergillus sp.* contamination of stockfeed and dairy products
Endogenous plant alkaloids	Natural plant defence chemicals
Fumonisin B_1	*Fusarium moniliforme* plus several less common species contamination of corn
Ochratoxin A	*Aspergillus ochraceus* and *Penicillium verrucosum* contamination of barley, wheat and many other commodities
Trichothecenes; T-2 and HT-2 toxin Deoxynivalenol (DON); Vomitoxin	*Fusarium graminearum, F. crookwellense* and *F. culmorum* contamination of wheat, barley and corn
Zearalenone	*Fusarium graminearum, F. crookwellense* and *F. culmorum* contamination of wheat and corn
CHEMICALS FORMED DURING FOOD PROCESSING	
Polycyclic aromatic hydrocarbons (PAHs)	Contaminant formed during processing (e.g. smoking)

5.2 Heavy Metals

5.2.1 Sources of contamination

Metals can potentially contaminate dairy products 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, dairy animals may directly consume soil.

Soil consumption by grazing cattle has been estimated at 0.5 kilograms per day (Van Hooft, 1995), however soil consumption is relative to the grazing pattern and may be more of an issue for beef cattle grazing dryer pastures close to the ground.

Metal contamination through processing may occur through metal pick-up from containers and metal cooking utensils. The bioavailability of trace elements in soil is about 1.5 times lower than in feed (Van Hooft, 1995).
In general, the transfer of heavy metals to milk is limited. Exceptions are the fat-soluble organic mercury and lead compounds. The highest transfer to milk is noted for lead and arsenic, followed by mercury and cadmium (Vito - LUC - RUG, 2003). In order to satisfy export requirements, these four heavy metals have been tested for in the AMRA survey.

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 dairy products may be increased when animals consume plants with high levels of arsenic. For instance, sheep and goats may graze plants with higher arsenic contents than cows. Old sheep dips could also be point sources of arsenic.

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 is a function of the concentration of cadmium in the feed and the amount of feed consumed. Moreover, uptake of soil during grazing (or soil contaminated feeds) is an additional factor contributing to total exposure of individual animals.

Lead can potentially contaminate dairy products through environmental contamination or through contamination of water supplies. Utensils containing lead, such as tin or pewter, may also cause contamination.
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.

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 of low levels of mercury by pasture-fed dairy cattle.

<u>Regulation of heavy metals in relation to dairy products</u>
For arsenic, cadmium and mercury there are no MLs in the Code (the Code) for milk or other dairy products. Dairy products are considered to be an insignificant dietary source of these heavy metals and therefore do not require a control, such as an ML.

Because of the increased sensitivity of infants for lead toxicity, the Code has an ML for lead in infant formula in standard 1.4.1 – Contaminants and Natural Toxicants. The maximum level (ML) of lead in infant formula is 0.02 mg/kg.

5.2.2 Arsenic

Hazard Identification and Characterisation

A risk assessment on arsenic was last performed by FSANZ[30] in Proposal 157 – Review of the maximum permitted concentrations of metal contaminants in food (ANZFA, 1999e). 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 vasodilation, then hyperpigmentation and hyperkeratosis with subsequent atrophy and degeneration of the skin leading over a period of time to the development of skin cancers.

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, 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, 1999d; ANZFA, 1999e).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has assigned a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg bw for inorganic arsenic (WHO, 1989a), 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

The arsenic content of tissues and body fluids is markedly influenced by the level of intake, but experiments in cows suggest there is a barrier to excessive mammary uptake, as milk concentrations were not increased by feeding diets containing 25 times the arsenic level in normal rations (Jensen, 1995). Transfer factors from feed to milk between 10^{-4}-10^{-5} kg/L (concentration in milk/concentration in feed, dry matter) have been reported (Van Hooft, 1995; Rosas *et al*., 1999; Perez-Carrera and Fernandez-Cirelli, 2005).

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

A dietary exposure assessment was conducted as part the 19th ATDS (ANZFA, 2001). The Survey reported no detection of total arsenic in feta cheese from cow's milk (limit of reporting 0.01 mg/kg), and mean levels below the limit of reporting (0.002 mg/kg) for feta cheese made from sheep's milk.

The 20th Australian Total Diet Survey (FSANZ, 2003) estimated exposure to total arsenic between 9-48% of the PTDI set for inorganic arsenic. Inorganic arsenic analysis was more expensive than total arsenic analysis. Therefore, only total arsenic was tested, which is an overestimate of inorganic arsenic. The 20th ATDS did not detect total arsenic in full fat milk and, while in cheddar cheese total arsenic was not detectable at 0.02 mg/kg (FSANZ, 2003) These results demonstrate consistent non-detectable or very low levels of total arsenic and as a consequence inorganic arsenic in Australian milk and milk products.

5.2.3 Cadmium

Hazard Identification and Characterisation

A risk assessment on cadmium was last performed by FSANZ[31] in Proposal 144 – Review of the maximum permitted concentration of cadmium in food (ANZFA, 1997). Cadmium has been most recently assessed by JECFA in 2003 (WHO, 2003). Cadmium has an extremely long biological half-life in man 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 man 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 (WHO, 1989b).

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 body weight per week (WHO, 2003).

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, 1993a).

31 As the Australia New Zealand Food Authority (ANZFA)

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 (WHO, 2001).

Dietary exposure

Cadmium can potentially be carried over into milk in lactating cows from intestinal absorption (Van Hooft, 1995). In a 2-week experiment where 3g cadmium (as $CdCl_2$) was administered to cattle, less than 0.022% was found in milk. The transfer factor from feed to milk for cadmium is reported to be between $2.3*10^{-5}$ –$5.8*10^{-5}$ kg/L (concentration milk/concentration dry matter in feed) (Van Hooft, 1995). In a study from Thailand, the levels of cadmium in milk were below the level of reporting and therefore, the transfer factor was reported to be zero (Parkpian *et al*., 2003a).

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. The survey data indicated cadmium was not detected in full cream milk, cheddar cheese. The 19th ATDS reported no detection of cadmium in feta cheese from cow's milk and mean levels below the limit of reporting (0.001 mg/kg) for feta from sheep's milk.

The 20th Australian Total Diet Survey did not detect cadmium in full cream milk, the limit of reporting for this analysis was 0.005 mg/kg (FSANZ, 2003) The maximum level of cadmium found in cheddar cheese was 0.007 mg/kg..

Cadmium residues in cow's milk were monitored in 112 samples in the 2001 – 2002 AMRA survey; there were no residue detections. In the 2003-4 NRS survey there were seven cadmium detections in sheep livers above the Australian Standard (ML) of 1.25 mg/kg. The residue levels ranged between 1.3 mg/kg and 2.4 mg/kg. Cadmium residues are a common finding in sheep offal, particularly in older animals across the southern states of Australia. A residue action level of 2.5 mg/kg for trace back purposes has been agreed between NRS and state and territory government regulatory authorities. None of the seven residue detections were above the action level, so trace backs were not done for these detections (DAFF 2005a).

In a study in India it was found that cadmium concentrations in buffalo milk were higher than levels in cow's milk (0.10 μg/L vs. 0.07 μg/L, respectively). The study authors explained the difference, because of the different fat content of buffalo milk (7.5%) in comparison to cow's milk (3.8%) (Tripathi *et al*., 1999a).

Research on Manchego cheese during traditional cheese making and ripening indicated that cadmium concentrations increased during pasteurisation and more noticeably during ferment addition, thereafter falling during coagulation. These differences are related to the moisture content during cheese manufacture, since no differences were found for dry weight.
There were no significant differences in cadmium levels between newly made and mature cheeses (Zurera-Cosano *et al*., 1997).

A French study looked at the levels of cadmium in cows and sheep milk after 10-weeks oral administration with cadmium. Before the oral administration of cadmium, the levels of cadmium in the milk were around 0.4 µg/L in ewes and <0.2 µg/L in cows. They found that almost all the cadmium was transferred from the milk into milk products (in particular into casein fractions) and the mean levels in ewes were 3.3 ± 1.4 µg/L and 2.5 ± 1.4 µg/L in cows (Mehennaoui *et al*., 1999).

5.2.4 Lead

Hazard Identification and Characterisation

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.

The absorption of lead from grass, hay and soil is around 5% and the gastrointestinal absorption of lead in cows is around 10%, of which 90-95% of lead is excreted in the faeces. The transfer factor of lead from feed into milk is reported between 10^{-4}-10^{-5} kg/L (concentration milk/concentration feed dry matter) (Van Hooft, 1995; Parkpian *et al*., 2003b).

A linear dose related excretion of lead from plasma into milk was found in rats and mice after intravenous injection and the lead concentration in milk was approximately 100 times higher than that in plasma 24 h after administration demonstrating efficient transport of lead into milk (Hallen *et al*., 1995). Oral feeding of lead acetate at the dose rate of 500 mg/day to limited number of lactating cows has been reported to significantly increase the milk lead excretion (Willett *et al*., 1994). However, the level of lead in milk samples from animals seven months after an acute episode of lead toxicosis was undetectable (Galey *et al*., 1990).

The concentration of lead in milk depends on the concentration of unbound lead in blood. The lead concentration in milk was found to be relatively constant up to blood levels between 0.2 and 0.3 µg/ml and increased sharply at higher blood lead levels in an accidental lead exposure over a period of 1 to 2 days through licking of burnt storage batteries by cows (Oskarsson *et al*., 1992). It has also been shown that contamination of lead resulted in blood lead concentrations exceeding 0.20 µg/ml and that the excretion of lead in milk from cows significantly increased. (Swarup *et al*., 2005).

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

The most widely used biomarker of exposure to lead is the concentration in blood (measured in µg/dL). The most critical effect of lead at low concentrations is reduced cognitive development and intellectual performance in children.

A number of studies in which various tests of behavioural performance were used have shown an association between blood lead concentration and reduced intelligence quotient in children exposed pre-and postnatally.

At blood concentrations below 10-15 µg/dL, the effect of confounding variables and limits to the precision of analytical and psychometric measurements increase the uncertainty of any estimate of effect. If a threshold does exist, it is unlikely to be detected because of these limitations.

However, there is some evidence of an association between cognitive deficits and exposure to concentrations even below 10 µg/dL. In conclusion, the toxicological review suggests that there is a small safety margin between the PTDI and the LOEL for children (ANZFA, 1999f).

JECFA (1993) established a Provisional Tolerable Weekly Intake of 25 µg/kg bw (equivalent to a PTDI of 3.5 µg/kg bw/day) for all age groups on the basis that lead accumulates in the body and an increase of the body burden should be avoided this level was reinforced in 2000 (WHO, 2000). This upper limit has been adopted by FSANZ as a provisional tolerable daily intake (PTDI) for the purposes of dietary modelling (ANZFA, 1999f). IARC has classified lead into group 2A (probably carcinogenic for humans) (IARC, 2004).

Dietary exposure

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 (ANZFA, 1999f). The modelling estimated that the mean dietary exposure for the Australian population, aged 2 years and older to be 2-6% of the PTDI.

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%). Dairy products were not a contributor to lead dietary intake.

The modelling estimated that the mean dietary exposure for children, aged 2 years, was 5-17% of the PTDI. The foods that contributed to the exposure of children and infants were milk (16%), pineapple juice (9%), apple juice (8%), sugar (8%), bread (8%), and tea (3%) for schoolchildren, and milk (24%), juice (21%), and bread (5%) for toddlers (WHO, 2000).

The 19th ATDS found a mean level of lead of 0.004 mg/kg in feta cheese from cow's milk and a mean level of 0.028 mg/kg in feta cheese made from sheep's milk. (ANZFA, 2001).

In the 20th Australian Total Diet Survey lead was not detected in full fat milk or cheddar cheese, with a limit of reporting at 0.01 mg/kg. Lead was also not detected in infant formula.

Lead residues in milk were monitored in 112 samples in the 2001 – 2002 AMRA survey; there were no residue detections. In the 2003-4 NRS survey, there was one detection of lead in cattle liver above the Australian Standard (ML) of 0.5 mg/kg for cattle. A residue action level for trace back has been set at 1 mg/kg and therefore no trace back action was required. There were two lead detections in livers above the Australian Standard (ML) of 0.5 mg/kg for sheep. A residue action level for trace back has been set at 1 mg/kg. None of the lead residue detections were higher than the trace back action level (DAFF 2005a).

In two studies in India it was found that lead concentrations in buffalo milk were higher than levels in cows milk (3.35 µg/L vs. 1.70 µg/L, respectively). It could be an indication that buffalo's have a higher tolerance for lead or the different fat content of buffalo milk (7.5%) in comparison to cow's milk (3.8%) (Tripathi *et al*., 1999b; Dwivedi *et al*., 2001).

In an Argentinean study, there was a significant difference ($p<0.05$) for the mean concentration in milk between young (<5 years, 17 µg/L) and old cows (>5 years, 34 µg/L), however there was a poor correlation between age and lead milk concentration ($r=0.038$) (Rubio *et al*., 1998).

In Canada there were no differences in lead content between whole, 2% butterfat and skim milks (Dabeka and McKenzie, 1987). In Kasar cheese from Turkey seasonal variation in lead content was found, with highest levels in winter months. The study authors suggested that this increase could be through differences in feed, in summer the cows eat pasture, while in winter the cows are fed silage (Yuzbasi *et al*., 2003). There were also differences between in lead content of samples from different cheese producers. These findings indicate that exposure to lead contamination in other countries may be different from Australia.

5.2.5 Mercury

Hazard Identification and Characterisation

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

The different chemical forms of mercury can exhibit quite distinct pharmacokinetic 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.

In mammals, methylmercury is almost completely absorbed in the gastrointestinal tract. Subsequently, in humans, about 90 percent of methylmercury absorbed is then excreted in the faeces. It is likely that the same patterns occur in dairy animals.

Methylmercury can be excreted at low levels in the breast milk of rats, humans, and guinea pigs (Yoshida *et al*., 1992; Sundberg and Oskarsson, 1992). The transfer factor from feed to milk is reported at 1.7*10-4 kg/L (concentration milk/concentration in dry matter in feed) (Van Hooft, 1995).

Sundberg and coworkers ((Sundberg *et al*., 1998) studied the elimination of radio labeled methylmercury in lactating and non-lactating mice exposed to methylmercuric chloride via a single intravenous injection at 0.5 mg Hg/kg body weight. A three compartment pharmacokinetic model was used to fit the data. The values for the methylmercury kinetic parameters were significantly higher in lactating than non-lactating mice: plasma clearance (93.5 and 47.1 mL/hour/kg, respectively) and volume of distribution (18,500 and 9,400 mL/kg, respectively). The terminal half-life of methylmercury in plasma was 170 hours for lactating and 158 hours for non-lactating mice. The milk to plasma concentration ratios for total mercury after methylmercury administration were lower than those seen with inorganic mercury, and varied between 0.1 and 0.7, with a mean of 0.20.

Mercury concentrations in milk were constant throughout the 9-day follow-up period post exposure. The results indicate that physiological changes during lactation alter the pharmacokinetics for methylmercury in mice (ATSDR, 1999).

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 (WHO, 2003).

In June 2003, JECFA evaluated new information 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 (WHO, 2003).

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, 1993b).

Dietary exposure

A dietary exposure assessment was conducted as part of the assessment of methylmercury 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. Dairy products did not contribute to methylmercury dietary intake.

The 19th ATDS reported no detection of mercury in feta cheese from cow's milk or from sheep's milk. The 20th Australian Total Diet Survey did not detect mercury in full fat milk, cheddar cheese, and table spread margarine samples (FSANZ, 2003).

Mercury residues in cow's milk were monitored in 112 samples in the 2001 – 2002 AMRA survey; there were no residue detections.

5.2.6 Risk characterisation for heavy metals

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

As discussed in the individual sections above, all four metals can result in serious adverse effects when consumed at high concentrations. However, when dairy animals consume these metals, a very small fraction is transferred into the milk. Data from various sources in Australia on the concentrations of these metals were non-detectable or at very low levels in milk and milk products. The data available are mainly from dairy products from cows. However, on the basis of physiology and good agricultural practices, it can be assumed that the levels of metal contaminants in dairy products from sheep, goats, buffalo or camels will be similar to levels found in dairy products from cows, i.e. very low or not detectable.

In conclusion, dietary exposure to arsenic, cadmium, lead and mercury from dairy products does not raise any public health and safety concerns.

5.3 Micronutrients

5.3.1 Essentiality role and sources

Micronutrients, are defined in physiological terms as substances that comprise less than 0.01% of the body mass.

Besides having an essential role, i.e. they are required in the human diet, the potential exits for over consumption of some micronutrients, thereby resulting in a public health and safety risk.

5.3.2 Iodine

The natural content of iodine in milk varies with the amount of iodine ingested by the cow, through drinking water and pasture. In pastures, the iodine content depends upon the concentration and nature of iodine in the soil (Dunsmore and Luckhurst, 1975). There are seasonal variations in iodine concentrations in milk, which are closely related to dietary intake; winter rations containing supplements, such as ethylene dihydroiodide result in considerable increases in milk iodine.

Although a significant amount of iodine present in milk comes from ingestion, iodine can also enter the milk through the use of iodine sanitisers on milking equipment and through the use of iodine teat dips and udder washes used to prevent mastitis.

Iodophors[32] have been used since 1962 as sanitisers by the dairy industry in both Australia and New Zealand (Thomson, 2004). Dairy products had been a major source of iodine in the New Zealand diet, but a move away from the use of iodophors has apparently resulted in lower iodine concentrations in dairy products (Thomson 2004). Iodine is still used in agricultural practice as a teat disinfectant post milking in some parts of Australia (Seal, 2004) Dairy Australia submission to P230, 2005), there are a large number of products registered for use by the APVMA register (APVMA 2005b), and water is naturally high in Queensland and parts of Australia, especially in summer (S. Rice, personal communication).

<u>Hazard identification and characterisation</u>

A risk assessment on iodine was most recently carried out by FSANZ in relation to Application A470 – Formulated Beverages.(FSANZ, 2005a).

A large number of human experimental, clinical, and epidemiological studies on the effects of excess iodine have been reported and reviewed in detail by both the Joint FAO/WHO Expert Committee on Food Additives (WHO, 1989a) and the US Agency for Toxic Substances and Disease Registry (ATSDR, 2004). These studies indicate that the primary effect of excess iodine is on the thyroid gland and regulation of thyroid hormone production and secretion.

Excess iodine can produce an enlargement of the thyroid gland (goitre) and/or affect the production of the thyroid hormones.

[32] Iodophors are iodine-liberating disinfectants which comprise of organic compounds containing iodine in a micellar cage of polyvinyl-pyrrolidone or nonoxyno-complex. When diluted, iodine is released and can exert its bactericidal properties. Commercial preparations contain 0.3 – 1.75% iodine. Of which 80 – 90 % is released upon dilution (Fischer *et al*., 2002).

A diminished production of thyroid hormones is referred to as hypothyroidism (and may be accompanied by goitre), while increased thyroid hormone synthesis and secretion by the thyroid gland is referred to as hyperthyroidism.

The human response to excess iodine can vary. Some individuals can tolerate large intakes (up to 50 μg/kg/day) while others may respond adversely to levels close to recommended intakes (3-7 μg/kg/day). Individuals responding adversely to relatively low intake levels often have an underlying thyroid disorder or have a long history of iodine deficiency.

A tolerable upper intake level of 1100 μg iodine/day for adults has been established by the US Institute of Medicine (US Institute of Medicine, 2001). This is the proposed level to be adopted in Australia by the National Health and Medical Research Council as part of their current review of Nutrient Reference Values (NHMRC, 2004). FSANZ has adopted this level as the upper level of intake (UL)[33] for the purpose of risk assessment for the general healthy population (FSANZ, 2005b).

In summary the ULs for iodine in the various age groups are:

1-3 years	**200 μg/day**
4-8 years	**350 μg/day**
9-13 years	**650 μg/day**
14-18 years	**1000 μg/day**
adults	**1100 μg/day**

It should be noted, however, that individuals with thyroid disorders or a long history of iodine deficiency might respond adversely at levels of intake below the UL. Therefore, the health risk for these individuals needs to be considered separately from the general population.

Dietary exposure

The iodine concentrations in food do not allow a determination of whether the iodine is from natural sources or as a result of contamination. However, an increase of around 30 μg iodine/L in milk was measured when an iodophor post-milking teat dip of 0.5% iodine was applied to Holstein cows (Galton, 2004). In a survey of the nutritional composition of Australian dairy foods co-coordinated by the Australian Dairy Corporation (now Dairy Australia), the iodine content of milk was 50 μg/L. This is the level of iodine thought to be present naturally without contamination from iodophors (Dunsmore and Luckhurst, 1975). The iodine content of key dairy products was also measured: 250 ml milk contains 12.5 μg of iodine, 200 g reduced-fat yoghurt contains 34 μg of iodine and 40 g Cheddar cheese contains 6 μg of iodine (Dairy Australia submission to P230, 2005).

The 22nd ATDS (FSANZ, 2004b) incorporated the iodine content of a broad sampling of dairy produce, for example: yoghurts, custard, cheese (Cheddar, Parmesan, Edam, Feta, cream, Mozzarella, Brie), milk (skimmed, powdered, flavoured, evaporated, condensed), cream (sour) and ice cream. Iodine levels in this survey were full fat milk (0.133 mg/kg), low fat milk (0.159 mg/kg), full fat fruit yoghurt (0.167 mg/kg), full fat ice cream (0.213 mg/kg), butter (0.039 mg/kg), and cheeses (0.153-0.229 mg/kg), (FSANZ, 2004b).

33 The upper level of intake is the highest average daily nutrient intake level likely to pose no adverse health effects to almost all individuals in the general population.

In 1998, the Victorian Dairy Industry Authority carried out a state-wide survey to monitor iodine levels in raw tanker milk. Of the 218 samples taken, none exceeded 500 µg/L, which was the Australian standard for iodine in milk until 1998. The majority of samples had iodine concentrations of 100 – 199 µg/L. The combined results for iodine levels for Victoria are shown in Table 17 (VDIA, 1999).

Table 17: Iodine content of raw milk tanker samples from Victoria (1st July 1998 – 30th June 1999) (VDIA, 1999)

Iodine levels (µg/L)	Frequency
< 100	103
100 – 199	150
200 – 299	10
300 – 399	0
400 – 499	0
≥500	0

A dietary exposure assessment was conducted as part of the assessment of iodine for Application A470 – Formulated beverages. The results of this assessment are presented in Table 18.

Table 18: Estimated dietary intakes of iodine in Australia, and percent of upper level of intake (UL) (FSANZ, 2005a)

Age group	Mean intake µG/DAY (%UL)	95th percentile intake µg/day (%UL)
2-3 years	106 (55)	206 (105)
4-8 years	109 (30)	217 (60)
9-13 years	130 (20)	276 (40)
14-18 years	142 (15)	338 (35)
≥19 years	116 (10)	276 (25)

The primary foods that contribute to dietary iodine exposure in the Australian population were dairy (67.8%), fruits (10.6%), cereal foods (5.6%), and seafood (5.3%) (FSANZ, 2005b)

Extent of iodine variability in milk

The main external influences on the level of iodine in milk are geographical variations and seasonal diets, in addition to the use of iodophors as sanitising agents of milking equipment and naturally high levels in water in certain parts of Australia.

In the U.K. seasonal variations can occur when iodine rich stock feed is given to dairy cattle during winter to compensate for reduced access to grazing pastures (FSA 2000). For example, winter milk was found to contain 210 microgram/kg while summer milk contained 90 microgram/kg. This seasonal variation may reflect the greater use of compound feeding stuffs during the winter months (FSA 2000). Iodine may be naturally present in the ingredients used in animal stockfeed or may be added via supplements. Iodine is included in compound stockfeed to protect animal health and incidentally it provides a source of iodine in human diets.

Iodophors have been used widely in the past as sanitising agents for teats and milking equipment, and may contribute significantly to the iodine content of milk. Australia, New Zealand and many other overseas countries have now reduced the use of iodophors, resulting in a lowering of milk iodine content.

However, some nations (e.g. United Kingdom) still maintain the practice of (high) iodophors use, which contributes to the global variability in milk iodine content.

Table 19 demonstrates some of the variability that can exist in milk iodine concentration on a global scale; only a selection of countries are provided due to the lack of information on international milk iodine concentrations. New Zealand data have been obtained from the 2003/4 Total Diet Survey (NZTDS) results, while information on Australia is available from Tasmania, where periodic monitoring is undertaken by two major milk processors, for the Northern Victorian District of the Goulburn Valley, and from the results of the 22nd Australian Total Diet Survey (FSANZ, 2004b).

Table 19: Annual Iodine Concentrations in Milk (μg/L)

	Minimum	Maximum	Mean
Australia (Tasmania) (Personal communications: Seal J, 2004)	110	440	265
Australia (Victoria) (Nestlé submission in response to IAR A528)	31	361	155
Australia (FSANZ, 2004; 22nd ATDS, 2004)	90	210	133
New Zealand (Vannoort, 2004)	41	235	86
United Kingdom (United Kingdom Food Standards Agency 2000)	184	426	315
Germany (Preiss 1997)	<100	150	115
International Mean (FAO/WHO 2002)	34	54	46

5.3.3 Selenium

Selenium in food is predominantly in the form of organo-selenium 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. Many plants such as *Astragalus* and *Stanleya* accumulate high levels of selenium, although most of these selenium-accumulating plants are unpalatable (Panter and James, 1990).

Due to deficiencies in Australian soils supplementation with selenium in agricultural or veterinary chemicals for example the oral drenches such as Cydectin Se, are registered for use in sheep and cattle (Pointon, 2004). While dairy products are considered to have low concentrations of selenium, the relatively large amount of milk consumed (as compared to solid foods) results in milk being a source of selenium intake.

Hazard Identification and Characterization

The safety of selenium was most recently assessed by FSANZ in Application A470 – Formulated Beverages (FSANZ, 2005a). Furthermore, NHMRC has established upper levels of intake for selenium in specific age groups (NHMRC, 2004).

In food animals, a study found that up to 18% of the selenium in an oral diet might be excreted in milk (Maus, 1980).

There is limited data about toxicity in humans but the most common outcomes are hair and nail brittleness and loss as well as gastrointestinal disturbance, skin rash, fatigue, irritability and nervous system abnormalities. Studies from China give a NOEL for adults of 800μg/day, which was consistent with one US study.

An Uncertainty factor of 2 is applied (US Institute of Medicine, 2000) to protect sensitive individuals because of gaps in data and incomplete knowledge, bearing in mind that the toxic effect of selenium is not severe but may not be reversible. The Upper Level of Intake (UL) is therefore set at 400µg/day for adults including pregnancy and lactation as there is no data to suggest increased susceptibility.

The UL for young infants was based on the studies showing that human milk concentrations of 60µg/L were not associated with adverse effects. This gives a NOEL of 47µg/day (7µg/kg body weight). An Uncertainty Factor of 1 is applied, as there is no evidence that maternal intakes associated with human milk in this range causes infant or maternal toxicity. As there is no evidence of increased toxicity in older children and adolescents, the ULs for these groups was estimated on a body weight basis from the younger infant data using the level of 7µg/kg body weight (NHMRC, 2004).

In summary the ULs for the various age groups are:

1-3 years	**90 µg/day**
4-8 years	**150 µg/day**
9-13 years	**280 µg/day**
14-18 years	**400 µg/day**
adults	**400 µg/day**

Dietary exposure

Where plants are deficient in selenium, milk selenium levels are reported at 5-30 ng/ml, moderate levels in plants are associated with milk levels of 30-66 ng/ml; concentrations of up to 1300 ng/ml were found in milk from cows living in seleniferous areas of South Dakota (Jensen, 1995). Selenium is present in cow's milk in concentrations in direct proportion to selenium intake (Panter and James 1990).

Most of the selenium in cow and goat's milk is found in the skim milk with only 2-10% being in the fat fraction. About 30% of the total selenium in goat milk is found in the whey, compared with over 70% in bovine milk, of which 80% is found with β-lactoglobulin (Jensen, 1995).

A dietary exposure assessment was conducted as part of the assessment of selenium for Application A470 – Formulated Beverages (FSANZ, 2005a). 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 15-35% of the UL and dietary exposure at the 95th percentile of 40-80% (ANZFA, 1995; FSANZ, 2005a)

The 20th Australian Total Diet Survey indicated the following primary foods that contribute to dietary selenium exposure in the Australian population, aged 2 years and older: chicken meat (19%), marine fish (11%), pork (10%) eggs (10%), wheat flour (5%) and milk and dairy (5%).

The 20th Australian Total Diet Survey data have indicated selenium levels in full fat milk (0.011 mg/kg), low fat milk (0.013 mg/kg), full fat fruit yoghurt (0.017 mg/kg), various cheeses (0.057-0.107 mg/kg), and full fat vanilla ice cream (0.0013 mg/kg), chocolate milk (0.066 mg/kg) (FSANZ, 2003).

5.3.4 Zinc

The zinc content of milk is not constant but influenced by a number of factors such as stage of lactation, nutritional status of the animal, and environmental contamination through drinking water or if galvanised containers were used for storage.

Hazard identification and characterisation

The safety of zinc was most recently assessed by FSANZ in Application A470 – Formulated Beverages (FSANZ, 2005a). Furthermore, NHMRC has established draft upper levels of intake for zinc specific age groups (NHMRC, 2004).

Studies of chronic and sub-chronic toxicity of zinc are well documented. Prolonged intakes of zinc supplements ranging from 50 mg/day up to 300 mg/day have been associated with a range of biochemical and physiological changes.

These changes include hypocupraemia, leucopaenia, neutropaenia, sideroblastic anaemia, decreased concentrations of plasma copper and decreased activity of the copper containing enzymes, superoxide dismutase and caeruloplasmin, altered lipoprotein metabolism and impaired immune function. Many of these biochemical and physiological changes are similar to those observed during copper deficiency. Nevertheless, there are problems with hazard identification in that these changes are not specific to copper deficiency and the clinical relevance of some is unknown.

Systemic evidence of copper deficiency in humans may be observed at doses of 150 mg/day in humans, but doses as low as 50 mg/day may indicate a threshold effects, as observed by changes in biochemical markers of copper deficiency (ANZFA, 1999h).

A LOEL of 60 mg/day is set, based on a study where copper status was evaluated after supplemental intake of 50 mg/day as zinc gluconate in 18 healthy female subjects (aged 25 to 40 years) for 10 weeks. Endothelial superoxide dismutase activity was significantly lower than pre-treatment values. An uncertainty factor of 1.5 was used to account for inter-individual variability in sensitivity and for extrapolation from a LOEL to a NOEL. Because reduced copper status is rare in humans, a higher uncertainty factor was not justified.

For children a study in infants fed 5.8 mg/L of zinc for six months did not reveal effects of zinc on serum copper or cholesterol concentrations or other adverse effects. This would result in an intake of 4.5 mg/day for infants 0 through 6 months of age. This NOEL was divided by an uncertainty factor of 1.0 to obtain an upper limit of 4 mg/day (rounded down) for infants 0 through 6 months. No adverse effects of zinc in children and adolescents could be found. Due to a dearth of information, the UL for young infants was adjusted for older infants, children and adolescents on the basis of relative body weight. Values have been rounded down (FSANZ, 2005a).

In summary the ULs for the various age groups are:

1-3 years	**7 mg/day**
4-8 years	**12 mg/day**
9-13 years	**23 mg/day**
14-18 years	**34 mg/day**
Adults	**40 mg/day**

Dietary intake
A dietary exposure assessment was conducted as part of the assessment of zinc for Application A470 – Formulated beverages (FSANZ, 2005a). The results of this assessment are presented in Table 24.

Estimated intakes were adjusted based on second day intake data from the NNSs. Dietary modelling has been conducted only for food intake. Intake through other sources (i.e. supplements and drinking water) was not included in the modelling.

Table 24: Estimated dietary intakes of zinc, and percent of UL (FSANZ, 2005a)

Age group	Mean intake mg/day (%UL)	95th percentile intake mg/day (%UL)
2-3 years	7.5 (110)	10.4 (150)
4-8 years	8.2 (70)	11.7 (100)
9-13 years	10.9 (45)	16.5 (70)
14-18 years	12.7 (35)	21.3 (65)
≥19 years	11.9 (30)	18.4(45)

The primary foods that contribute to dietary zinc exposure in the Australian population, aged 19 years and older, were meat, poultry, and game products and dishes (35%) cereals and cereal products (14%), milk products (13%), cereal based products and dishes (10%), and vegetable products and dishes (10%) (ABS, 1995).

In Australia the following concentrations of zinc in various dairy products are reported: milk, cow 3 mg/L; milk, goat 4 mg/L; milk powder 30-39 mg/kg; various cheeses (cream, Feta, Mozzarella, Parmesan, blue vein, Edam, Gouda, Neufchatel, Cheddar, Swiss, Ricotta, Cottage) 2-65 mg/kg; yoghurt 4-7 mg/kg; ice cream 5-7 mg/kg; cream 0-4 mg/kg (FSANZ, unpublished data).

Overseas, concentrations of zinc in milk from various dairy animals are reported as cow 4 mg/L, buffalo 0.2-0.3 mg/L, goat 3-6 mg/L and for sheep 1-2 mg/L (Jensen, 1995).

According to the International Dairy Federation (International Dairy Federation, 1992) the zinc concentration in milk will hardly raise if zinc is added to the diet. Zinc concentrations of 4.2, 6.7 and 8.0 mg/l in milk are reported at concentrations of 44, 372 and 692 mg zinc/kg dw in the feed. Further raising of the zinc concentration in the food up to 1279 mg/kg dw did not lead to a further raise in the concentration in milk (Van Hooft, 1995).

5.3.5 Risk characterisation of micronutrients

An evaluation of iodine, selenium and zinc was performed to establish whether there are potential public health and safety risks with high level consumption of these micronutrients present in dairy products.

In addition to having an essential role, there is a potential for over consumption of some micronutrients, thereby resulting in a public health and safety risk.

5.3.5.1 Iodine

Most high consumer population groups, except for the 2-3 year olds (105% UL) are estimated to have intakes of iodine below the UL (FSANZ, 2005a). Due to the use of 24-hour dietary survey data, which tends to over-estimate habitual food consumption amounts for high consumers, it is likely that the 95th percentile dietary intake is an over-estimate. Overall, the potential to exceed the UL, even for 2-3 year olds, is considered to be low.

Comparison of estimated intakes with the UL is not appropriate when considering the health risk for individuals with thyroid disorders or a long history of iodine deficiency, as typically they respond adversely to levels of intake that fall below the UL and, in some cases, at levels that approximate normal dietary intakes. Such individuals may therefore potentially be at risk even from natural fluctuations in the iodine levels in foods.

In conclusion, dairy products contribute significantly to the intake of iodine. The current levels of iodine in dairy products do not raise public health and safety concerns.

5.3.5.2 Selenium

While selenium derived from dairy products makes a contribution to selenium intake, the level of exposure was significantly below the UL. The current levels of selenium in dairy products do not raise public health and safety concerns.

5.3.5.3 Zinc

Dairy products contribute approximately 13% of the overall zinc intake in the population. Recent modelling has indicated that children in Australia, aged 2-8 years, may be exceeding the UL for zinc, both at the mean and at the 95th percentile dietary intake. For these calculations, intake from other sources, i.e. contamination from galvanised containers and intake from supplements have not been included. For adults estimated zinc intakes are below the UL (FSANZ, 2005a).

Recent modelling has indicated that children in Australia, aged 2-8 years, may be exceeding the UL for zinc, both at the mean and at the 95th percentile dietary intake. For adults estimated zinc intakes are below the UL (FSANZ, 2005a).

The UL for children was based on levels in infants that did not reveal effects of zinc on serum copper concentrations or other adverse effects. Due to a dearth of information, the UL for young infants was adjusted for older infants, children and adolescents on the basis of relative body weight.

In conclusion, while there may be a potential risk of exceeding the UL for some sub-population groups, milk is not a major contributor to the zinc intake. Therefore, it is concluded that there are no public health and safety concerns with the current levels of zinc in dairy products.

Overall conclusion

There are no public health and safety concerns with the current levels of iodine, selenium and zinc in dairy products. Milk is a source of iodine, selenium and zinc, and therefore, has a role in preventing deficiencies for these essential micronutrients in the community.

Milk and milk products contribute significantly to the intake of iodine, and in a lesser extent to the intake of selenium and zinc.

5.4 Organic Compounds

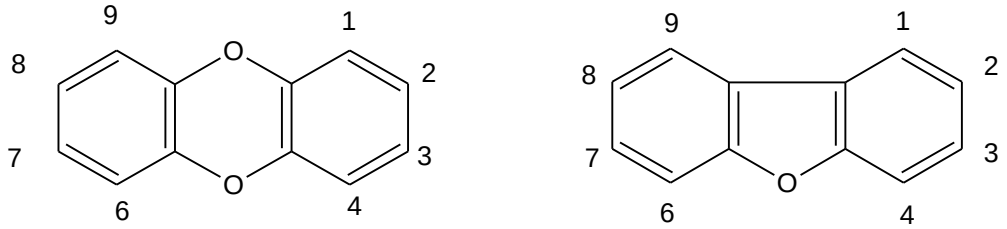
5.4.1 Dioxins and dioxin-like polychlorinated biphenyls

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, 2004a); (NDP, 2004). The Code does not contain an ML for dioxins.

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 (NDP, 2004).



Dibenzo-*p*-dioxin

Dibenzofuran

Figure 3: 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 (NDP, 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 (NDP, 2004). High fat dairy produce is therefore amenable to higher dioxin levels and detection in milk therefore acts as a signal for potential subsequent problems due to environmental contamination.

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 PCDD/Fs and PCBs have been used previously. However, the most recent review of TEFs was that of the World Health Organisation (WHO) in 1998 (van den Berg *et al*., 1998). Under the 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 (NDP, 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 so-called 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 (NDP, 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 (NDP, 2004). IARC concluded that TCDD is carcinogenic to humans (IARC, 1997).

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

Exposure Evaluation - National Dioxin Program

The collection of milk samples for the National Dioxin Program (NDP) was co-ordinated by Dairy Australia. In this study 19 composite milk samples were analysed (Table 16). As there were isolated instances of exposure shown in the beef cattle results, it is possible that a particular dairy herd could be similarly exposed. However, it is likely that residues in milk would be lower than those detected in beef fat due to continual excretion via the milk. Furthermore, residues in milk consumed would be further reduced by dilution with milk from other herds.

Table 16: Australian data for dioxin and furan residues compared against the EU standard (NDP, 2004)

Species	EU Standard Maximum pg TEQ/g*	Mean** result from NDP study (%)	Number of samples
Beef	3	0.56 (18.6%)	109
Milk	3	0.43 (14.5%)	19
Sheep	3	0.57 (19.1%)	45

* on a fat basis

** mean results are upperbound concentrations expressed as pg TEQ/g. Values in parentheses are expressed as a percentage of the EU standard for that species or commodity.

The EU standard in EU Regulation (EC) No. 2375/2001 only refers to dioxins/furans and dioxin-like PCBs are not currently included. These results indicate that Australian levels for dioxins/furans are < 20% of the EU standard.

A summary of the mean PCDD/F concentrations for dairy products used in the dietary modelling is shown in Table 17. Individual composite sample PCDD/F and PCB results are summarised in a FSANZ Technical Report (FSANZ, 2004a).

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.

For infants aged 9 months the major contributors to PCDD/F exposure were infant formula (containing non dairy fat *i.e.* plant-derived oils) (82%) and milk and dairy products including cheese, ice cream and infant dessert (5%).

For toddlers (2-4 years), the major contributors to PCDD/F exposure were milk and dairy products including cheese and ice cream (55%). Taking the whole population (2+ years) into account, milk and dairy produce contributed 31% of the PCDD/F dietary exposure.

Table 17: Mean levels of PCDD/F in food (FSANZ, 2004a)

	Number of composite samples	PCDD/F	
		Lower bound pg/g FW	Upper bound pg/g FW
Butter	10	0.011	0.20
Milk chocolate	1	0.0029	0.044
Milk, whole	13	0.0010	0.0065

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.

Risk Characterisation

Dioxins enter the food chain when animals eat contaminated plants or inhale smoke from burning organic matter. The dioxins are then absorbed in the animal fat, increasing in concentration as they migrate up the food chain. The consumption of animal products with high fat content, including dairy products can therefore theoretically increase human exposure to dioxins.

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 (NDP, 2004). For infants aged 9 months, the mean estimated exposure to dioxins was in the range of 11.8 and 60.8 pg TEQ/kg bw/month and for all Australians aged 2 years or older, the mean upper bound monthly intake of dioxins is 15.6 pg TEQ/kg bw/month. The relatively high exposure for infants is due to their high food consumption relative to body weight. Overall, these levels are significantly below the TMI of 70 pg TEQ/kg bw/month.

Dairy products are a relatively high contributor to the total dietary exposure of the Australian population to dioxins and dioxin-like compounds, however the levels overall are well within the JECFA PTMI.

Both Australian and New Zealand milk and butter have relatively low PCDD/F concentrations in milk and butter compared to other areas of the world (Table 18); 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 (Table 19; FSANZ, 2004a).

Table 18: Comparison of mean PCDD/F concentrations in selected foods from different areas of the world (FSANZ, 2004a)

	Mean PCDD/F (pg TEQ/g lipid)						
	Australia	New Zealand[1,2]	UK	Netherlands[3]	Europe[1]	Asia[1,4]	North America[1]
	(NDP, 2004)	(MFE 1998)	(FSA 2003)	(Freijer et al 2001)	(Codex 2003)	(Codex 2003)	(Codex 2003)
Milk	0.04-0.23	0.019-0.16	0.46-0.47	0.57	0.3-2.5	0.30-1.8	0.3-0.9
Butter	0.013-0.23	0-0.095	-	0.68	-	-	-

[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.

Table 19: 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, 2004a	0.9-10.2	2.8-5.4	3.7-15.6
New Zealand[2]	(Ministry for the Environment, 1998; Ministry for the Environment, 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 person.

Conclusion

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

5.4.2 Polychlorinated biphenyls

Unlike dioxins, PCBs do not occur naturally in the environment; they are derived from man-made sources which were manufactured for approximately 50 years for use as components of insulating fluids in transformers and other electrical equipment (NDP, 2004).

The safety of polychlorinated biphenyls (PCBs) was last assessed by FSANZ in Proposal 158 – Review of the maximum permitted concentration of non-metals in food (ANZFA, 1999c).

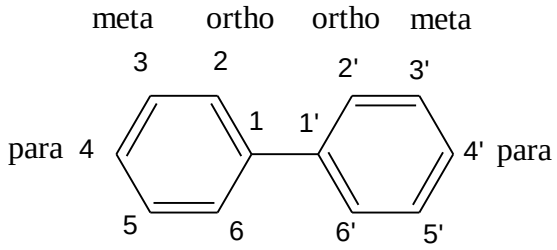


Figure 4: Structure of biphenyl

The PCBs are structurally similar to the PCDDs and PCDFs (dioxins) and include 209 congeners, from the monochloro congener through to the fully chlorinated decachloro congener; the basic aromatic nucleus is shown in Figure 4.

Like the PCDD/PCDFs, the biological effects of PCBs are very dependent on both the degree of chlorination and on the position of the chlorine atom around the aromatic nuclei (i.e. whether they are *ortho*-, *meta*-, or *para*- to the phenyl-phenyl bridge at carbon-1).

Food Regulation

As part of Proposal P158 - Review of the maximum permitted concentrations of non-metals in food – a risk management strategy that included a ML for total polychlorinated biphenyls in milk and milk products was included in Table to clause 3 – Maximum level of non-metal contaminants in food - Standard 1.4.1 – Contaminants and Natural Toxicants (Table 20). Considering the uncertainty surrounding the potential toxicity of PCB's, their persistence within the environment and the necessity to achieve low PCB levels, the ML was set to include total polychlorinated biphenyl concentrations in food i.e. including dioxin-like PCBs.

Table 20: Maximum levels of PCBs in food

Column 1	Column 2 (mg/kg)
Polychlorinated biphenyls, total	
Mammalian fat	0.2
Poultry fat	0.2
Milk and milk products	0.2
Eggs	0.2
Fish	0.5

Hazard Identification and Characterisation

Animal feeding studies, mostly using rodents, have shown that the range and severity of the toxic effects of PCBs is correlated with the PCB congener/mixture used.

The long-term toxic effects of relatively high levels of PCBs include changes in liver enzyme activity and increased liver weights in rats; adverse reproductive effects and neurotoxicity have also been observed in rodents.

The choice of a particular NOEL for human health risk assessment should be identified for the most sensitive effect in the most sensitive species. JECFA (WHO, 2000) has designated non-human primates as the species most sensitive to the toxic effects of PCBs and has assigned a NOEL of 0.04 mg/kg bw/day, based on the general toxicity of Aroclor 1242 in monkeys. However, the limitations of the available data and the toxicological differences in PCB mixtures that were used in animal feeding studies has made it difficult to establish a value for tolerable intake for humans.

Dietary Exposure

For toddlers (2-4 years), the major contributors to PCB exposure were fish (49%) and milk and dairy products (30%). Taking the whole population (2+ years) into account, milk and dairy produce contributed 11% of the PCB dietary exposure (FSANZ, 2004a). No data is available for dietary exposure to PCBs in non-breast-fed infants. Mean levels of PCBs in foods are shown in Table 21.

Table 21: Mean levels of PCBs in food (FSANZ, 2004a)

		PCB	
	Number of composite samples	Lower bound pg/g FW	Upper bound pg/g FW
Butter	10	0.017	0.070
Milk chocolate	1	0.0048	0.012
Milk, whole	13	0.0013	0.0060

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.

A small number of PCBs were included in the yearly AMRA survey from 1998 – 2003 (Table 22), and no residues were found in milk or finished products. PCBs were not detected in milk products in the Australian Market Basket Survey, or the New Zealand Total Diet Survey.

In the 2003-4 NRS survey, PCBs were analysed in cattle, sheep, goat, buffalo and camel meat and no residues were detected.

Both Australian and New Zealand milk and butter have relatively low PCB concentrations compared to other areas of the world (Table 23); bearing in mind that there are differences in analytical methodologies and calculation of the reporting of TEQs.

Table 22 Comparison of PCB, Test Results (ADASC, 2005)

Survey Year	PCBs	
	No. tested	No. >ML
1998/1999	327	0
1999/2000	189	0
2000/2001	204	0
2001/2002	112	0
2002/2003	109	0
2003/2004	77	0

Risk Characterisation

Toxicological evaluation of PCBs is complicated by many factors, the first of which is the paucity of data concerning human exposure to, and the effects of, PCBs. Much of the animal toxicity data are based on testing mixtures that contain many PCB congeners with varying degrees of chlorination and different stereochemical structures. Differences in toxicity between PCB congeners may also be associated with specific metabolites and/or their specific intermediates.

Oral exposure to PCBs is associated with adverse effects in animals; the most consistent and pronounced is the occurrence of liver tumours in rodents. However, the available human data (mainly from accidental exposures) is equivocal in respect of an association between PCBs and increased cancer mortality.

In summary, a range of surveys has indicated that PCBs are either not detected or detected at very low levels in the Australian and New Zealand food supply. The low level of dietary exposure is well below the reference health standard. It is concluded that there are no public health and safety concerns associated with residues of PCBs in dairy products.

Table 23: Comparison of mean PCB concentrations in selected foods from different areas of the world (FSANZ, 2004a)

	Mean PCBs (pg TEQ/g lipid)						
	Australia	New Zealand[1,2]	UK	Netherlands[3]	Europe[1]	Asia[1,4]	North America[1]
	(NDP, 2004)	(MFE 1998)	(FSA 2003)	(Freijer et al 2001)	(Codex 2003)	(Codex 2003)	(Codex 2003)
Milk	0.04-0.11	0.027-0.15	0.34-0.43	0.69	0.2-1.8	-	0.5
Butter	0.021-0.086	0.15-0.15	-	0.96	-	-	-

[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.

5.4.3 Organochlorines

Organochlorine pesticides are generally condensed organic compounds in which chlorine averages 60% of the molecular weight. Persistent organochlorines such as DDT, Dieldrin, Heptachlor and Hexachlorobenzene (HCB) have not been available for use in Australia since the 1970s. However, they are still present in soils where they were used for spot and broad acre pest control. Although these compounds have been de-registered for use, full risk assessments have been carried out and extraneous residue limits (ERLs) set by FSANZ for a range of food commodities that have the potential to be exposed to organochlorines during their production, including milk.

Grazing livestock can ingest soil or crops contaminated with environmentally persistent compounds such as DDT and Dieldrin and as such, these organochlorines are considered environmental contaminants. These compounds have been de-registered in Australia for many years as agricultural pesticides, but due to their persistent nature in the environment, particularly in soil, low concentrations may be identified from time to time. Extraneous residue limits (ERLs) are established to account for residue due to previous use.

Overall conclusion

Seven years of AMRA survey data have indicated that there are no environmental residues of organochlorines or PCBs in milk (500 – 1050 analyses carried out) *i.e.* there is 100% compliance with respective MRLs. Furthermore, no residues of heavy metals were found in milk over this period (112 analyses carried out).

It can therefore be concluded that dietary exposure to environmental contaminants from dairy products does not raise public health and safety concerns.

5.5 Plant, fungal and bacterial toxins

The susceptibility of stockfeed to contamination by plant, fungal and bacterial toxins will vary according to geographic location. Critical controls to ensure that stockfeed is free from toxins combines on-farm controls for pasture management (GAP), in addition to vendor declarations from suppliers of supplementary feed.

5.5.1 Classification

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. There are dual concerns with natural contaminants: they may cause detrimental effects on animal health and subsequent production losses and also, some toxins can pass the blood-milk barrier and be present in low concentrations in the milk.

Some of the more common naturally occurring toxins, their sources and an indication of whether the toxin is carried over into milk and milk products, are listed in Table 24. This report has focussed mainly on those natural toxins, which are carried over into milk and are potentially of concern to human health.

Table 24: Naturally occurring toxins of plant, fungal and bacterial origin

Type	Toxin	Pathogen	Source/Host	Carry-over	Ref.
Endogenous plant toxin	Pyrrolizidine alkaloids	[a]N/A	Forage plants and weeds (e.g. comfrey, Patterson's curse, heliotrope)	**Yes**	(Cheeke, 1995); (FSANZ, 2001c)
″	Indole alkaloids (and hordenine)	N/A	*Phalaris spp*. (e.g. canarygrass)	No	(Cheeke 1995)
″	Oxalates	N/A	Tropical grasses (e.g. bufflegrass, pangolagrass, settaria, kikuyugrass)	No	(Cheeke 1995)
″	Cyanide	N/A	Tropical forage grasses (e.g. sorghum)	No	(Cheeke 1995)
″	Photosensitising agents e.g. Steroidal saponins, hypericin	N/A	St. Johns Wort; Buckwheat and other pasture grasses	No	(Cheeke 1995)
″	Saponins, oxalates	N/A	Kikuyu grass	No	(Cheeke 1995)
″	Dicoumarol	N/A	Sweet vernal grass, sweet clover	No	(Dwyer *et al*., 2003)
″	Quinolizidine alkaloids	N/A	Lupins	No	(FSANZ, 2001a)
Bacterial toxin	Corynetoxin	*Rathayibacter toxicus*	synergy of bacterium-nematode-grass (e.g. annual ryegrass)	**Yes (limited evidence)**	(Cheeke 1995); (Edgar, 1994)
Mycotoxin	Aflatoxin	*Aspergillus sp.*	Forage and stored grains e.g. corn, sorghum, peanuts, cottonseed and cottonseed meal	**Yes**	(ANZFA, 1999a)
″	Ochratoxin	*Aspergillus sp. and Penicillium sp.*	Forage and stored grains	**Yes**	(JECFA, 2001c)
″	Fumonisin	*Fusarium sp.*	Forage and stored grains, particularly corn and sorghum.	**Yes**	(Cheeke 1995);(JECFA, 2001b)
″	Trichothecenes	*Fusarium sp*	Forage and stored grains, particularly wheat and corn	**Yes**	(Cheeke 1995)
″	Zearalenone	*Fusarium sp.*	Forage and stored grains	**Yes**	(Cheeke 1995); (EFSA 2004b)
″	Cyclopiazonic acid	*Penicillium spp., Aspergillus spp.*	Cereal grains	**Yes**	(EMAN 2005); (Dorner *et al*., 1994); (Finoli *et al*., 1999)
″	Phomopsins	*Phomopsis leptostromiformis*	Lupin	No	(FSANZ, 2001b)
″	Indole-diterpene neurotoxins	*E.g. Acremonium lolii*	Perennial ryegrass pasture	No	(Cheeke 1995)

Type	Toxin	Pathogen	Source/Host	Carry-over	Ref.
″	Sporidesmin	*Pithomyces chartarum*	None - direct infection from fungal spores	No	(Cheeke 1995)
″	Ergot alkaloids	*Claviceps purpurea*	Grain, grass	No	(EMAN 2005)
	Patulin	*Penicillium spp., Aspergillus spp., Byssochlamys spp.*	Fruit, vegetables, cereal grains and silage.	No	(EMAN 2005)
	Citrinin	*Penicillium spp., Aspergillus spp.*	Cereal grains	No	(EMAN 2005)
″	Moniliformin	*Fusarium spp.*	Cereal grains	No	(EMAN 2005)
″	Sterigmatocystin	*Aspergillus sp.*	Cereal grains	No	(EMAN 2005)
″	Paxilline and *N*-formylloline	*Neotyphodium (endophytic)*	*Echinopogon* spp – indigenous Australasian grass	?	(Miles *et al*., 1998)
Other mycotoxins [b] – *Aspergillus clavatus* and *Aspergillus clavatus* toxins, Citreoviridin; lesser-known *Fusarium* toxins e.g. beauvericin, enniatin, fusaproliferin; Griseofulvin, Nitropropionic acid; Kojic acid; Penicillic acid; *Penicillium roquefortii* toxin; Viomellein; Vioxanthin; Xanthomegnin; Waleminols. gliotoxins, mycophenolic acid, PR-toxin, penitrem A, roquefortines A, B and C, sterigmatocystin and cyclopiazonic acid [C]					(EMAN 2005)

[a] not applicable

[b] less studied mycotoxins found in animal feed and known to effect cattle health. Some toxins however can co-occur with other toxins, for example viomellein has been found in conjunction with ochratoxin A, and could possibly also be carried over into milk.

[c] these toxins have all been found to be associated with cheese spoilage. Although potential hazards associated with these toxins have been cited, in many cases lack of data on their occurrence in foods precludes a risk evaluation.

Plant Toxins

A literature review (as summarised by (Colegate *et al*., 1998)) of the transfer of plant-derived toxins to the milk of lactating grazing animals (Panter and James 1990) has identified the following important issues:

- Contaminated milk, sufficient to cause overt toxicoses in suckling young or humans, can be obtained from an asymptomatic animal.
- Physico-chemical properties of the toxins may lead to favoured distribution and concentration in milk.
- The complexity of milk (emulsified fats in an aqueous solution of protein and minerals) makes it a suitable sink for virtually any toxin that is bound to plasma proteins, freely circulating in the plasma or dissolved in blood lipids.
- Chronic, low level, repetitive exposure of animals to toxins may lead to accumulation in the milk, and may result in a chronic, low level, repetitive exposure of humans to the toxins.
- Young animals and young children may be more at risk to milk-borne, plant-associated toxins since they may experience greater exposure and may not be able to detoxify or eliminate the toxins as efficiently as adults.

- Some toxins are preferentially eliminated via the mammary gland and may be bound to milk protein or occur in the aqueous phase or milk fat.
- Modern methods of pooling and processing milk will dilute toxin concentrations but increased risk exists when the milk comes from a few animals such as on a family farm.
- Chronic damage to organs, such as the kidney or liver, as a result of ingestion of toxic plants may affect the ability of the lactating animal to detoxify the xenobiotics and thereby increase transfer via the milk.

Diseases resulting from the consumption of mycotoxins are called mycotoxicoses. In dairy cattle, mycotoxicoses may be expressed through reduced milk production, poor performance among fresh cows and increased incidence of disease. There usually is intermittent diarrhoea and, frequently, reduced or erratic feed intake. Symptoms may be wide-ranging and not specific. They might include: reduced feed intake or feed refusal; an undernourished appearance; rough hair coat; subnormal production; increased abortions or embryonic mortalities; silent heats or irregular oestrus cycles; expression of oestrus in pregnant cows; and decreased conception rates. Some of the general toxicity effects of mycotoxins are summarised in Table 25.

Table 25: Effect classification of mycotoxins found in Australia

Main Effect	Fungal source	Toxins
Hepatotoxicity	*Penicillium* spp.	Rubratoxins
		Luteoskyrin
		Cyclochlorotine
	Diaporte spp.	Phomopsins
	Aspergillus spp.	Aflatoxins
	Pithomyces spp.	Sporidesmin
Nephrotoxicity	*Penicillium* spp	Ochratoxins
		Citrinin
Neurotoxicity	*Penicillium* spp	Penitrems
		Lolitrems
		Patulin
		Citreoviridin
	Acremonium spp.	Ergopeptines
		Lolitrems
	Claviceps spp.	Ergopeptines
Oestrogenic effects	*Fusarium* spp.	Zearalenols
		Zearalenone
Cytotoxicity	*Fusarium* spp.	Trichothecenes
		Nivalenols (e.g. DON, Vomitoxin)
		T-2 toxin
		HT2 toxin
Multiple effects	*Penicillium* spp; Aspergillus spp.	Cyclopiazonic acid

5.5.2 Aflatoxins

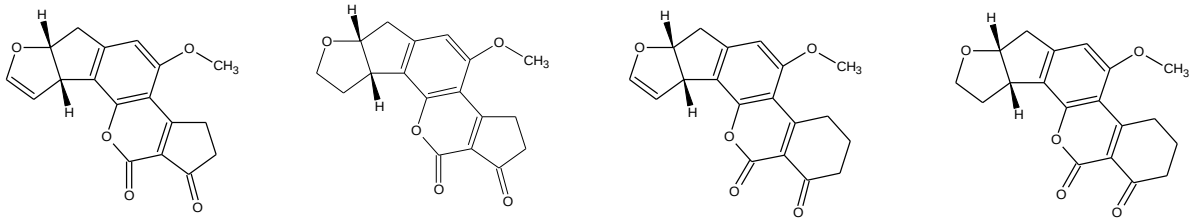
The safety of aflatoxins was last assessed by FSANZ in Proposal 158 – Review of the maximum permitted concentration of non-metals in food (ANZFA, 1999a; ANZFA, 1999b). Aflatoxins are a group of naturally occurring toxic secondary metabolites produced primarily by two species of ubiquitous Aspergillus 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. The use of peanut meal, corn or sorghum in dairy rations are regarded as particularly susceptible to aflatoxin contamination (Dr J. Pitt, personal communication.). Conditions favouring aflatoxin development include drought stressed, insect-damaged feed stored at high temperatures (25 – 32^{o}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; EFSA, 2004b). Aflatoxin dietary intake in humans mainly arises from contamination of maize and groundnuts and their products (JECFA, 1998a). The chemical structures of aflatoxins B_1, B_2, G_1 and G_2 are given in Figure 5.

Aflatoxins M1, M2 and M4 are commonly known as milk aflatoxins, and may be found in cattle, sheep or goat milk after the animal has ingested feed containing aflatoxins B1, B2 or B4 respectively (JECFA, 1998a). The milk aflatoxins are hydroxy-metabolites of aflatoxins B1, B2 and B4 respectively. Both aflatoxins M2 and M4 occur in milk at much lower concentrations compared to aflatoxins M1, and are thus considered as of less public health significance.

There is a linear relationship between the amount of aflatoxin B_1 ingested daily and the level of aflatoxins M_1 in the milk. Milk aflatoxins retain the toxic properties of the parent compound, but do not have the same potency; about 1.5% of aflatoxin is excreted as the metabolite M1 and the concentration of aflatoxins B_1 in milk is approximately 1/300 of the concentration of aflatoxins B1 in the stockfeed (IPCS, 1998).



Aflatoxin B_1 Aflatoxin B_2 Aflatoxin G_1 Aflatoxin G_2

Figure 5: 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, 1998a). 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, 1998a). 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, 1998a).

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 (JECFA, 1998a; JECFA, 1998b).

Aflatoxin M1 has toxicological properties comparable to those of aflatoxin B_1, but has a carcinogenic potency of one or two orders below that of aflatoxin B_1 (IPCS, 1998). EFSA has set a Maximum Limit (ML) for aflatoxin M1 in milk at 0.05 μg/ kg, and 0.025 μg/ kg for infant formulae, respectively, aiming to reduce human exposure to the lowest achievable level (EFSA 2004a). Codex, however have set an ML of 0.5 μg/ kg for aflatoxin M1 in whole milk. In Australia, there is no ML for aflatoxins in milk and the ALARA principle applies.

Historical data (1987 – 1992) on the presence of aflatoxin M1 in Australian milk samples (ANZFA, 1999a), was collated by The Australian Mycotoxin Data Centre (AMDC). There were ten positive samples found within 227 samples (4.4% positives), these were mainly found in spray dried milk powder (Table 26). In recent AMRA surveys (2000 – 2004) Aflatoxin M1 was not detected in milk samples (vat and tanker) or in finished products.

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; ANZFA, 1999b).

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.)

Although most of the aflatoxin levels recorded in the international literature as residues in single feed, mixed dairy concentrates and tank milk were low (<0.1 – 16 μg/kg), there have been more recent reports of higher aflatoxins levels in animal feeds originating from Europe, with levels ranging from 25 – 40 μg/kg (Vallone and Dragoni, 2005).

Table 26: Aflatoxin M1 (mg/L) in Australian milk product samples (ANZFA, 1999b)

Reporting date	Product	No. samples	No. +ve	% +ve	Range	Min.	Max.	Ave.
Sept. 1990	Milk powder	10	1	10		0.2	0.2	0.2
June 1992	Milk powder	42	5	12		0.2	0.4	0.3
June 1987	Milk	3	3	100		0.26	0.52	0.39
June 1987	Milk powder	1	1	100	0.2	1.5	1.5	1.5
various	Milk	54	0		0.2 – 0.4			
	Dried milk	73	0		0.26 – 0.52			
	Dried skim milk	10	0		1.5			
	UHT milk	25	0					
	Cheese	8	0					
	Goat's milk	1	0					
	Total	227	10	4.4	0.2 – 1.5	0	1.5	-

The carry-over rate for aflatoxins from contaminated feeds into milk of dairy cows is 1 – 2% on average, however this is considered to be considerably higher in high yielding cows. Changes in the plasma-milk barrier and the consumption of significantly higher amounts of concentrated feeds (exposed to European environmental conditions) are thought to contribute to the higher carry-over rates of aflatoxins of 6% (EFSA 2004a). Estimated concentrations of aflatoxin M1 in milk varies for different animal species. Under worst-case conditions, high yielding sheep, goats and camels could potentially have twice the carry-over of aflatoxin M1 in their milk, as compared to dairy cows and buffalo milk could contain up to four times the amount of aflatoxin M1 as cows milk (EFSA 2004a).

In the AMRA survey, aflatoxin M1 testing commenced in 2002/2003, and continued during 2003/2004. During that period, 143 samples were tested with no residue detections identified. The 2003/2004 AMRA survey included 50 samples from targeted areas as well as 39 random milk samples. There is no routine testing for other mycotoxins in the AMRA survey as it is unlikely that they are present in significant levels, due to effective management practices.

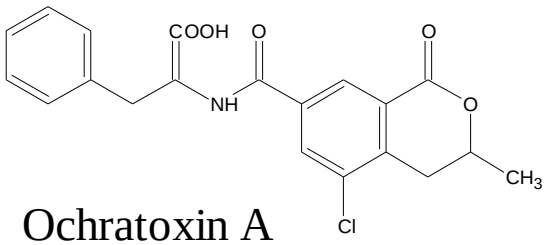
Risk characterisation

Aflatoxins are regarded as human carcinogens, the intake of which should be reduced to levels as low as reasonably achievable. While secondary exposure to aflatoxins through consumption of milk products derived from cattle fed aflatoxin-containing feed can occur, the levels found are very low.

In conclusion, there are no public health and safety concerns with the very low levels of dietary exposure to aflatoxin M1 from dairy products.

5.5.3 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). The chemical structure of ochratoxin A is given in Figure 6.



Ochratoxin A

Figure 6: Chemical structure of Ochratoxin A

Hazard Identification and Characterisation

Ochratoxin A is slowly absorbed from the gastrointestinal tract. It is distributed in a number of species via the blood, mainly to the kidneys, with lower concentrations found in liver, muscle and fat. The major metabolite of ochratoxin A in all species examined is ochratoxin α. Ochratoxin α and other minor metabolites that have been identified are all reported to be less toxic than ochratoxin A.

Ochratoxin A have been shown to be nephrotoxic in all mammalian species tested (Bakker and Pieters, 2002). The main target is the renal proximal tubule, where it exerts cytotoxic and carcinogenic effects. Significant sex and species differences in sensitivity to nephrotoxicity were evident, in the order pig>rat>mouse. Carcinogenesis was observed at doses higher than those that caused nephrotoxicity in rodents.

IARC has classified Ochratoxin A into group 2B (possibly carcinogenic to humans – sufficient evidence in animals, and inadequate data in humans) (IARC, 1993c).
JECFA recently reviewed Ochratoxin A and retained the previously established PTWI of 100 ng/kg bw per week pending results of on-going studies on the mechanisms of nephrotoxicity and carcinogenicity. JECFA concluded that the new data raised further questions about the mechanisms by which Ochratoxin A causes nephrotoxicity and renal carcinogenicity and the interdependence of these effects.

In reaching this conclusion, JECFA noted the large safety factor applied to the NOEL for nephrotoxicity in deriving the PTWI, which corresponds to a factor of 1500 applied to the NOEL for carcinogenicity in male rats, the most sensitive species and sex for this end-point (JECFA, 2001c).

Ochratoxin A is also immunotoxic and teratogenic at higher than nephrotoxic doses. Pigs, dogs and poultry are particularly sensitive to the nephrotoxicity and a NOEL has not been established in pigs and dogs. Ruminants are less sensitive due to degradation of ochratoxin A to the less toxic ochratoxin α by the rumen microflora, although sheep have a lower capacity to degrade ochratoxin A than other ruminants (EFSA 1990), (Hohler *et al*., 1999).

Accumulation of ochratoxin A occurs in blood, liver and kidney, and significantly lower residue concentrations have been found in muscle tissue, fat and milk (EFSA 1990).

In the 20th ATDS, ochratoxin A was not detected in any of the following foods: breads, biscuits, rice, oats, processed wheat bran, breakfast cereals, instant coffee, peanut butter, almonds and milk chocolate.

Risk characterisation

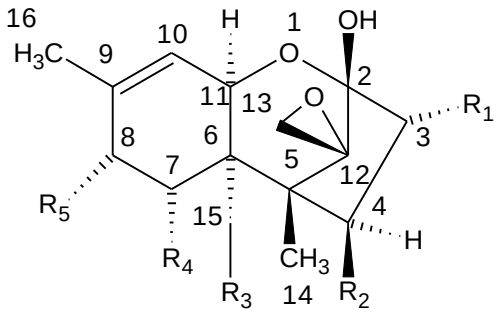
Ochratoxin A has been shown to be nephrotoxic in all mammalian species tested. JECFA established a PTWI of 100 ng/kg bw per week pending results of on-going studies on the mechanisms of nephrotoxicity and carcinogenicity. There is little evidence that humans are exposed to ochratoxin in significant amounts in most diets, however more monitoring and exposure data would be required to enable a definitive risk characterisation to be made.

In conclusion, there are no public health and safety concerns in relation to levels of dietary exposure to ochratoxin A from dairy products.

5.5.4 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. Most reports describe type A: T-2 and HT-2 toxin; type B: DON and NIV, trichothecenes and will be the focus of this review. The chemical structures of the trichothecene mycotoxins T-2, HT-2, DON and NIV are given in Figure 7.

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 produce 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 7: 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. At present, there is no evidence of human cancer cause by trichothecenes (IPCS 1990).

In an epidemiological study, reporting human food poisoning caused by infected wheat in India in 1989 which affected an estimated 50 000 people, a NOEL of 0.44 μg/kg bw was estimated. 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. However, samples were collected four months after the outbreak, and the exposure was not limited to DON but included other toxins which leads to gross uncertainties in the estimated NOEL (SCF, 1999).

Although T-2 toxin, HT-2 toxin, DON and NIV appear to cause similar effects at the biochemical and cellular level and there are similarities in toxic effects, there are also substantial differences in the spectrum of toxic effects *in vitro*. 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/day) only and confirmed the t-TDI for nivalenol (t-TDI = 0.7 μg/kg bw/day) and the combined t-TDI for T-2 toxin and HT-2 toxin (t-TDI = 0.06 μg/kg bw/day) (SCF, 2002e).

There is no data available regarding trichothecene residues in Australian dairy products. Although trichothecene toxins such as DON and T-2 can be carried- over into milk products (IPCS 1990), there is little residue data for residue levels of these toxins found in milk or milk products due to cattle eating mouldy feed.

Risk characterisation

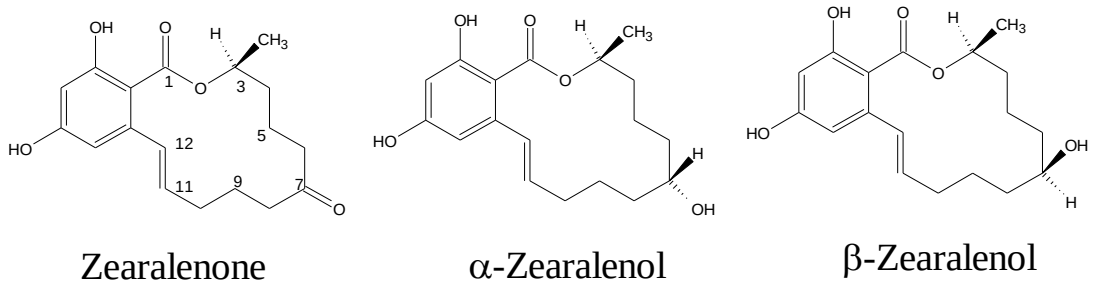
On the basis of the data available there is a possible association between trichothecene exposure and episodes of human disease expressed as gastrointestinal symptoms. Secondary exposure to trichothecene toxins through consumption of dairy products derived from cattle fed trichothecene-containing feed, presents a negligible risk to the consumer. Carry over of DON to food products of animal origin are not thought to be of concern as animals refuse feed when mycotoxins are present in high concentrations, and DON undergoes rapid metabolism and elimination in livestock species.

In conclusion, there are no public health and safety concerns in relation to levels of dietary exposure to trichothecene toxins from dairy products.

5.5.5 Zearalenone

Zearalenone is a non-steroidal estrogenic mycotoxin (SCF, 2000b) that can be produced by several field fungi including *Fusarium graminearum* (*Gibberella zeae*), *F. culmorum, F. cerealis*, *F. equiseti* and *F. semitectum*. The main metabolites of zearalenone are α-and β-zearalenol and the glucuronide conjugates of both the parent compound and its metabolites (JECFA, 2000). Zeranol residues can be differentiated by the presence or absence of zearalenone metabolites. If Zeranol occurs with other zearalenone metabolites it is more than likely due to the ingestion of *Fusarium* spp. – from infected pasture, or grain, or plant material containing zearalenone by the cattle. The chemical structures of the zearalenone (ZEA) and α- and β- zearalenol (ZOL) are given in Figure 8.

α-zearalenol has been previously assessed by JECFA as a veterinary medicine.



Zearalenone α-Zearalenol β-Zearalenol

Figure 8: 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 like decreased fertility, increased embryolethal 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 (Kuiper-Goodman *et al*., 1987; JECFA, 2000).

Pigs and sheep appear to be more sensitive than rodents (SCF, 2000a). Dairy cattle herds with low fertility were found to have higher levels of blood and urinary zearalenone and its metabolites, due to feeding on pastures with levels of about 400 ppb of the mycotoxins (Whitlow, 2002).

In humans, zearalenone has been measured in endometrial tissue from 49 women and found at a concentration of 48 ± 6.5 ng/ml tissue from 27 women with endometrial adenocarcinoma, at 170 ± 18 ng/ml in tissue from 11 women with endometrial hyperplasia, and at concentrations below the limit of detection in tissue from 11 women with normal proliferative endometrium. Zearalenone was not detected in eight samples of hyperplastic and five samples of neoplastic endometrial tissue (Tomaszewski *et al*., 1998).

Zearalenone or zearalenol was suspected to be the causative agent in an epidemic of premature thelarche in girls aged six months to eight years, which occurred in Puerto Rico between 1978 and 1981, as these compounds were detected in blood plasma. The authors reported that homogenates of locally produced meat gave strong responses in a cytosol receptor assay with rat uterus, indicating the presence of substances that bind to oestrogen receptors, although the United States Food and Drug Administration later failed to detect any of the oestrogen growth promoters used in food. The involvement of natural sources of estrogenic compounds, such as some plant metabolites and mycotoxins, has not been ruled out (SCF, 2000b). A statistically significant correlation was found between the pubertal changes and the consumption of meat products and soya-based formula, but the association explained only 50% of the investigated cases, and the authors suggested better diagnosis and reporting or some unsuspected factor accounted for the reported increase in precocious pubertal changes (Freni-Titulaer *et al*., 1996).

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).

There is no data available regarding zearalenone residues in Australian dairy products although in the 2003-4 NRS survey, Zeranol was monitored in cattle and sheep meat and no residues were detected (DAFF 2005a).

Carry-over of zearalenone, α- zearalenol and β zearalenol into dairy products can occur though and low level of detection has been reported in milk and cheese (Hagler, 1980). In the U.K., for example, zearalenone was detected in 3% of conventional retail milk samples at levels ranging from 1.2 to 5.5 μg/L (EC 2003).

Dietary exposure

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 ng/kg bw to 420 ng/kg bw/day. Bread and other cereal products were the most prominent sources of exposure (EFSA 2004b).

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

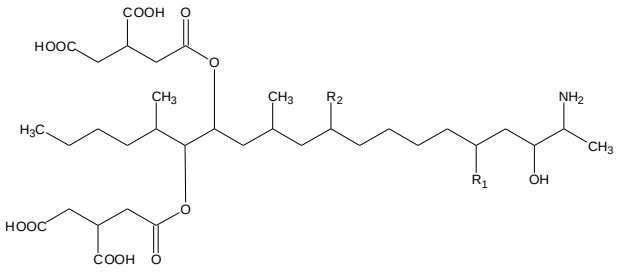
Risk characterisation

Zearalenone is a non-steroidal estrogenic mycotoxin implicated in numerous mycotoxicoses in farm animals, especially pigs. Estimated average dietary exposure internationally is below the PMTDI of 0.5 μg/kg bw/day. Susceptibility varies amongst species and limited experimental studies indicate that, after pigs, sheep are more sensitive to the adverse effects of zearalenone. Secondary exposure to zearalenone through consumption of products derived from dairy animals 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 dairy products.

5.5.6 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, 2001b). 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 9.



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

Figure 9: 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 pregnant rats and rabbits, very low concentrations of fumonisin B_1 were recovered in the uterus and placenta. No fumonisin B_1 was found in the foetuses, indicating an absence of placental transfer.

There was little evidence of significant transfer during lactation, and fumonisins do not appear to be metabolised *in vitro* or *in vivo* (JECFA, 2001a; JECFA, 2001b).

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, 2001b).

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, 2001b).

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, 2001b).

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, 2001b).

While the acute toxicity if fumonisins is low, it is the known cause of two diseases that occur in domestic animals with rapid onset: equine leukoencephalomalacia and porcine pulmonary oedema syndrome.

Both of these diseases involve disturbed sphingolipid metabolism and cardiovascular dysfunction. Overall, cattle appear to be less susceptible to fumonisins compared to other species.

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 exceptionally 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 (IPSC, 2000), in milk, fumonisin B1 was found in only very few samples (<0.006%) at levels close to 5ng/ml (Maragos and Richard, 1994) and is not significantly transferred into milk from short-term dietary exposure (Prelusky *et al*., 1996).

Dietary exposure

Maize is the only commodity that contains significant amount of fumonisins (IPCS 2000b). 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, 2001b).

Fumonisin B_1 may be found in low concentrations in dairy products but should not contribute significantly to human dietary exposure. Furthermore, fumonisins are poorly absorbed, rapidly excreted and not metabolised in animal systems.

Risk characterisation

Fumonisin B_1 is carcinogenic in mice and rats and induces fatal diseases in pigs and horses at levels of exposure that humans encounter. Fumonisin B_1 has been associated with sporadic gastrointestinal disorders in humans and, for populations whose diet is based on maize, there are correlative studies linking fumonisins and oesophageal cancer (IPCS 2000b). Secondary exposure to fumonisin B_1 through consumption of dairy products derived from dairy animals fed fumonisin B_1-containing feed is very low and represents a negligible risk to the consumer as there is little carry-over of the toxin into milk.

In conclusion, there are no public health and safety concerns in relation to levels of dietary exposure to fumonisin B1 from dairy products.

5.5.7 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 milk, cheese and butter (Dorner *et al.,* 1994).

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 Ca2+ so it could pose a particular risk to humans taking drugs such as calcium antagonists designed to carefully control calcium homeostasis (EMAN 2005).

Studies of the effect of CPA are also reported on broiler chicks. The observed toxicity may be masked or caused by other co-occurring mycotoxins of which aflatoxins and T2-toxin have been cited (EMAN 2005)and also ochratoxin A (Gentles *et al*., 1999). The induction of toxicity seen in exposure to ochratoxin alone and CPA alone indicates that these two mycotoxins express their toxicity by different mechanisms.

CPA toxicity was expressed mainly through increased relative weights of the proventriculus and increased activity of creatine kinase. The combination of ochratoxin and CPA was characterised by increased relative weights of the liver, kidney, pancreas and proventriculus; decreased concentrations of serum albumin, total protein and increased concentrations of triglycerides and uric acid (Gentles *et al.,* 1999).

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.

It has similar pharmacological properties to the anti-psychotic drugs, chlorpromazine and reserpine, in mice and rabbits. Near lethal doses of 11 to 14 mg/kg body weight induce continuous involuntary tremors and convulsions. It may be able to produce similar effects in humans (EMAN 2005).

CPA imine is a related metabolite that occurs in culture but is considered to be much less toxic than the parent compound.

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 micro g/kg/day or 700 micro g/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).

Dietary exposure

CPA has been detected in Europe at levels up to 10 mg/kg or higher in cheese, milk , stockfeed (maize, millet, peanuts, pulses, hay) and mixed feeds. 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).

When lactating ewes were administered 5 mg/kg /bw/day CPA for two days, the effect on the ewes was rapid and milk production and feed intake dropped within 24h.

After 48h, milk production had dropped to 20% and animals had increased respiration rates and body temperatures. CPA was found in the milk (236 ng/g after day 1) and concentrations rose after the second dose. CPA remained detectable in the milk for up to 9 days (Dorner *et al.,* 1994).

In an assessment of moulds isolated from the rind of Taleggio cheese, twenty-seven strain of Penicillium were isolated and all produced CPA (Finoli *et al.,* 1999). Although it the toxin can migrate to the core of the cheese, the highest concentrations were found in the rind.

Risk characterisation

The occurrence of CPA in milk and dairy produce is potentially of concern due to its high toxicity to major organs and due to its interference with the uptake and release of Ca2+; 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 very low, possibly as it occurs in the same products susceptible to aflatoxin contamination, and is therefore indirectly controlled by regulations in place for the aflatoxins More data would be required to complete a full risk characterisation of CPA.

5.5.8 Corynetoxins

Corynetoxins are a group of closely related tunicamycin-like toxins produced by pathogenic plant bacteria, *Rathayibacter toxicus (*also known as *Clavibacter toxicus*, formerly *Corynebacterium spp*.). The disease associated with corynetoxin toxicity is known as annual ryegrass toxicity (ARGT) (Edgar 1994) and is almost unique to Australia due to the specific type of grasses grown for pasture. Large areas of Western Australia and South Australia and, to an unknown extent, areas of Victoria, New South Wales and Queensland are susceptible to infection with *R. toxicus* or *Anguina spp.* or both. Corynetoxins, extracts of toxic seed heads and toxic ryegrass have proven lethal to all animal species exposed naturally, or tested, including sheep, cattle, horses, donkeys, pigs, guinea pigs, rats, mice and chickens.

There is a history of ARGT in Australia since 1955 and cattle and sheep losses have been reported in large numbers since then. Corynetoxicosis of livestock grazing on infected *Agrostis avenacea* caused Flood plain staggers on the flood plains of the Darling river in northern New Sough Wales between spring 1990 and autumn 1991. Over this period 1722 cattle, 2466 sheep and 11 horses died on 31 farms (Davis *et al*., 1995). On average, about 30,000 sheep and several hundred cattle die each year from ARGT in the wheat belt of Western Australia (Allen, 2002). Although the bulk of dairy cattle are reared on the coastal belt of WA, they would be at risk if they were fed toxic hay or toxic grain as supplement (J. Allen, personal communication).

The contamination of hay and straw by corynetoxins is a major concern for Australian hay and straw exports, and the Department of Agriculture Fisheries and Forestry have recently released information on a standard for minimising risk of corynetoxin contamination of hay and straw for export (DAFF 2005b).

Hazard Identification and Characterisation

Corynetoxins cause livestock poisoning through a relatively complex association between a grass, nematode, bacterium and a bacteriophage.

Germinating grass seedlings, including annual ryegrass (*Lolium rigidum*), annual beardgrass (*Polypogon monospeliensis*) and blown grass (*Agrostis avenaceae*) are infected with a nematode (*Anguina sp*.), which carry out their life cycle in the flower heads of the developing grasses (McKay and Ophel, 1993). The bacterium *R toxicus* may be carried by the nematode and produces toxins, during senescence of the pastures, which can then persist in the dry pasture during the spring and summer. Animals grazing such pastures may become intoxicated, characterised by a stagger, although the toxicity of the pasture decreases in autumn with new grass growth. The symptoms of ARGT in livestock closely resemble those of Bovine Spongiform Encephalitis (also known as mad-cow disease) i.e., intermittent episodes of cerebral convulsion superimposed on varying degrees of cerebella dysfunction, and often result in the death of the infected livestock. It is thought though that toxin is only produced if *R. toxicus* is infected with a bacteriophage (Ophel *et al*., 1993).

The corynetoxins are a family of at least eight separate glycolipid molecules (Vogel *et al*., 1981), which are closely related to the tunicamycin group of antibiotics in structure and function (Edgar *et al*., 1982). Structurally, corynetoxins are made up of uracil, N-acetylglucosamine, an 11 carbon sugar (tunicamine) and a fatty acid. The sugar moieties of these toxins are essential for toxicity.

The corynetoxins are potent, irreversible, transition state analogue inhibitors of *N*-acetylglucosamine-1-phosphate transferase (GPT). Since this enzyme catalyses the initial step in the biosynthesis of the dolichol-linked oligosaccharide chains destined for *N*-linking to proteins, the corynetoxins block N-linked glycoprotein synthesis (Jago *et al*., 1983) and consequently have high, general mammalian toxicity**,** and similarly inhibit peptidoglycan synthesis in the cell wall of bacteria. Reduced fibronectin levels are thought to be a cause of the breached blood-brain barrier, impaired cardiovascular function and decreased peripheral circulation and oxygen utilisation seen in corynetoxin poisoned animals (Berry *et al*., 1980). A lethal oral dose of corynetoxins for sheep, cattle or pigs is between 1 and 5 mg/kg. The primary organs of corynetoxin toxicity are the central nervous system and the vascular system.

Much information on the toxicity of the corynetoxins is derived from studies with tunicamycin, which has close structural similarity to corynetoxins and has a similar mechanism of action as the corynetoxins. Tunicamycin produces similar clinical disease in sheep and rats, and has similar toxicity. Depending on the sensitivity of the animals and on the source form of the toxin, the oral lethal dose for tunicamycin or corynetoxins varies between approximately 1 mg/kg and 5.6 mg/kg. For acute toxicity at least, sheep, cattle and pigs are all equally susceptible. The approximate lethal dose for tunicamycin or corynetoxins given by subcutaneous injection to sheep is 20-40 μg/kg. Reported lethal doses by parenteral (subcutaneous and intraperitoneal) administration for nursling rats, adult male rats and adult female rats are 110-160, 350 and 450 μg/kg, respectively (Allen, 2002).

The corynetoxins are cumulative toxins and the total lethal dose is the same whether given as a single dose or as repeated smaller doses up to 2 months apart. This cumulative effect is more obvious in sheep than rats (Jago and Culvenor, 1987).

Although the effects of large doses of corynetoxins have been described (Jago and Culvenor 1987), the effects of long term, low level exposure to these toxins in the diet or environment are unknown.

However, because the corynetoxins are cumulative in their action there is reason to suspect this type of exposure may pose a risk to human and animal health (Colegate *et al*., 1998).

In addition to being fatally toxic to animals, sheep and cattle can apparently ingest up to ¾ of a lethal dose and still appear clinically unaffected. There is a lag time of about 3 or 4 days between ingestion of a lethal dose and the onset of clinical signs. These factors can contribute to apparently normal, but intoxicated animals, being presented for slaughter (MLA, 2003).

Dietary exposure

The major areas of exposure of livestock to pasture-based contaminated feed are in the southern regions of Western Australia and South Australia. All domestic food and food product animals can potentially be exposed to corynetoxins in their feed supply. This includes pasture based feed , grains and fodder based feeds.

Since infected annual ryegrass is a common weed in grain crops, humans can be exposed, in a primary manner, to corynetoxins by inhalation of dust associated with grain harvesting, transportation and processing, or by ingestion of food products from contaminated grain, especially when a local, contaminated crop is sourced by the consumer for home processing. If an animal is primarily exposed to corynetoxins, then humans could be exposed in a secondary manner if the food products derived from the exposed animal are ingested.

Initial data (Stewart *et al*., 2004) indicates that the corynetoxin analogue, tunicamycin, can be translocated to muscle tissue in addition to liver, kidney and heart. However there was no reduction in GPT levels in the livers of rats nursing from tunicamycin-treated dams; this preliminary research indicated that corynetoxins are not carried-over to milk of lactating rats.

Risk characterisation

There have been no instances of human clinical symptoms being ascribed to exposure to corynetoxins. The corynetoxins have an affinity for cellular membranes and thus are cumulative in their action. The clinical and sub-clinical effects of long term, low level exposure to the cumulative corynetoxins are currently unknown. The limited data available indicates that carry-over of corynetoxins into milk is unlikely. Further data is required before a risk characterization can be completed.

5.5.9 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* (Patterson's curse), *Senecio spp.* (ragwort), *Symphytum* spp (comfrey). and *Crotalaria retusa* (rattleweed). The *Sympthytum* spp. is deliberately ingested 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 although plants producing PAs are uncommon in improved pastures used in dairy production.

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 MLs for pyrollizidine alkaloids in food have been established.

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 man to hepatocellular injury, cirrhosis and veno-occlusive disease.

The pyrroles react with macromolecules in the cells in which they are either formed or gain access leading to the formation of S-bound protein adducts and DNA crosslinking.

The pyrones have been shown to have mutagenic activity, mainly in *Drosophila* and many have been shown to be carcinogenic, mainly in the rat. There is no evidence of pyrollizidine alkaloid-induced cancer in humans (FSANZ, 2001c).

In laboratory and domestic animals, marked anti-mitotic activity due to the pyrones has been demonstrated but this is not a prominent feature of their toxicity in humans. The main pathological feature of this effect in animals is in the liver, and less so in other tissues.

In humans, the major toxicological effect of chronic exposure to PAs is 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. More specifically, PAs found in goats milk were shown to produce hepatoxic effects in rats (Goeger *et al*., 1982).

In relation to milk from domestic animals, it is likely that no more than about 0.1% of the ingested alkaloid base will be excreted in milk. PAs and PA N-oxides are known to be excreted in cow's milk, but due to milk bulking, it is unlikely that significant exposures would come from this source.

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. There is currently no data to indicate whether PAs occur in oilseed crops. 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.

Further research on the mechanisms of PA-induced hepatotoxicity may clarify the apparent differences in species specificity. 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. It is unlikely that significant exposure to PA would come from dairy products as dairy pasture is managed in order to exclude plants with high PA contents, and, if present, the carry-over of PAs into milk is very small. In conclusion, on this basis there are unlikely to be safety concerns in relation to dietary exposure to PAs from dairy products. However further data would assist in further characterising the public health and safety risk.

5.5.10 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). Pregnant cows have the greatest risk of giving birth to calves with crooked calf disease when the concentration of the teratogen anagyrine is highest and the cows are in the susceptible 40-75 day gestation period when ingesting the plant (Keeler R.F. *et al*., 1976). In addition, milk production is reduced in cows fed lupins, this could be partly due to a reduced true protein supply to the small intestine. Smaller studies have also indicated that *Lupinus formosus* caused clinical toxicoses in cows. In Australia, generally only 2% of dairy rations would include lupin (Dairy Australia, personal communication).

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, where the major alkaloid is sparteine. Traditional consumption of debittered lupins in Europe suggests a dose of 0.35 mg/kg/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/day (FSANZ, 2001a).

Exposure Assessment

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 milk, however there is currently no data available on the levels of lupin alkaloid in milk.

Risk Characterisation

There is no data available on potential carry-over of lupin alkaloids into milk and therefore the potential public health and safety risk cannot be characterized.

5.5.11 Phomopsins

The phomopsins are a family of mycotoxins produced by the fungus *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<6µg to 360µg/kg and levels as high as 4522µg/kg in seed have also been detected. However, the overall dietary exposure of dairy cattle to lupins is 2% of their total diet.

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 and milk. 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 excreted in milk it is unlikely to be a risk to public health and safety as lupins are not a major feed source for dairy cattle.

5.5.12 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 scelrotia, are derivatives of lysergic acid and fall into three groups, ergotamine, ergotaminine and clavines (EFSA 1990).

The ML for ergot is set at 500 mg/kg in cereal grains.

Hazard Identification and Characterisation

Ergotism in man is relatively uncommon 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). Ergot infection of grains, such as sorghum, has been found to reduce milk production; for example, cattle fed infected grain at 1% concentrations reduced milk yield by 30% after 5 weeks. The suggested maximum tolerable sclerote levels in dairy cow feed (whole diet) is 0.3% as these concentrations did not affect milk, if cows were on a full grain ration, the limit has to be reduced to 0.1% sclerotes (DPI 2005).

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. In Europe, rye bread has often been linked to outbreaks of ergotism. Ergot alkaloids are not transferred to the milk of cows consuming ergot (EMAN 2005).

Risk characterisation

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

5.6 Water as a source of chemical contaminants

As part of the on-going food safety programme established by the Australian dairy industry, an investigation by the National Milk Harvesting Centre was commissioned to determine if the use of water on dairy farms posed any risk to the food safety of milk (DPI, 2003). This process was based on the water safety plan suggested in the WHO's guidelines for drinking water quality (draft 3rd edition).

All water-milk contact pathways had low risk scores and the pathway with the greatest risk to the food safety of milk was milking plant flush with a risk score of 6.3 from a maximum possible score of 125 (~5%).

Using a complex model that utilises the specific biotransfer factors for each potentially contaminating chemical the maximum limit for chemical contamination from contact with water was calculated as between zero and 1.2%. However, the presence of contaminants at these levels would be undetectable.

Mercury and the organic solvent 1,2-dichlorobenzene (1,2- DCB) were identified as having the greatest potential contribution to contamination of milk through water contamination. Natural release of mercury into drinking water is extremely low, but contamination can result from industrial emission or spills.

Mercury has been estimated to be present at in extremely low amounts below the detectable limit of 0.0001 mg/L (NHMRC 2004) in milk through water contamination. 1,2-DCB is used primarily as a chemical intermediate for dyestuffs and pesticides, however it has not been found in Australian drinking water; when farming is situated adjacent to industrial areas its presence could arise from inadvertent spills, atmospheric deposition or by contact with contaminated soils, as has been found overseas.

Water food safety hazards would be higher resulting from industrial/manufacturing use, however, Dairy primary production is not in vicinity of industrial areas, and if water does become contaminated e.g. from a chemical spill, this will be highly visible (e.g. fish kill) and entry of the water into dairy processing would be averted.

Algal blooms in waterways may also be a source of toxins, for example, microcystin, cylindrospermopsin and saxitoxin (Briand and Humbert, 2003). Dramatic intoxification events have occurred in Australia due to toxins released from algal blooms, for example in 1992, 10,000 livestock died along the Darling river from a massive bloom of the Neurotoxic cyanobacteria, *Anabaena circinalis* (Falconer, 1998).

Whilst algal toxins pose a problem for cattle, there is no evidence that the microcystin-LR toxin is carried-over to milk (Feitz A.J. *et al*., 2002) and therefore is of negligible risk to the consumer. There is no data available regarding the carry-over of other algal toxins into milk. Saxitoxin is associated with paralytic shellfish poisoning and is only regulated by FSANZ with regard to bivalve molluscs (under Standard 1.4.1).

In conclusion, no food safety risks were identified with the use of water in dairy farms.

5.7 Miscellaneous

5.7.1 Radionuclides

Australia, in common with all countries in the world, has received fallout from atmospheric nuclear weapons tests conducted by various nations.

Generally fallout has been substantially less in Australia than for countries in the northern hemisphere but there have been small contributions from French atmospheric tests in the Pacific in the 1970s and from British tests in Australia in the 1950s.

The Australian Radiation Protection and Nuclear Safety Agency (ARPANZA) have issued a statement confirming the radiation-safe status of foodstuffs, including milk and milk products, in Australia[34] and AQIS routinely monitors dairy products for the presence of Caesium and Strontium for export certification.

Routine fall-out monitoring has been undertaken in all Australian states since the mid 1950s and detected minimal levels of radioactivity from nuclear testing in the northern hemisphere. During the British testing in Australia extensive monitoring was undertaken. Following cessation of French atmospheric nuclear weapons in the Pacific in 1974, fallout deposition decreased rapidly until the present, where levels are at or below the minimum detectable in air. Monitoring since the Chernobyl accident showed no increase in fallout deposits and all indications are that essentially no fallout from Chernobyl occurred in Australia.

[34] (www. arpanza.gov.au).

Extensive testing since the Chernobyl accident of many agricultural products (inducing foodstuffs) exported from Australia confirms that the radioactivity levels are negligible and significantly less than the 10 Becquerels per kg measured as Caesium 134 and Caesium 137. ARPANSA routinely analyses radioactivity in a range of food products and they are found to be effectively free of any radionuclide contamination

In 1989, Codex established guidelines for radionuclides in foods following accidental nuclear contamination for use in international trade(Codex 1989). In the event of such a nuclear accident occurring, Codex has prescribed a list of agricultural and semi-natural countermeasures. The International Commission on Radiological Protection has also established intervention levels for different foods; this is calculated using the reference level of dose for an accident (5 mSv) as a function of the mass of food consumed (kg) and the dose per unit intake factor (Sv/kg). In dairy produce (milk and cream), the intervention levels for adults of isotopes of strontium (^{90}Sr), iodine (^{131}I), alpha emitting isotopes of Plutonium (Pu) and trans Pu-elements, and all other radionuclides of half-life >10 days, notably ^{134}Cs and ^{137}Cs, are 125, 500, 20 and 1000 Bq/kg respectively (Pates 2000). Due to the sensitivity of infants, separate guidelines have been established; the intervention values for ^{90}Sr, ^{131} I, and ^{136} Cs are 160, 1600 and 1800 Bq/L respectively (WHO 1988).

In conclusion, there are no public health and safety concerns associated with dietary exposure to radionuclides from the consumption of milk and milk products. The occurrence of an incident in a country with active radionuclear industries would influence this assessment.

5.7.2 Development of antimicrobial resistance

The development of antimicrobial resistance is a relatively recent problem, which impinges on animal and human health as well as antibiotic usage patterns.

Antibiotics kill most, if not all, of the susceptible bacteria, but may leave behind – or select, in biological terms –bacteria that have developed resistance, which can then multiply and thrive. Pathogenic bacteria that were formerly susceptible to an antibiotic can develop resistance through changes in their genetic material.

These changes can include the transfer of DNA from resistant bacteria, as well as spontaneous changes, or mutations, in a bacterium's own DNA. The DNA coding for antimicrobial resistance (AMR) can be located on either the chromosome or plasmid of a bacterium. Plasmid-based resistance is transferred more readily than chromosomal-based resistance.

Once acquired, genetically determined AMR is passed on to future generation and sometimes to other bacterial species. The dose of antimicrobial and length of time bacteria are exposed to the antimicrobial are factors affecting whether the resistant bacteria population will dominate. Low doses of antimicrobials administered over long periods of time to large groups of animals, such as doses used for growth promotion in animals, favour the emergence of resistant bacteria.

The prophylactic use of antimicrobials (i.e. medications administered for lengthy periods, associated with claims of improved feed efficiency and growth promotion) has been a cause for concern regarding the induction of AMR.

Through the activities of the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) and the Expert Advisory Group on Antimicrobial Resistance (EAGAR) (see Appendix 12), the registration of several antibiotics, have been withdrawn, or are under review. The streptogramin, Virginiamycin is used therapeutically in feed premix for cattle for the treatment of acidosis.

However, the labelling instructions have recently been revised for dairy cattle usage by the APVMA (APVMA 2003b). No other antimicrobials are registered for in-feed administration for dairy cattle.

AMR and dairy pathogens

In addition to many international reports of AMR as a result of the treatment of mastitis (for example, (Kirk *et al*., 2005), (Makovec and Ruegg, 2003);(Wallmann *et al*., 2003)), the incidence of AMR through antibiotic usage in the Australian dairy industry has been monitored in Queensland since 1999 (Stephens 2003). Table 8 detail results collated by the Toowoomba Veterinary Laboratory between 1999 and 2000. In samples taken from bovine mastitic milk, one third of *S. aureus* isolates were found to be resistant to ampicillin and penicillin, whilst a small number were resistant to novobiocin (Table 27). Over the past three years, there have not been significant increases in antimicrobial resistance and AMR for tetracycline, ampicillin and penicillin has plateaued (C. Stephens, personal communication).

The antibiotics detailed in Table 27 with high and/or multiple AMRs to dairy pathogens, are considered to be of low importance according to the EAGER Importance Rating (EAGAR 2003).

In conclusion, there is no evidence that the use of antimicrobials in the dairy industry contributes significantly to the development of AMR.

Table 27: Antibiotic susceptibility testing of *Staphylococcus aureus* isolated from bovine mastitic milk between 1999 and 2001 (Stephens 2003)

Antibiotic	Amp10	CXM30	DA2	N30	NV30	OB5	P10	TE30
Number of strains tested	121	107	121	121	120	121	121	121
Number of strains resistant	40	0	0	0	3	0	40	0
Strains resistant %	33.1	0	0	0	2.5	0	33.1	0

AMP10 = ampicillin (10μg)
CXM30 = cefuroime (30 μg)
DA2 = clindamycin (2 μg)
N30 = neomycin (30 μg); novobiocin (30μg)
OB5 = cloxacillin (5μg)
P10 = penicillin (10 i.u.)
TE30 = tetracycline (30 μg)

5.7.3 Sanitisers and cleaning agents

Milk handling and processing results in milk soils and deposits which comprise mainly of minerals, lipids, carbohydrates and proteins. In addition, other potential contaminants in

milk-handling equipment include dust, micro-organisms and lubricants. Thus, cleaning and disinfection are critical aspects of GMP in the dairy industry. Sanitisers and cleaning agents are regulated as Agvet chemicals by the APVMA.

Sanitising reduces micro-organisms to acceptably low numbers, unlike sterilisation, which destroys all microbial life. Sanitisers are applied to surfaces that have already been cleaned in order to kill micro-organisms that have survived the cleaning and/or equipment storage process. Steam, hot water and chemical sanitisation can be used in dairy plants and chemical sanitisers are commonly circulated through milk-handling equipment (Reinemann, 2003).

Dairy cleaning agents may be acidic or alkali compounds. The primary function of acidic compounds is to dissolve inorganic (mineral) deposits, while alkali compounds are used primarily to dissolve organic deposits (fat and protein). Other constituents are added to amplify the acid/alkali removal processes, for example, chlorine is often added to alkaline detergents as a peptising agent to aid in protein removal and to improve the rinse-ability of the detergent. Cleaning in place chemicals typically are caustic soda (at 0.8% strength) and nitric acid (0.6% strength).

A typical washing cycle of milking equipment and tankers consists of the following steps: immediate pre-rinse with clean cold water, a hot detergent wash using a caustic soda-based product , a second cold wash to remover all traces of detergent and finally a sanitising rinse with peracetic acid or hypochlorite, may be used.

The most commonly used disinfectants are chlorine-containing compounds, such as chlorhexidine and hypochlorite, as well as quaternary ammonium compounds (e.g. benzalkonium chloride) and hydrogen peroxide. Iodophors are infrequently used in Australia nowadays (see Section 5.3.2).

Sanitisers can also potentially cause post-milking contamination of milk. However, residues of detergents and disinfectants/sanitisers in milk on the farm and at the dairy plant level are prevented by following HACCP monitoring programmes which ensure cleaning, disinfection, draining and rinsing procedures are carried out optimally. However, sanitizer contamination may potentially occur in milk and milk products at very low concentrations as indirect and incidental food contaminants (FSANZ 2005).

6. Potential risks from processing activities post farm gate

6.1 Biogenic amines

Biogenic amines (BAs) are low molecular weight organic bases, which result from the amino acid decarboxylase activity of micro-organisms (Leuschner and Hammes, 1998); (Stratton *et al*., 1991). In cheese, BAs are produced during ripening as the casein is slowly degraded by proteolysis. Biogenic amines are classified as: aromatic biogenic amines (octopamine, dopamine, tyramine, serotonin, histamine, β-phenylethylamine and tryptamine); diamines (putrescine and cadaverine); and polyamines (agmatine, spemidine, and spermine). The characteristic structures of common BAs are shown in Figure 10.

The European Union, the U.S. FDA and several other countries have set regulatory levels in the range of 50 – 200 mg histamine/kg fish (EU, 2005); (Fletcher *et al*., 1998). In Australia, the Code regulates histamine levels in fish and fish products, and the level of histamine must not exceed 200 mg/kg.

Little is known regarding the toxicological dose of other BAs; for tyramine and phenylethylamine, upper limits of 100-800 mg/kg and 30 mg/kg have been set (Scheurer and Rödel, 1995). In rats, the no-observed adverse effect level for tyramine, putrescine and cadaverine was 180 mg/kg of body weight (Til *et al*., 1995).

Although cheese may contain exceptionally high levels of histamine and other BAs, (>2000 mg/kg), tolerance limits have not been set.

Hazard Identification and Characterisation

BAs occur in a wide variety of foods, such as fish, meat and cheese products, wine and other fermented foods (Izquierdo-Pulido *et al*., 1997). Amine production has been associated with protective mechanisms of micro-organisms against acidic environments (Vanderkerckove, 1977) however human health problems may result from the ingestion of foods containing relatively high levels of certain BAs (Ekici *et al*., 2002); (Sancak *et al*., 2005)).

For example, "cheese syndrome" and histamine intoxication are related to increased tyramine and histamine levels respectively.

The adverse effects of these BAs include nausea, respiratory distress, heart palpitations, headache, hyper or hypotension and hypertensive crises due to the interaction with monoaminoxidase inhibitor drugs (MAOI) (Gonzalez de Llano *et al*., 1998), or in individuals with genetic or acquired diaminoxidase deficiency. These reactions can be potentiated by other BAs, such as putrescine, cadaverine, spermine and spermidine (Stratton *et al.,* 1991). Furthermore, in healthy individuals, the diamines, putrescine or cadaverine are not considered to be toxic, although they can potentiate the toxicity of histamine (Bardocz, 1995).

After fish, cheese is the second most commonly implicated food associated with histamine poisoning (Pinho *et al*., 2004). Most of the cases in which large amounts of amines are produced in cheeses have been attributed to lactic acid bacteria and E*nterobacteriaceae* (often used in starter cultures), with decarboxylating activity (Joosten and Northolt, 1987);(Sumner *et al*., 1985). In dairy products, there is a differential distribution and range of concentrations of BAs according to the type and source of that product (Table 28).

Ripened cheeses consistently show the highest levels of BAs, particularly tyramine, cadaverine and putrescine (e.g. maximum level of 611.7 mg/kg putrescine), whereas milk, curd, whey and unripened cheeses had no detectable BAs in many cases, regardless of whether goat or cow milk was tested. BA levels in rennet however were higher (e.g. 69.3 mg/kg tyramine) (Novella-Rodriguez *et al*., 2000); (Novella-Rodriguez *et al*., 2002).

Food poisoning, as a result of biogenic amine production, in particular histamine, is relatively well documented in fish (as summarised by (Lehane and Olley, 1999); OzFoodNet working group, 2002 and 2004; (ESR, 2001)), however there is only sparse mention made of outbreaks of food poisoning specifically due to BAs in dairy products (Sumner *et al.,* 1985); (Sancak *et al.,* 2005).

However, outbreaks of histamine-related food poisoning due to cheese have occurred in New Zealand. The incidences involving cheeses include Swiss, Cheddar, Gruyere and Cheshire cheese. Of the six cases of dairy-related histamine poisoning, the histamine content was 187 mg/100g, and was found to be due to control point failure, i.e. abnormally long storage period with possible incorrect temperature stability ((ESR, 2001)).

Recent research using double-blind placebo testing and open food challenges have shown that biogenic amines present in many cheeses can be a significant trigger of food intolerance reactions amongst patients presenting with symptoms such as urticaria, headaches and gastrointestinal problems (A.Swain and R. Loblay, Allergy Unit, RPA Hospital, Sydney, personal communication).

Dietary exposure

Different cheese types show widely varying BA concentrations, with higher levels for harder, mature cheeses (Table 30; (Novella-Rodriguez *et al.,* 2002); (Fernández-García *et al*., 2005)). This is a reflection of the degree of proteolysis and subsequent free amino acid levels (Sumner *et al.,* 1985), water activity, pH, NaCl concentration and microbiological profile (Novella-Rodriguez *et al.,* 2002); (Pinho *et al.,* 2004); (Galgano *et al*., 2001).

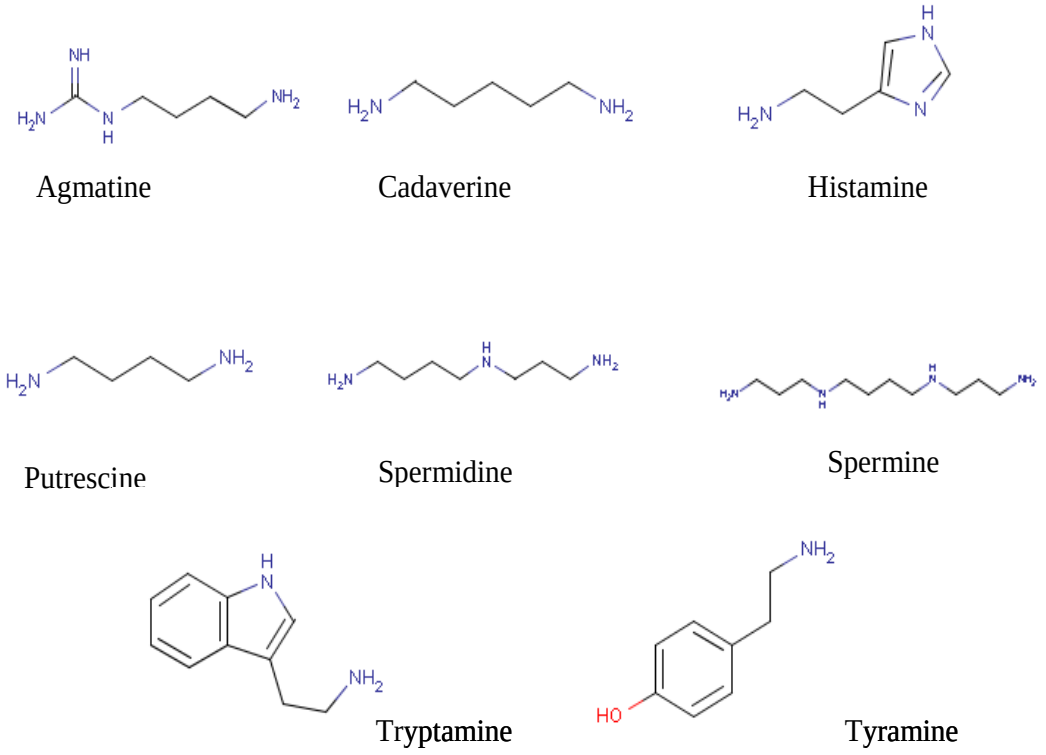


Figure 10: Chemical Structures of some biogenic amines detected in dairy products

Cheeses with comparable microbiological profiles also differ greatly in their BA concentrations (Schneller *et al*., 1997). In addition, the concentration of different BAs varies in different portions of the cheese; for example, at day 18 of ripening, cadaverine concentration is much higher in the rind of Camembert cheese than in the centre of the cheese (Engel *et al*., 2005).

In a comparison of raw-milk vs. pasteurised-milk semi-soft cheeses, the BA profile was also vastly different (Schneller *et al.,* 1997). Semi-soft cheeses produced from pasteurised milk showed much lower total BA concentrations compared to semi-soft cheeses made from raw milk (51 – 1096 mg/kg compared to 1011 – 3133 mg/kg).

The highest histamine concentration was found in a raw milk cheese (573 mg/kg); the highest total BA concentration (4817 mg/kg) was also detected in a raw-milk cheese that had been stored for 36h.

It has been reported that in order to elicit any symptoms of toxicity, according to the health and age of the individual, ingestion of 70–1000 mg of histamine in a single meal is necessary (Taylor *et al*., 1982). It is further assumed that an intake of 100 mg histamine will usually evoke poisoning symptoms (Joosten, 1988).

Table 28: Biogenic amine content (mg/kg) in different commercial dairy products[1] (adapted from (Novella-Rodriguez *et al.*, 2000)

Biogenic amine	Milk [1]	Yoghurt[1]	Unripened cheese [1]	Ripened cheese[1]	Pasteurised milk[2]	Rennet[2]	Curd[2]	Whey[2]
TY[3]	nd	nd	nd – 0.51	nd – 241.9	nd	69.3	2.0	0.65
PU	nd	nd	nd – 1.43	nd – 611.7	nd	37.07	0.89	0.31
CA	nd	nd – 0.27	nd – 1.49	4.2 – 215.3	0.11	48.98	0.67	0.22
HI	nd	nd	nd	2.21 – 163.6	nd	10.47	0.98	0.28
TR	nd	nd	nd	nd – 45.1	nd	4.6	1.32	nd
PHE	nd	nd	nd	nd – 29.0	nd	5.16	nd	nd
AG	nd – 0.18	nd – 0.39	nd	nd – 22.0	nd	2.43	0.28	0.21
SD	0.16 – 0.18	nd – 0.43	0.39 – 0.82	nd – 43.0	0.35	4.38	1.77	0.24
SM	nd	nd – 0.34	nd – 1.12	nd – 18.7-	0.96	4.83	2.23	nd

[1] samples were taken from Spanish retail stores and represented different commercial brands

2 goats milk-derived products

[3]. TY = tyramine, PU = putrescine, CA = cadaverine, HI = histamine, TR = tryptamine, PHE = β-phenylethylamine, AG = agmatine, SD = spermidine, SM = spermine

[3] range (minimum – maximum) nd= not detected

Table 29: Biogenic amine contents of different cheeses

Cheese	Histamine (mg/kg)[3]	Tyramine (mg/kg)	Putrescine (mg/kg)	Cadaverine (mg/kg)	Reference
Traditional Turkish cheeses[1]	0 - 2180	-	-	-	(Ekici *et al*., 2002); (Sancak *et al.,* 2005)
Cheddar (mild)	1 – 108	-	-	-	(Antila *et al*., 1984)
Cheddar and other hard cheeses e.g. Emmenthal[2]	352 - 1720	23 - 520	18 - 254	21 - 254	(Aygün *et al*., 1999)
Swiss	nd – 2500	-	-	-	(Antila *et al.,* 1984)
Hispanico (hard Spanish cheese)	20	103 - 191	-	-	(Fernández-García *et al.,* 2005)
Roquefort and Blue	t - 409	-	-	-	(Antila *et al.,* 1984)
Soft cheeses (e.g. Camembert, Romadur)[2]	4 - 296	4 - 324	5 - 441	5 - 635	(Aygün *et al.,* 1999)
Edam and other semi-hard cheeses e.g. Gouda[2]	4 - 122	7 - 220	7 - 282	7 - 80	(Aygün *et al.,* 1999)
Edam (fresh)	4.0	-	-	-	(Antila *et al.,* 1984)
Edam (ripened).	1.7	-	-	-	(Antila *et al.,* 1984)
Montasio (semi-hard Italian cheese)	5.5 – 378.1	35 – 373.5	2.0 – 286.5	0.3 – 30.2	(Innocente and D'Agostin, 2002)
Cottage	nd	-	-	-	(Antila *et al.,* 1984)1984.

[1] Turkish cheeses included Beyaz, Kasar, Tulum, Civil and Otlu

[2.] cheese samples purchased from food shops in SE Germany; hard cheeses (n=31); semi-hard cheeses (n=14) and soft cheese (n=5).

[3] range (minimum – maximum)

t = trace; nd = not detected; - = no data

Exposure to biogenic amines has not been investigated by FSANZ. Exposure to BAs from dairy products is most likely to be through eating hard, mature cheeses (Table 29), which comprise approximately 0.1 – 0.27% of the total diet up to the age of eighteen, and 0.05 – 0.07% of the adult diet (FSANZ, 2003).

Raw milk cheeses are also a source of BAs, but currently there is no information regarding exposure patterns in Australia.

Histamine is only monitored on a random basis in imported fish products. There was a recent incident related to tinned anchovies requiring a food recall in Australia; higher than acceptable levels of histamines were found in the product.

Risk Characterisation

Under certain conditions biogenic amines may be present in mature cheeses in high enough concentrations to induce toxic symptoms . However, the overall importance of dietary BAs as a potential human health risk is still somewhat controversial. For example, the production of BAs may be confused with allergenic symptoms too as the clinical symptoms of such pseudoallergenic reactions are indistinguishable from IgE-mediated allergenic reactions (Melnick *et al*., 1997). This may well lead to the under-reporting of dairy-related food poisoning due to biogenic amine production.

As a general conclusion, the levels of BAs in dairy products are safe for most people but there may be potential problems for high consumers and for some individuals due to intolerances induced by BAs. Finally, a complete risk characterisation cannot be carried out due to poor understanding of hazard characterisation and lack of data regarding exposure levels.

6.2 Fungal by-products

The public health and safety aspects of food-borne bacterial toxins, such as enterotoxins, Shiga toxins and Veroxytotoxins, have been addressed in the Microbiological Risk Profile. Fungal toxins such as gliotoxins, mycophenolic acid, PR-toxin, penitrem A, roquefortines, sterigmatocystin and cyclopiazonic acid (see section 3.2.3.1), which would most likely have originated from infected stockfeed or other environmental sources (i.e. water or air), have also been found in the moulds associated with cheese. A recent study on 122 cheese samples from goat and sheep milks, produced in Southern Italy, revealed high levels (44.3%) of contamination with potentially toxigenic species of *Penicillium*, *Aspergillus* and *Fusarium*, despite there being no superficial (sensory) signs of contamination. The most contaminated cheeses were the medium and long ripened samples (46.3% and 32.2% respectively), and the industrial cheeses (59.1%). The artisan cheeses were the least contaminated (26.8%) (Montagna *et al*., 2004). Although potential hazards associated with these toxins have been cited, in many cases lack of data on their occurrence in foods precludes a risk evaluation.

Toxins produced by *Penicillium roqueforti*

Penicillium roqueforti is a common saprophytic fungus that is widespread in nature and can be isolated from soil, decaying organic substances and plant parts. It is mainly used in the production of blue cheeses, such as Roquefort, Stilton and other blue cheeses. The U.S. Environmental Protection Agency has produced a final risk assessment on one of the key fungal toxins found in cheese, *P. Roqueforti*, which is summarised below (US EPA 2005).

There is considerable evidence to indicate that most strains of *P. roqueforti* are capable of producing harmful secondary metabolites (alkaloids and other mycotoxins) under certain growth conditions (Peberdy, 1985);(Sharpell, Jr., 1985). These mycotoxins include isofumigaclavin C, penicillic acid, PR toxin, patulin, botryodiploidin and roquefortine. The effects noted with ingestion of these mycotoxins are mutagenesis and tumorigenesis as well as extensive liver, kidney and nerve damage. Although there is a lack of documented cases of human toxicity, studies have shown that in the laboratory industrial strains of *P. roqueforti* can produce mycotoxins (Wei *et al*., 1985; Betina, 1989). However, the endpoints that are noted and the doses at which the effects are observed frequently are based on LD50 studies and omit references to No Observable Effect Level (NOEL) dosages (US EPA 2005). Amongst the toxins produced by *P. roqueforti,* roquefortine, PR toxin and mycophenolic acid are most commonly found in cheeses and subsequently more complete toxicological studies have been carried out on them.

Roquefortine

Hazard identification and characterisation

Roquefortine is an indole mycotoxin. It is produced by *P. roqueforti* and some other *Penicillium* species, namely *P. notatum*, *P. oxalicum*, *P. communi*, *P. corymbiferum*, *P. expansium* and *P. urticae* (Scott, 1984); (Arnold *et al*., 1987) reported an LD50 of 169 mg/kg in male and 184 mg/kg in female CR57 mice and 189 mg/kg in male and 184 mg/kg in female Swiss Webster mice.

Schoch (Schoch *et al*., 1984) conducted mutagenicity studies by the Ames test on six strains of *P. roqueforti* used commercially for the production of mould ripened cheese. Neither the fungus or roquefortine showed any mutagenic activity by the Ames test (Frank *et al*., 1977; Schoch *et al.,* 1984) fed a suspension of *P. roqueforti* to rats by gavage over their life span and showed that there was no evidence of a possible carcinogenic effect.

Dietary exposure

Low concentrations of roquefortine C were found in Roquefort type blue cheese by Ohmomo (Ohmomo *et al*., 1977) and Scott and Kennedy (Scott and Kennedy, 1976) found concentrations of roquefortine up to 6.8 mg/kg in samples of market blue cheese. Ware et al. (Ware *et al*., 1980) reported average levels of 0.42 mg/kg of roquefortine in 12 samples of blue cheese and of 0.045 mg/kg in two samples of blue cheese dressing. Roquefortine seems to be produced by most strains of *P. roqueforti* isolated from blue cheese or used as cheese starters (Scott *et al*., 1977).

Risk characterisation

It is unlikely that blue cheese is a potential acute human health hazard given the amounts of roquefortine present.

PR Toxin

Hazard identification and characterisation

PR toxin is one of the most acutely toxic metabolites known to be formed by *P. roqueforti* (Scott, 1981). The oral median lethal dose was 115 mg/kg. Within 10 minutes of an oral dose of about 10 mg (160 mg/kg) animals experienced breathing difficulties which persisted to death (Wei *et al*., 1973). Oral doses above about 130 to 160 mg/kg body weight were fatal to 60g rats in 36 hours or less.

Gross pathology consisted of swollen, gas filled stomach and intestines, while histological changes included congestion and oedema of lung, brains and kidney with degenerative changes in liver and kidney and haemorrhage in the kidney as well.

Chen et al. (Chen *et al*., 1982) studied the toxic effects of PR toxin in a range of animals. Toxic effects in mice and rats included abdominal writhing, decrease of motor activity and respiration rate, weakness of the hind leg and ataxia. It was concluded that PR toxin produced acute toxic effects in animals via an increase of capillary permeability and direct damage to lungs, heart, liver, and kidneys. Despite clear toxicological effects of ip introduced PR toxin, rats administered 0.5 mg PR toxin orally procapite/prodic for two months showed no visible effect. Mutagenicity of PR Toxin was demonstrated by Ueno et al. (Ueno and Ueno, 1978). Polonelli et al. (Polonelli *et al*., 1982) carried out preliminary studies on possible carcinogenic effects of PR toxin in rats, however the results were inconclusive.

The acute toxicities of the PR derivatives were considerably lower than that of the parent compound (Scott and Kanhere, 1979). They conclude that both PR toxin and PR imine are unstable in blue cheese and believe that the agents responsible for destruction of PR toxin formed during ripening of the blue cheese are most likely amino compounds. PR toxin enters into reactions involving its aldehyde function to form crosslinks between DNA and protein (Moulé *et al*., 1980). It also inhibits in vitro transcriptional capacity of nuclei isolated from the liver of male Wistar rats when the compound is administered in vivo. The toxin inhibited both the RNA polymerase systems responsible for ribosomal RNA synthesis and heterogenous nuclear RNA synthesis. PR toxin inhibited the in vitro activities of rat liver DNA polymerases alpha, beta and gamma, as well (Moulé *et al.,* 1980); (Lee and Wei, 1984).

Exposure Assessment

PR toxin has been reported in cheese, mouldy grains and silage (EMAN 2005) although little data is available regarding the levels found.

Risk Characterisation

It is unlikely that PR toxin in cheese is a potential acute human health hazard given that t it is unstable.

Mycophenolic Acid

Mycophenolic acid is a metabolite produced by many strains of *P. roqueforti* and by a few other species of *Penicillium* (La Font et al., 1979). Although Engel et al. (Engel *et al.,* 2005)only found that 25% of all *P. roqueforti* strains produce mycophenolic acid. It has antibiotic activity against bacteria and dermatophytic fungi and also interferes with viral multiplication (Planterose, 1969). Mycophenolic acid is also used in liver transplantation as an immunosuppressive agent.

Hazard identification and characterisation

The toxicity of mycophenolic acid for mammals appears to be low: LD50 in rats is 2,500 mg/kg and 500 mg/kg IV; in mice the LD50 is 700 mg/kg and 450 mg/kg IV (Wilson, 1971). The oral LD50 of 700 mg/kg in mice placed mycophenolic acid in the U.S. Environmental Protection Agency's moderately toxic category. Chronicity tests of daily oral doses of 80 and 320 mg/kg for one year did not cause apparent signs of toxicity in rabbits (Adams *et al*., 1975). However, rats given daily oral doses of 30 mg/kg died within 9 weeks and rhesus monkeys receiving 150 mg/kg daily developed abdominal colic, bloody diarrhoea, weight loss and anaemia after two weeks (Carter *et al*., 1969).

Thirty-five human patients who received high oral doses of mycophenolic acid (2.4 g to 7.2 g daily) had some adverse reactions, including cramps, nausea and diarrhoea, and mutations were induced in a mouse mammary carcinoma cell line with mycophenolic acid (Marinari *et al*., 1977); (Umeda *et al*., 1977).

Exposure Assessment
Mycophenolic acid has been reported in cheese (EMAN 2005), and in particular in blue cheese (Lafont *et al*., 1979) where levels of up to 4 mg/g dry culture was determined.

Risk Characterisation
A full risk characterisation cannot be carried out due to lack of data, but there is considered to be low toxicity in mammals.

In summary, the risk associated with *P. roqueforti* lies with its production of a range of mycotoxins, which have been studied to varying degrees. Some of these mycotoxins have been shown to be produced by *P.roqueforti* strains used for cheese production and some have been detected in small amounts in the cheese itself. PR toxin and roquefortines appear to be the most toxic of the mycotoxins produced by *P.roqueforti*. PR toxin, one of the most potent mycotoxins, is unstable and deteriorates rapidly, so apparently under normal production conditions it does not pose a health risk. Roquefortine has been recovered from blue cheese at low levels and there have been no reported adverse effects from consumption of the cheese. The composition of medium used to make cheese and the length of time and conditions of the fermentation lead to highly variable results with respect to the composition and amounts of mycotoxins produced. In general, mycotoxins are produced in media with a high carbon to nitrogen ratio.

6.3 High heat treatment

6.3.1 Polycyclic aromatic hydrocarbons

The term ‘polycyclic aromatic hydrocarbons’ (PAHs) commonly refers to a large class of organic compounds containing two or more fused aromatic rings made up of carbon and hydrogen atoms. PAHs are soluble in many organic solvents and are highly lipophilic. They are chemically rather inert (IPCS 1998a; IPSC, 1998b).

Raw food does not normally contain high levels of PAHs. Processing procedures, such as smoking and drying, and cooking of food is commonly thought to be the major source of contamination by PAH (SCF, 2002d; SCF, 2002b). Depending on a number of parameters: time, fuel used, distance from the heat source and drainage of fat, type of cooking (grilling, frying, roasting), cooking results in the production in the food of a number of compounds including PAHs.

Hazard Identification and Characterization
The acute toxicity of PAHs is moderate to low. The well characterized PAH, naphthalene, showed oral and intravenous LD_{50} values of 100-500 mg/kg bw in mice and a mean oral LD_{50} of 2700 mg/kg bw in rats. The values of other PAH are similar (IPCS 1998b).

PAHs have been studied extensively in assays for genotoxicity and cell transformation; most PAHs are positive in some genotoxicity assays. The only compounds for which negative results were found in all assays were anthracene, fluorene and naphthalene.

Owing to inconsistent results, phenanthrene and pyrene could not be reliably classified for genotoxicity.

Comprehensive work on the carcinogenicity of PAHs shows that 17 of 33 studied are, or are suspected of being carcinogenic. Only benzo-[a]-pyrene has been adequately tested using dietary administration (SCF, 2002a).

In humans the majority of studies available have examined occupational exposure to PAHs via inhalation, and in a few studies, via dermal exposure. Most of the reports are on exposure to mixtures of PAHs, which also contained other potentially carcinogenic chemicals, in occupational or environmental situation (SCF, 2002c).

Dietary exposure

FSANZ does not have data regarding the exposure of the Australian population to PAHs. The intake of individual PAH from food has been estimated to be 0.10-10 µg/day per person. Cereals and cereal products are the main contributors to the intake of PAH from food because they are a major component of the total diet (IPCS 1998a; IPSC, 1998b). A Swedish study has found that smoked and grilled foods show the highest PAH levels though they make only a modest contribution to total PAH dietary intake, since they are minor components of the usual diet (Larsson, 1986; IPSC, 1998b; IPCS 1998b). However, it should be noted that smoked and grilled food may contribute significantly to the intake of PAH if such foods are part of the usual diet.

PAH in dairy products have been detected in milk (Cavret *et al*., 2005) and in smoked cheese (e.g. (Osborne and Crosby, 1987)). For example, the benzo pyrene content of a smoked Italian Provola cheese was 1.3 µg/kg (Lintas *et al*., 1979), and concentrations of 0.01 – 5.6 µg/kg fresh weight fluranthene, benz anthracene, benzo phenanthrene, benzo pyrene, benzo perylene and indeno pyrene were found in a smoked cheese sample. PAH have also been found in the U.K. in unsmoked cheeses at levels of 0.01 – 0.06 µg/kg (McGill *et al*., 1982) and in British butter and cream samples (Dennis *et al*., 1991). PAHs have also been associated with ash (Yoshida R *et al*., 2003) and therefore may potentially be present in ash cheeses.

Risk Characterisation

Data linking dietary exposure of polycyclic aromatic hydrocarbons to possible human health risks are inconclusive. Some PAH are likely to be genotoxic carcinogens – with no known level of safe exposure. Estimated average dietary exposure for the Australian population is unavailable. Exposure is expected to be highly variable and linked to processing practices however, overall exposure from food is likely to be low. Though there is potential risk due to carcinogenic properties of some PAHs, particularly benzo[a]pyrene and as such exposure should be as low as reasonably achievable, the contribution of PAHs in the diet to the development of human cancer is not considered to be high (IPCS 1998b)

In conclusion, dietary exposure from the consumption of PAH in dairy products represents a negligible risk to the consumer.

7. Chemicals used in further processing

Further processed food products can utilise a range of chemicals such as food additives, processing aids and packaging options to create niche market products. The Standards applicable to the regulation of chemical used in further processed dairy products include;

- Standard 1.3.1 – Food Additives
- Standard 1.3.3 – Processing Aids
- Standard 1.3.4 – Identity and Purity
- Standard 1.4.3 – Articles and Materials in Contact with Food

7.1 Food Additives

Food additives are commonly used in processed dairy products. FSANZ regulates food additives through Standard 1.3.1 – Food Additives. A food additive is any substance not normally consumed as a food in itself and not normally used as an ingredient of food, but which is intentionally added to food to achieve one or more of the technological functions specified in Table 30. A food additive, or its by-products, may remain in the food.

Food additives should always be used in accordance with GMP. As a guide to assist manufacturers in compliance with this provision, the standard cites the Codex Alimentarius Commission Procedural Manual (CAC, 1999), which sets out the following relevant criteria for use in assessing compliance with GMP:

(a) the quantity of additive added to food shall be limited to the lowest possible level necessary to accomplish its desired effect;
(b) the quantity of the additive that becomes a component of food as a result of its use in the manufacture, processing or packaging of a food and which is not intended to accomplish any physical, or other technical effect in the finished food itself, is reduced to the extent reasonably possible; and,
(c) the additive is prepared and handled in the same way as a food ingredient.

Substances added to food in accordance with the Code must also meet appropriate specification for identity and purity. Standard 1.3.4 – Purity and Identity – details the specifications for permitted food additives. A substance must comply with a reference in;

(a) Food and Nutrition Paper 52 Compendium of Food Additive Specifications Volumes 1 and 2, including addenda 1 to 9, published by the Food and Agriculture Organisation of the United Nations in Rome (1992); or
(b) the fourth edition of the Food Chemicals Codex published by the National Academy of Sciences and the National Research Council of the United States of America in Washington, D.C. (1996), including supplements published to take effect on 1 December 1997, 31 March 2000 and 31 December 2001; or
(c) the Schedule to this Standard.

If no relevant specifications exists in one of these documents, a secondary tier of reference documents comprising other recognised national standards or pharmacopoeia.

Table 30: Technological functions which may be performed by food additives

Functional class *sub-classes*	Definition
Acidity regulator acid, alkali, base, buffer, buffering agent, pH adjusting agent	alters or controls the acidity or alkalinity of a food
Anti-caking agent anti-caking agent, anti-stick agent, drying agent, dusting powder	reduces the tendency of individual food particles to adhere or improves flow characteristics
Antioxidant antioxidant, antioxidant synergist	retards or prevents the oxidative deterioration of a food
Bulking agent bulking agent, filler	contributes to the volume of a food without contributing significantly to its available energy
Colouring	adds or restores colour to foods
Colour fixative colour fixative, colour stabiliser	stabilises, retains or intensifies an existing colour of a food
Emulsifier emulsifier, emulsifying salt, plasticiser, dispersing agent, surface active agent, surfactant, wetting agent	facilitates the formation or maintenance of an emulsion between two or more immiscible phases
Firming agent	contributes to firmness of food or interact with gelling agents to produce or strengthen a gel
Flavour enhancer flavour enhancer, flavour modifier, tenderiser	enhances the existing taste and/or odour of a food
Flavouring (excluding herbs and spices and intense sweeteners)	intense preparations which are added to foods to impart taste and/or odour, which are used in small amounts and are not intended to be consumed alone, but do not include herbs, spices and substances which have an exclusively sweet, sour or salt taste.
Foaming agent Whipping agent, aerating agent	facilitates the formation of a homogeneous dispersion of a gaseous phase in a liquid or solid food
Gelling agent	modifies food texture through gel formation
Glazing agent coating, sealing agent, polish	imparts a coating to the external surface of a food
Humectant moisture/water retention agent, wetting agent	retards moisture loss from food or promotes the dissolution of a solid in an aqueous medium
Intense sweetener	replaces the sweetness normally provided by sugars in foods without contributing significantly to their available energy
Preservative anti-microbial preservative, anti-mycotic agent, bacteriophage control agent, chemosterilant, disinfection agent	retards or prevents the deterioration of a food by micro-organisms
Propellant	gas, other than air, which expels a food from a container
Raising agent	liberates gas and thereby increase the volume of a food
Sequestrant	forms chemical complexes with metallic ions
Stabiliser binder, firming agent, water binding agent, foam stabiliser	maintains the homogeneous dispersion of two or more immiscible substances in a food
Thickener thickening agent, texturiser, bodying agent	increases the viscosity of a food

A review of the technological functions regulated in Standard 1.3.1 indicates some functional classes, such as propellants, intense sweeteners and raising agents are unlikely to be relevant to dairy products. The Standard, through Schedule 1, have specified permitted uses of food additives by food type for dairy products. The permissions for dairy products relate mainly to preservative and colouring functions.

There is anecdotal evidence that small dairy manufacturers may add homeopathic chemicals to their dairy produce, for example, cider vinegar and plant extracts; in general these are unregulated products.

Benzoic acid

Benzoic acid is one of the oldest chemical preservatives used in the cosmetic, drug and food industries. It occurs naturally at low levels (~0.2 mg/kg) in a range of foods including dairy products (milk, cheese, yoghurt) (IPCS 2000a).

Although benzoic acid is not approved for use as an additive in the manufacture of dairy products, other than dairy (and other fat) based deserts, there is potential for the natural levels of benzoates to concentrate in fermented dairy products. This could potentially be a problem when exporting a dairy product to a country that prohibits the use of benzoic acid as a preservative.

Hazard Identification

Benzoates were evaluated by JECFA in 1996 (WHO, 1997), where the ADI for benzoic acid and its calcium, potassium and sodium salts, expressed as benzoic acid equivalents, of 0-5 mg/kg bw was maintained.

The ADI of 0-0.5 mg/kg bodyweight established by JECFA for benzoic acid and its salts is based on a long-term exposure study in rats. The NOEL was established at the highest dose tested (500 mg/kg bodyweight per day) where no adverse effects were observed. Signs of toxicity were observed in more recent short-term studies at higher dose levels. In establishing the ADI, a safety factor of 100 was applied to the NOEL to take into account species differences and individual human variation.

Dietary exposure

A dietary exposure assessment was conducted as part of the assessment of benzoates for the 21st ATDS (FSANZ, 2005c). The mean estimated dietary exposure to benzoates was less than 50% of the ADI for all population groups assessed. The mean estimated dietary exposure for the population aged two years and over, representing mean lifetime exposure, was approximately 15% of the ADI for males and approximately 10% of the ADI for females. The 95th percentile estimated dietary exposures to benzoates exceeded the ADI for young boys (approximately 140%) and young girls (approximately 120%) aged 2-5 years, and was equivalent to the ADI for schoolboys aged 6-12 years. All other population groups were below the ADI for 95th percentile estimated dietary exposures. The 95th percentile estimated dietary exposure to benzoates for the population aged two years and over, representing lifetime exposure for a high consumer of benzoates, was approximately 60% of the ADI for males and approximately 50% of the ADI for females.

The major foods contributing to dietary exposure to benzoates for young children aged 2-5 years were cordial, non-cola soft drinks and orange juice. For all other age groups assessed, non-cola soft drinks were the greatest contributor to dietary exposure to benzoates. In the 21st Australian Total Diet Survey benzoic acid was detected in various cheeses (3-11 mg/kg), while there was no analysis for milk.

Risk characterisation

The mean estimated dietary exposure to benzoates for all population groups was well below the ADI, indicating that for the majority of the population, there is no public health and safety risk from the consumption of a balanced diet which includes foods containing benzoates.

Dairy products are not a major contributor to the overall exposure of benzoic acid and therefore, benzoic acid dietary exposure from the consumption of dairy products presents a negligible risk to the consumer.

7.2 Processing Aids

Substances can be used in the processing of foods to fulfil a technological purpose relating to a treatment or process, but do not perform a technological function in the final food. For the purposes of the Code these substances are known as processing aids. Examples relevant to dairy products include the use of hydrogen peroxide.

Processing aids are regulated through Standard 1.3.3 – Processing Aids. For the purposes of the Standard a processing aid is a substance used;

(a) in the processing of raw materials, foods or ingredients, to fulfil a technological purpose relating to treatment or processing, but does not perform a technological function in the final food; and

(b) in the course of manufacture of a food at the lowest level necessary to achieve a function in the processing of that food, irrespective of any maximum permitted level specified.

Unless expressly permitted in this Standard, processing aids must not be added to food.

Similarly to food additives, the quality of processing aids is regulated through provisions in Standard 1.3.4 – Purity and Identity. Chemicals used as processing aids listed in the Standard 1.3.3 – Processing Aids - are grouped by functional classes. Permitted usage by functional classes include:

- Generally permitted processing aids;
- Antifoaming agents;
- Catalysts;
- Decolourants, clarifying and filtration agents;
- Desiccating preparations;
- Ion exchange resins;
- Lubricants, release and anti-stick agents;
- Carriers, solvents and diluents;
- Processing aids permitted in packaged water used as an ingredient in other foods;
- Bleaching agents, washing and peeling agents;
- Extraction solvents;
- Processing aids with miscellaneous functions;
- Enzymes of animal origin;
- Enzymes of plant origin;

- Enzymes of microbial origin; and,
- Microbial nutrients and microbial nutrient adjuncts.

The Processing Aid Standard is currently under review (Proposal P276 Review of Enzyme Processing Aids and Proposal P277 – Review of Processing Aids (other than enzymes). The review will address the following:

- safety of currently permitted processing aids;
- removing any obsolete processing aids; and
- correct errors, remove anomalies and improve consistencies within the Code.

It is not anticipated that the structure of Standard 1.3.3 – Processing Aids - will be changed.

The review of Standard 1.3.3 might result in some changes which could be relevant for the Dairy Standard, but is not expected to have a major impact.
The regulation of certain processing aids, for example hydrogen peroxide, benzoyl peroxide and lactoperoxidase system are further clarified below.

Hydrogen peroxide
Hydrogen peroxide is a very effective bactericidal agent (Fox *et al*., 2000). In some European countries and America, hydrogen peroxide is used as an alternative to pasteurisation in certain hard cheeses, though the use is not practiced commercially to any great extent. There is some evidence for the efficacy of using hydrogen peroxide treatments to inactivate penicillin residues in milk (Hanway *et al*., 2005).

The World Food and Agriculture Organisation permit addition of hydrogen peroxide to milk at concentrations of 0.05 – 0.25%, on the condition that all the hydrogen peroxide remaining in the milk after processing is converted by catalase into O_2 and H_2O (Tarhan, 1994).

In Australia, hydrogen peroxide (H_2O_2) is an approved processing aid (Standard 1.3.3 – Processing Aids – Table to clause 12) and may be used as a bleaching, washing and peeling agent in the course of manufacture of all food with a maximum permitted residue level of 5 mg/kg (0.005%). It is not, however, permitted for use as a preservative in Standard 1.3.1. As a processing aid, the hydrogen peroxide may be used to fulfil a technological purpose relating to treatment or processing, but not to perform a technological function in the final food. Thus, there is no permission for the use of hydrogen peroxide as a chemical alternative to pasteurisation in the Australian dairy industry, even though in the past, in a few instances State Dairy Authorities have identified the use of hydrogen peroxide in both cream and brine and appropriate corrective/enforcement actions were taken.

<u>Hazard identification and characterisation</u>
The dissociation of hydrogen peroxide is a violent and exothermic reaction. The systemic effects of hydrogen peroxide result from its interaction with catalase in the tissues with liberation of oxygen and water as it decomposes. Ingestion results in gastrointestinal irritation, the severity of which depends on the concentration. There is also a risk of gas embolism. A number of deaths have been reported in the literature. In most cases the exposure were to concentrated solutions of 30 to 40% (IPCS, 1998; IPSC, 1998a).

Oral ingestion of 3% hydrogen peroxide solutions (household strength) generally does not result in severe toxicity but may result in vomiting, mild irritation to mucosa and burns in the mouth, throat, oesophagus and stomach. Ingestion of higher concentration, e.g. >10%, can result in more dangerous sequelae such as burns to mucus membranes and gut mucosa (ATSDR, 2004).

Most cases of ingestion of hydrogen peroxide result in only mild effects. Persistent exposure to low levels of hydrogen peroxide is unlikely to cause chronic toxicity as hydrogen peroxide is rapidly detoxified in the body.

International Agency for Research on Cancer (IARC) considered that there was inadequate evidence for carcinogenicity in human (Group 3) (IARC, 1999).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) had evaluated the safety of hydrogen peroxide in 1965, 1973 and 1980 respectively. JECFA considered that ingestion of small amounts of hydrogen peroxide, that may be present in food, would produce no toxicological effects due to rapid decomposition of the chemical by the enzyme catalase of the intestinal cells (FAO, 1967; WHO, 1974; WHO, 1980). No ADI was allocated.

The antimicrobial effects and limitations of hydrogen peroxide use have been summarised in a report commissioned by FSANZ (Juffs, H and Deeth, H, 2005).

Lactoperoxidase system

Lactoperoxidase is a porphyrin-containing peroxidase secreted by the mammary gland. Lactoperoxidase catalyses the oxidation of thiocyanate (SCN^-) to hypothiocyanate by using hydrogen peroxide.

The lactoperoxidase system (LPS) is a major contributor to the antibacterial activity of milk (Kussendrager and Van Hooijdonk, 2000). The antibacterial system functions by the generation of oxyacids that react with protein sulfhydryls in bacterial cell walls, essentially terminating metabolism. LPS has been shown to function effectively as a bacteriocide against several Gram negative bacteria and is bacteriostatic against Gram positive bacteria (Aimutis, 2002).

In bovine milk, lactoperoxidase is the second most abundant enzyme (after xanthine oxidase) and its concentration is approximately 30 mg/l, constituting about 0.5% of the whey proteins. Unlike other anti bacterial proteins, lactoperoxidase levels are relatively low in colostrums, but increase to a maximum level 3-5 days postpartum. In order for this naturally occurring enzyme to exert an antibacterial effect, it requires the presence of both hydrogen peroxide and thiocyanate.

LPS is used in some instances in the preservation of dairy products; it's function is enhanced in the presence of thiocyanate and glucose oxidase (Van Hooijdonk *et al*., 2000).
Bovine lactoperoxidase is thermostable and is not affected by pasteurisation.
The International Dairy Federation has published guidelines for preservation of milk using the lactoperoxidase system.

LPS is not an approved sanitising agent for Australian dairy products, although bovine derived lactoperoxidase is permitted in Standard 1.3.3 as a processing aid for meat surfaces.

Codex Alimentarius allows for the use of LPS in countries where pasteurisation and refrigeration facilities are not available for milk processing and storage. The Codex Alimentarius Commission does not consider LPS to be of toxicological concern at the prescribed levels for those countries where it is permitted for use.

Benzoyl peroxide

Benzoyl peroxide (measured as benzoic acid) releases hydrogen peroxide and is permitted in Standard 1.3.3 as a processing aid for bleaching, washing and as a peeling agent to a maximum level of 40 mg/kg. Benzoyl peroxide is not permitted for use as a food additive in Australian dairy products.

Benzoyl peroxide is permitted in the USA as a bleaching agent to a maximum level of 100 mg/kg for whey powder, and as such may be found as a constituent in imported products from the USA, if the level in the final food does not exceed 40 mg/kg; there are no risks associated with its consumption at the permitted level.

7.3 Packaging

The major role of dairy packaging is to retard product deterioration by preventing microbial recontamination and excessive chemical deterioration. Fluid products are especially susceptible to microbial, enzymatic and chemical spoilage. In addition, packaging must also provide containment, facilitate use, identify products and appeal to customers.

Protection of product flavour

A specific issue relating to packaging and dairy products is the ability of dairy products to adsorb taints from primary packaging (in contact with the product) and from contaminating chemicals disseminated in the surrounding air-space of a dairy product.

Packaging can allow the transfer of odours such as stored food (e.g. onions) or distribution odours (e.g. diesel or fresh paint fumes). Packaging made from substandard materials or overheated during formation can transfer odours to products. For example, inks can transfer solvent odours to products and polymer-based materials can transfer plastic-like odours if over heated. In addition, exposure to light can cause the formation of "light-struck" off-flavours (Hotchkiss and Meunier-Goddik, 2003).

Regulation of packaging materials

FSANZ regulates food contact uses of primary packaging materials through Standard 1.4.3 – Articles and Materials in Contact with Food. The Standard regulates food contact materials in general terms. The Standard does not specify individual packaging materials for food contact or how they are 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 of America (USA) and European Economic Community (EEC) directives relevant to the manufacture and use of plastics.

Where a public health and safety concern is identified, maximum levels may be established in Standard 1.4.1 – Contaminants and Natural Toxicants. Examples include the maximum levels set for tin (all canned food), acrylonitrile and vinyl chloride (all food) in association with packaging materials.

Currently AS 2070 prohibits the use of recycled plastic materials in plastic materials for food contact use therefore only virgin plastics are used for packaging in Australia.

Migration of chemicals into dairy produce
Although prescription of specific plastic packaging is beyond the scope of the Code, it is pertinent to note that there has been evidence of the migration of chemicals from packaging used to wrap dairy produce.

Cling-film is used both for wrapping foods at the retail level (for example, cheese) as well as for use in the home for a variety of applications. Plasticisers are added to polymers such as PVC to confer different degrees of flexibility in the production of cling-film. Plasticisers are mainly highly lipophillic, organic esters of low molecular weight with a potential to migrate from the packaging material into the packaged food, thereby becoming contaminants. Two such plasticizers are di-(2-ethylhexylexyl)adipate (DEHA) and di-(2-ethylhexyl)phthalate (DEHP). DEHA has replaced the phthalates in thin plasticized PVC food packaging films, due to reports of the induction of testes toxicity and antiandrogenic effects of DEHP (Dalgaard *et al*., 2003). The EU states that DEHA has low acute toxicity, is not genotoxic and does not cause irritation.

Although plasticisers are not an issue in Australia, in other countries, both DEHA and DEHP have been reported as contaminants of dairy produce (Page and Lacroix, 1995); (Castle *et al*., 1987). In a U.K. survey, milk and cream showed very low levels of DEHP (< 0.01 – 3 mg/kg), whereas cheese and butter levels were as high as 114 mg/kg total phthalate in some cases (Sharman *et al*., 1994). The migration of DEHA from food-grade PVC film, containing 28.3% DEHA, into hard and soft cheeses was found to be dependent upon contact time, fat and moisture contents, and consistency of the cheese samples (Goulas *et al*., 2000). The presence of cheese rind also greatly reduced the migration of DEHA in Edam and Kefalotyri (Goulas *et al.,* 2000).

Internationally there are intermittent reports of the migration of other chemicals from food packaging. For example, during the past year there were reports from Europe that the chemical isopropylthioxanthone, commonly used in printing ink on some food packaging, was also present at very low levels in some liquid infant formula products (EFSA, 2005).

7.4 Distribution and Transport

After manufacture, dairy products remain vulnerable to chemical contamination from the environment and containers. Cross-contamination of taints (FSANZ 2005) and traces of chemicals from other foods during transport of mixed loads or from lubricants, refrigerants or paint, for example, may occur during transport. Furthermore, as milk and dairy products are perishable, they must be transported without undue delay to prevent the introduction of contaminants and the growth of toxin-producing pathogens.

Food Safety Programs are in place in order to manage potential contamination during distribution. Thus dairy food transport vehicles, equipment and vessels are designed, constructed and maintained to prevent the introduction of contaminants and temperature increase (that is, maintaining a temperatures $\leq 5^{o}C$). Cleaning and sanitising of dairy food carriers is a key aspect of the Food Safety Program, which relies upon the use of water of suitable quality.

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GLOSSARY

ADASC	Australia New Zealand Dairy Authorities' Standards Committee
ADI	Acceptable Daily Intake
AgVet	Agricultural and Veterinary
AMR	Antimicrobial Resistance
AMRA Survey	Australian Milk Residue Analysis Survey
APVMA	Australian Pesticide and Veterinary Medicines Authority
AQIS	Australian Quarantine and Inspection Service
ARGT	Annual Ryegrass Toxicity
ARPANZA	Australian Radiation Protection and Nuclear Safety Agency
ATDS	Australian Total Diet Survey
BA	Biogenic Amine
BST	Bovine Somatotropin
CCU	Central Coordinating Unit
CIJIG	Commonwealth Interdepartmental JETACAR Implementation Group
Codex	Codex Alimentarius Commission
CPA	Cyclopiazonic acid
1,2-DCB	1,2-dichlorobenzene
DEHA	Di-(2-ethylhexyl)adipate
DEHP	Di-(2-ethylhexyl)phthalate
DFSV	Dairy Food Safety Victoria
DON	Deoxynivalenol (trichothecene mycotoxin)
EAGAR	Expert Advisory Group on Antimicrobial Resistance
EC	European Commission
EEC	European Economic Community
EPA	Environmental Protection Agency (US)
ERL	Extraneous Residue Limit
EU	European Union
EU SCOOP	European Union Scientific Cooperation
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration (US)
FSANZ	Food Standards Australia New Zealand
GAP	Good Agricultural Practise
GM	Genetically Modified
GMP	Good Manufacturing Practice
GPT	*N*-acetylglucosamine-1-phosphate transferase

HACCP	hazard analysis critical control point
HCB	Hexachlorobenzene
HDPE	High Density Poly Ethylene
HGP	Hormonal Growth Promotants
IARC	International Agency for Research on Cancer
IV	intravenous
JECFA	Joint FAO/WHO Expert Committee on Food Additives
JETACAR	Joint Expert Technical Advisory Committee on Antibiotic Resistance
JMPR	Joint Meeting on Pesticide Residues
LD_{50}	Lethal Dose for 50% of the experimental animals tested
LDPE	Low-density Poly Ethylene
LOEL/LOAEL	Lowest Observed Effect Level/Lowest Observed Adverse Effect Level
LOD	Limit of Detection
LOQ	Limit of Quantification
LOR	Limit of Reporting
LPS	Lactoperoxidase System
ML	Maximum Level
MOU	Memorandum of Understanding
MRL	Maximum Residue Limit
NARM Program	National Antibacterial Residue Minimisation Program
NDP	National Dioxins Program
NHMRC	National Health and Medical Research Council
NIV	Nivalenol (trichothecene mycotoxin)
NOEL	No Observed Effect Level
NORM Program	National Organochlorine Residue Management Program
NRA	National Registration Authority
NRS	National Residue Survey
OECD	Organization for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
OIE	World organisation for animal health
PA	Pyrrolizidine Alkaloid
PAH	Polycyclic Aromatic Hydrocarbon
PBDD	Polybrominated dibenzodioxins
PBDF	Polybrominated dibenzofurans
PCB	Polychlorinated biphenyls
PCDD	Polychlorinated dibenzodioxins
PCDF	Polychlorinated dibenzofurans

PET	Poly ethyleneterephthalate
PISC	Primary Industries Standing Committee
PMTDI	Provisional Maximum Tolerable Daily Intake
PPP	Primary Production and Processing
PR	Penicillium Roqueforti
PTDI	Provisional Tolerable Daily Intake
PTWI	Provisional Tolerable Weekly Intake
PVC	Polyvinylchloride
QA	Quality Assurance
rBST	Recombinant Bovine Somatotropin
SCF	Scientific Committee on Food
SDA	State Dairy Authority
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TDI	Tolerable Daily Intake
TEF	Toxicity Equivalency Factor
TEQ	Toxic Equivalence
TI	Tolerable Intake
TMI	Tolerable Monthly Intake
UL	Upper Level of Intake
USA	United States of America
VRE	Vancomycin Resistance Enterococci
WHO	World Health Organization
WHP	Withholding Period
ZEA	Zearalenone
ZOL	Zearalenol

VII APPENDICES

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APPENDIX 1

Impact of processing on dairy product safety

1.1 Milk and cream

1.1.1 Description

Milk is defined in Standard 2.5.1 of the Code as "the mammary secretion of milking animals, obtained from one or more milkings for consumption as liquid milk or for further processing but excludes colostrums".

In this Dairy Risk Profile, milk refers to the fluid form of milk derived from cow, sheep, goats, buffaloes, camels, horses and other mammalian animals, and available for human consumption through retail sale in Australia.

Milk may be sold in many forms, including whole milk, skim milk, low-fat milk, flavoured milk and other modified milks. Some of these products require the removal of the fat portion as cream. Under Standard 2.5.2, cream is defined as "a milk product comparatively rich in fat, in the form of an emulsion of fat-in-skim milk, which can be obtained by separation from milk". Cream is produced from whole milk by skimming or other separation means.

Milk is subjected to a range of processing operations before being sold. Typical processes include standardisation or formulation of milk, which may include: separation steps such as filtration, centrifugation, and sometimes clarification; homogenisation; and various forms of heat treatment such as thermisation, pasteurisation, sterilisation and UHT (ultra-high temperature) processing. The key processing operations are shown in Figure 1.

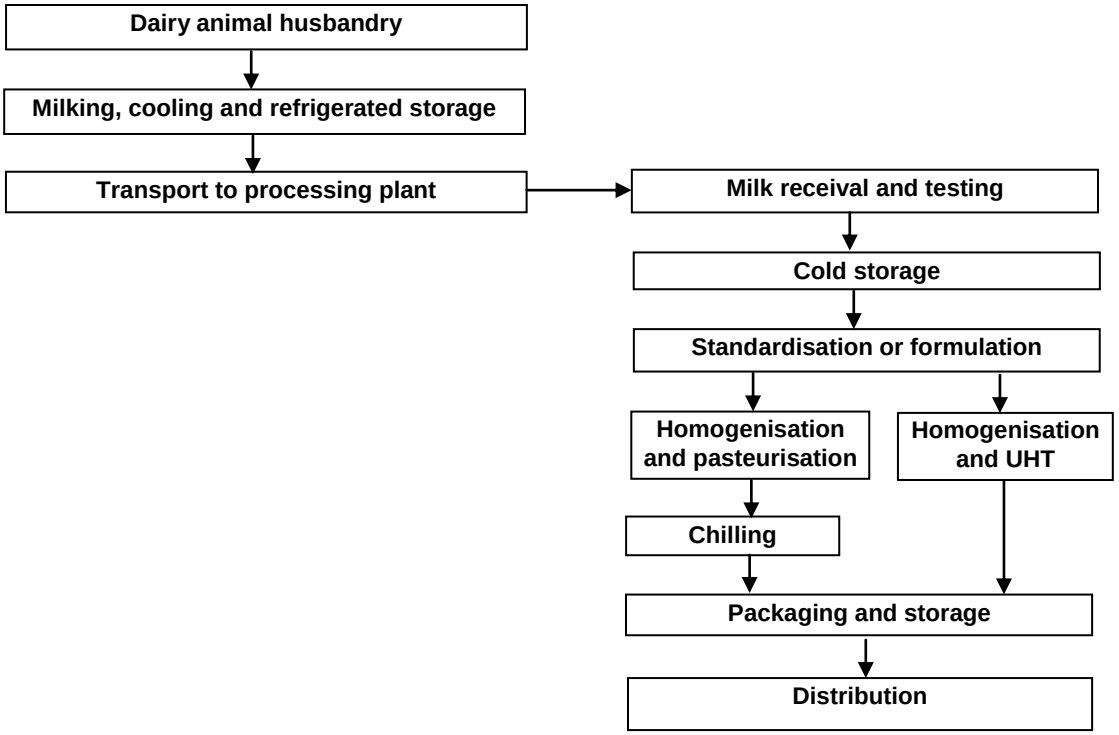

Figure 1: Indicative processing of fluid milk and cream products

Figure 1 simplifies the major steps in the processing of liquid milk products. The raw milk for low fat, skim milk and modified milk products is subjected to centrifugal separation to remove some of the fat phase. Flavoured milks have the addition of flavours and colouring agents, which are sometimes added post-pasteurisation, while ultra-high temperature processing (UHT) results in milk products which are shelf-stable and do not require refrigeration or chilling.

The cream that is separated from whole milk may be used in the production of liquid cream products, butter, and anhydrous milk fat. The cream may be subjected to additional processes including vacuum pasteurisation, which involves heat treatment and deodorisation of the fat.

1.1.2 The Microbial Flora of Milk

The microbial status of raw milk is influenced by various factors associated with milk production on farm. These factors impact on both the numbers of micro-organisms present in raw milk and the type of bacterial flora. Generally, few bacteria are present in milk drawn from the udder of a healthy animal, but bacteria may enter milk if it is drawn from an infected animal or if it is contaminated by unhygienic milking practices and poor milk handling.

Bramley and McKinnon (1990) identified the main groups of micro-organisms comprising the microflora of raw milk as follows:

Table 1: Major flora of raw milk

Group	Incidence (%)
Micrococci *e.g. Micrococcus*, *Staphylococcus*	30-99%
Streptococci *e.g. Enterococcus*	0-50%
Asporogenous Gram +ve rods *e.g. Corynebacterium, Microbacterium, etc*	<10
Gram –ve rods *e.g. Klebsiella, Escherichia, Enterobacter, Pseudomonas, etc*	<10
Sporeformers *e.g. Bacillus* spores or vegetative cells	<10
Miscellaneous *e.g. Streptomycetes,* yeasts, moulds, etc	<10

Various pathogenic micro-organisms may also be associated with raw milk. These include organisms shed by an infected animal (pathogens will predominate in milk from mastitic cows) or organisms that enter the milk from contaminated equipment and poor milking hygiene. Surveys of raw cow's milk, mainly conducted overseas, have detected *Aeromonas* spp., *B. cereus*, *Brucella* spp., *Campylobacter* spp., *Coxiella burnetii*, pathogenic *E. coli*, *L. monocytogenes*, *Mycobacterium* spp., *Salmonella* spp., *S. aureus*, *Streptococcus* spp. and *Y. enterocolitica*. Raw goat's and sheep milk have been shown to contain *Aeromonas* spp., *Brucella* spp., *Campylobacter* spp., pathogenic *E. coli*, *L. monocytogenes*, *Mycobacterium* spp., *S. aureus* and *Y. enterocolitica.* In Australian surveys, potential pathogens detected in raw cow's milk have included *Aeromonas* spp. and *S. aureus*. Detections in raw goat's milk have included *E. coli*, *L. monocytogenes*, and *Y. enterocolitica* (Appendix 3).

Overseas surveys of cream have detected *Aeromonas* spp. and *Shigella* spp., while Australian surveys have indicated the presence of *E. coli* and *Salmonella* spp. (Appendix 3).

1.1.3 Effect of milk processing on the growth and survival of microbial pathogens

To minimise microbiological activity, raw milk harvested from animals at dairy farms is cooled rapidly to temperatures of 7°C or lower within 2 hours of milking (White, 2003). In Australia, milk is cooled to 4°C.

After a period of storage on farm, raw milk is transported by insulated bulk milk tankers to milk processing facilities. Traditionally, milk transportation was conducted in the early part of the day to minimise the impact of heat from sunlight, but nowadays milk collection may occur at any time during the day or night and insulated tankers minimise the impact of ambient temperatures on milk.

When the raw milk reaches the processing plant, it is sampled and its status assessed by measuring parameters such as temperature, presence of antibiotics, somatic cell counts, standard plate count, bactoscan, fat content, etc. Information on the microbial content of milk is helpful in judging its sanitary quality and the conditions of production, and is used in payment schemes that reward producers of high quality raw milk. The raw milk is normally transferred to large insulated storage silos and maintained at temperatures of less than 4°C before decisions are made about how it will be processed.

Milk for drinking is usually homogenised, a process where the milk fat globules are physically reduced in size and then remain in suspension throughout the milk for long periods of time. In unhomogenised milk, fat globules may coalesce to form a compact cream layer. Homogenisation has little effect on the microbiology of fluid milk, but may predispose the fat to oxidation reactions that affect its quality, hence homogenisation occurs simultaneously with pasteurisation. Practically all drinking milk in Australia is homogenised, preventing the milk from separating and giving it a more uniform colour.

Pasteurisation involves heat treatment of milk with the aim of ensuring a microbiological safe product, as well as to extend the shelf-life during refrigerated storage. Milk pasteurisation may be carried out either as a batch holding heat treatment or a high-temperature-short-time (HTST) heat treatment. The batch process involves low-temperature-holding for 30 minutes or longer at temperatures of approximately 63°C. This has been largely replaced by HTST treatment at temperatures of ≥72°C for at least 15 seconds. The Code states: Milk must be pasteurised by:

(a) heating to a temperature of no less than 72°C and retaining at such temperature for no less than 15 seconds and immediately shock cooling to a temperature of 4.5°C; or
(b) heating using any other time and temperature combination of equal or greater lethal effect on bacteria; where dairy products contain elevated levels of fat or solids, the specified temperature is increased to compensate for the protective effect of these fat and solids on micro-organisms.

These specifications are sufficient to reduce populations of vegetative bacterial pathogens to a level considered safe for public health. The pasteurisation process used by processors of milk often employs temperatures and times in excess of 72°C for 15 seconds. This is to provide a higher margin of safety and to extend the shelf-life of liquid milk.

From survey data on industry pasteurisation practices in Australia HTST treatment of milk for liquid milk products was mostly in the range of 74-78°C for 15-30 seconds[35].

Pasteurisation processes for cream products utilise higher temperatures because of the protective effects of fat on micro-organisms. From survey data on industry pasteurisation practices in Australia the pasteurisation temperatures and times for table cream varied in the range from 75-80°C for 20-30 seconds[36]. Internationally recognised heat treatments for pasteurisation of cream is 65°C for 15 seconds for cream with 10-20% fat, and 80°C for 15 seconds for cream with >20% fat[18]. Thickened cream has thickeners such as alginates and/or carragenans added before pasteurisation.

The same survey data indicates that batch pasteurisation is still widely used in Australia for the pasteurisation of raw cows' milk used in the manufacture of dairy products such as cheese, cream, ice cream and yoghurt, particularly by the smaller processors, many of whom are processing milk in on-farm situations. However, batch pasteurisation accounts for only a small percentage of all milk pasteurised in Australia. Temperatures and times of heat treatment for batch pasteurisation range from 62-90°C and from 15 seconds to 30 minutes[18].

Further detail on pasteurisation and conditions used in Australia are described in Section 10.

Pathogens such as *Salmonella*, *Campylobacter*, *Staphylococcus*, pathogenic *E. coli* (particularly enterohaemorrhagic *E. coli*), *Y. enterocolitica* and *Listeria monocytogenes* which may be present in raw milk are inactivated by pasteurisation. However pasteurisation will not destroy heat stable enterotoxins such as those produced by *Staphylococcus aureus*, if the organism has grown and produced enterotoxin in raw milk prior to pasteurisation. Inadequate chilling of raw milk is one of the key factors for the build up of *Staphylococcus* enterotoxins (ICMSF, 1998).

Pasteurisation also has the advantage of destroying many of the spoilage micro-organisms present in raw milk, especially psychrotrophic bacteria which may proliferate during low temperature storage of liquid milk products. Pasteurisation, however, cannot be relied upon to destroy some of the more heat resistant bacteria (thermodurics) or bacterial spores produced by bacteria of the Genera *Bacillus* and *Clostridium*. After pasteurisation, milk and milk products still contain low numbers of thermoduric micro-organisms such as *Micrococcus* and *Enterococcus* species and some lactic acid bacteria. For this reason, pasteurised milk and milk products have a limited shelf-life even when stored at refrigeration temperatures (ICMSF, 1998).

To minimise growth of the surviving microbes, and to minimise post-process recontamination the steps of cooling pasteurised milk, filling and packaging, and refrigerated storage of pasteurised milk and cream must be well managed. Pasteurised milk is particularly vulnerable to post-pasteurisation contamination, and asepsis and good hygiene is essential for preventing contamination by pathogenic micro-organisms and for defending its shelf-life.

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35 Pasteurisation times and temperatures are from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

36 Pasteurisation times and temperatures are from the report to FSANZ **Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products (**Juffs, H and Deeth, H, 2005)

The shelf life of milk is influenced by the number of psychrotrophic bacteria that survive pasteurisation or subsequently contaminate the pasteurised product and grow at low temperatures in the liquid during storage. Although these contaminants are initially present in low numbers they can, under certain conditions, grow quickly and produce enzymes that break down protein and fat and generate off flavours and odours. The typical shelf life for pasteurised milk is from 7-14 days, although there are seasonal and regional variations.

UHT processing of milk involves heating milk at a temperature higher than 130°C with a holding period of 1-10 seconds with subsequent aseptic packaging. Usually the temperature and time combination is 138-145°C for 3-5 seconds (Deeth et al., 2003). Sterilisation treatment of milk is similar to that of UHT but at a higher temperature and is usually applied to condensed milk. The term '*sterilisation*', as used here, refers to commercial sterility of the milk or milk product. Milk and milk products of commercial sterility are not absolutely sterile in microbiological terms. However, those micro-organisms and spores that may survive the sterilisation treatment are incapable of development under normal conditions of storage (Hersom et al., 1980). Temperature and time combinations for the sterilisation of milk and milk products range from 105-120°C for 10-40 minutes (Hinriches et al., 2003).

Both UHT treatment and sterilisation destroy bacterial endospores (Deeth et al., 2003). Milk and milk products after UHT treatment or sterilisation can be stored without refrigeration for extended periods of time.

Available epidemiological data indicates that illness resulting from the consumption of pasteurised milk and cream is rare, although outbreaks involving *Campylobacter* spp., *Salmonella* spp., *E. coli* O157:H7, *L. monocytogenes* and *Yersinia* spp. have been linked to consumption of pasteurised milk (See Appendix 2). These outbreaks have usually been traced to inadequate pasteurisation and/or post-pasteurisation contamination and/or temperature abuse (ICMSF, 1998) and not to any failure of the pasteurisation process.

Surveys conducted overseas on pasteurised milk have indicated the presence of *Aeromonas* spp. and *B. cereus* in Turkey. Australian surveys have indicated the presence of *Aeromonas* spp. which was introduced during subsequent handling of the milk and *Yersinia* spp. (Appendix 3).

1.2 Cheese

1.2.1 Description

The term cheese covers over 1,000 varieties of fermented dairy products with significant variations in their flavour, texture and appearance. The process of converting liquid milk into cheese involves a series of steps that are modified to produce a cheese of the desired characteristics.

Cheeses manufactured from the milk of all species, including bovine, ovine and caprine animals undergo similar processing steps. The production of all cheese varieties generally follows a similar process comprising the following general stages:

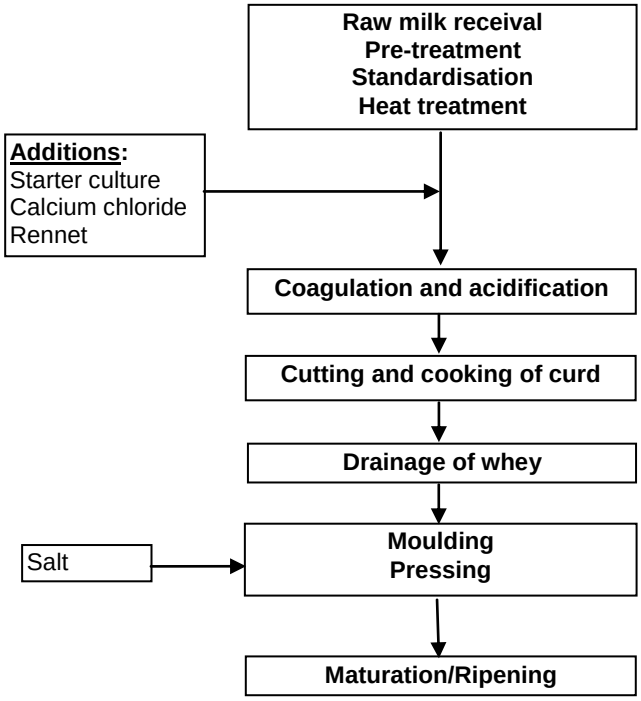

Figure 2: Overview of major steps in the manufacture of Cheddar-style cheese

Starter culture and rennet are added to milk resulting in the production of a cheese curd through a process of coagulation and acidification. The curds are usually cut and with mild (38-43ºC) heating there is separation of the whey, which is drained from the curds. The curds are salted before they are pressed into moulds and then stored under controlled conditions to ripen the cheese.

The actual processes vary enormously between cheese types. Some cheeses are manufactured without the use of rennet, while others are acidified by the addition of acid. Different starter cultures impart different physical and organoleptic properties on the cheese. Calcium chloride solution is added when milk is calcium deficient to assist cheesemakers. Calcium assists the milk coagulation process for rennet set cheeses. In some fresh cheese, the curds are not heated resulting in less expression of whey and a moister final product. Cheese curd may be washed with water, dry salted or brine salted, pressed or unpressed. The moisture content is influenced by the pH, salt concentration, amount of pressing, maturation conditions, etc. The addition of mould spores is required for surface ripened cheeses such as brie and camembert, while spores are incorporated in the curd for interior and surface ripened blue cheeses. During maturation a range of conditions are used, with particular attention to the temperature of the ripening rooms.

Historically, cheese was made from raw milk, and this practice still prevails amongst traditional cheese makers in countries such as France, England, and Italy. However heat treatment of raw milk reduces the populations of micro-organisms, both pathogenic and spoilage, before cheese making commences and provides the cheese maker with greater control over the microbial flora in the milk. Typically this involves pasteurisation, but heating milk to 64-70°C for 15-20 seconds *i.e.* thermisation is gentler than pasteurisation and does less damage to some of the intrinsic enzymes in the raw milk.

These enzymes are considered relevant in the development of specific cheese flavours similar to those in cheeses made from raw milk (ICMSF, 1998).

Production of raw milk cheese is generally not permitted in Australia. The Code (Clause 2 of Standard 1.6.2) prescribes that cheese made with thermised milk must undergo a maturation period of 90 days, at a temperature of no less than 2°C, from the date of manufacture. Alternatively, cheese can be made with pasteurised milk, *i.e.* milk subject to heat treatment at 72°C for 15 seconds or any equivalent of this temperature and time combination.

Several different schemes are used to classify cheeses. Cheeses may be grouped according to manufacturing or processing procedures, consistency or rheology (softness or hardness), country of origin, general appearance (size, shape, colour, surface ripening), source of milk, and chemical analysis (See Table 7).

Table 2: Classification of cheeses by type and moisture content

Cheese Type	Moisture	Description/Style
Very hard	<36%	Ripened by bacteria *e.g.* Parmesan, Asiago, Romano
Hard	<42%	Ripened by bacteria, without eyes *e.g.* Cheddar
		Ripened by bacteria, with eyes *e.g.* Emmental, Gruyère
Semi-soft	43-55%	Ripened principally by bacteria *e.g.* Gouda, Edam, Provolone
		Ripened by bacteria and surface micro-organisms *e.g.* Limburger
		Ripened principally by interior blue mould *e.g.* Roquefort, Stilton, Danablu
Soft	>55%	Ripened by surface mould *e.g.* Brie, Camembert
		Unripened (also referred as fresh cheese) *e.g.* Cottage cheese, Quark, Cream cheese
		Salt cured or pickled *e.g.* Feta

1.2.2 Microbial pathogens of major concern

Cheese has been the vehicle in a number of outbreaks of food-borne illness, involving pathogenic micro-organisms such as *S. aureus, Bacillus* spp., *Salmonella, L. monocytogenes, E. coli, Shigella, Cl. botulinum* and *Brucella* spp. (Cogan, 2003; ICMSF, 1998). A full list of the outbreaks resulting from cheese consumption is provided at Appendix 2.

Evidence from outbreak investigations suggests that illness resulting from consumption of cheese is often the result of faulty controls in cheese production; use of contaminated starter cultures or contaminated ingredients; post-pasteurisation contamination; or mishandling during transportation and/or distribution.

In microbiological surveys conducted overseas and Australia, a number of potential pathogens have been detected in cheeses made from pasteurised milk, namely *L. monocytogenes* and *S. aureus*. Additional pathogens have been detected in raw milk cheese (*B, cereus*, *Brucella* spp., pathogenic *E. coli* and *Y. enterocolitica*). Detections of *Bacillus* spp. and *L. monocytogenes* have occurred in pasteurised milk cheeses in surveys conducted in Australia (Appendix 3).

1.2.3 Effect of cheese processing on the growth and survival of microbial pathogens

Several factors act as hurdles influencing the growth and or survival of pathogenic micro-organisms in cheese. These include the amount of heat applied at various stages during the manufacture of cheese; the extent of acidification by the starter culture, salt levels, reduced water availability resulting from salting and ripening/maturation, production of bacteriocins by starter cultures, and the effect of selected food additives used in cheese making.

Survival of pathogenic micro-organisms is also dependent on the status of the organisms including their initial population, their physiological condition and their characteristics such as tolerance of low pH, salt, reduced water activity, heat, and resistance to bacteriocins produced by lactic acid bacteria, etc.

While each of these factors has an effect, it is their combined effect that has the greatest impact on the growth or survival of microbial pathogens in cheese.

1.2.3.1 Effect of temperature and time

Heat treatments in cheese making include pasteurisation, thermisation, and cooking of the curd. Of all the factors, heat treatment applied to raw milk (*e.g.* pasteurisation) is the most important factor in survival and growth of potential microbial pathogens in raw milk used for cheese making (Cogan, 2003). Pasteurisation of raw milk is sufficient to reduce populations of vegetative bacterial pathogens to a level that is considered safe for public health (Cogan, 2003). Spores of *Cl. botulinum* may be present in milk and survive pasteurisation, but interactions between water activity, salt, pH and production of antimicrobial agents by starter cultures in the cheese, usually prevent germination or growth.

Thermisation is a milder heating process than pasteurisation and, on its own is unlikely to lead to a safe milk product. As such, extended ripening or maturation is required to reduce the level of microbial pathogens to an extent that is considered safe for public health. The extent of ripening or maturation varies according to the type and characteristics of the cheese, particularly its final pH, water activity, salt content and type and concentration of additives.

The temperature employed to facilitate coagulation in cheese making is referred to as the curd setting temperature. Curd setting temperature usually matches the optimum growth temperature of the starter culture, which varies between 32-37°C (Broome et al., 2003). Setting temperature has little role in reducing the level of microbial pathogens in cheese and may contribute to an increase in the population of microbial pathogens in the case of a failure of the starter culture, *e.g.* due to poor viability of the starter or the presence of a phage or antibiotics in the milk, etc. These factors may all contribute to slow growth of the starter, but may not slow the growth of microbial pathogens present in the milk.

Curd cooking refers to a heat treatment in cheese making that is aimed at stopping the growth of the starter culture and facilitate the contraction of the curd and expulsion of whey (syneresis). Curd cooking temperature varies according to the type of cheese and the way acidification is carried out. For soft and semi-soft cheese, the temperature ranges from 30-38ºC (Banks, 2003; van den Berg et al., 2003); for hard cheese, curd cooking ranges from 38-55ºC (Bachmann et al., 2003); and for acid/heat coagulated cheeses such as Cottage cheese, Cream cheese, Quark, Queso Blanco, Ricotta, Mascarpone and Paneer cheese, curd cooking can be as high as 90°C (Fox, 2003; Lucey, 2003). Where the cooking temperature is below 40°C, there is a good chance that microbial pathogens will grow, until the acidity of the curd becomes sufficiently high.

The combination of the curd cooking temperature and time applied in making hard cheeses and acid/heat coagulated cheeses may be sufficient to inactive most, if not all, of the vegetative cells of microbial pathogens present in the cheese.

1.2.3.2 Effect of pH

Selection of a starter culture that is a fast acid producer is vitally important in cheese making. If the starter culture (usually added at 10^6-10^7 cfu/mL) immediately dominates the population of micro-organisms in the milk, the chance of an increase in the population of pathogenic micro-organisms in the milk is substantially reduced. This is due to the dominance of starter culture in utilising the available nutrients in milk and the inhibitory effect of declining pH increasing organic acids.

In case of a starter culture failure due to infection by bacterial phage or inhibitory substances in milk, such as residues of antibiotics, pathogenic and spoilage micro-organisms may dominate the microbial population (Cogan, 2003). This can lead to undesirable consequences, such as the build up of *Staphylococcus* enterotoxins that are likely to remain in the cheese.

Reaching the appropriate end point pH in acidification plays a critical role in reducing the level of microbial pathogens in cheese making. As illustrated by Cogan (2003), the level of *Salmonella* is reduced to zero during ripening of a Cheddar cheese if the pH of the cheese is 5.23 or lower, but remains constant throughout the 160 days of ripening when the cheese pH is 5.7.

During cheese maturation/ripening, cheese pH changes very slowly, and only a minor increase may occur during a long ripening time. The pH of surface-ripened cheeses, such as Camembert, Brie, Blue, and Tilsit, generally increases as a result of production of ammonium ions on the surface of the cheese. In other cases, the increase of cheese pH is a result of oxidation of lactate to water and carbon dioxide, which forms bicarbonate ions (Cogan, 2003).

1.2.3.3 Effect of salt on microflora

The level of salt (%w/w) in cheese ranges from approximately 0.7-6%. Salt, along with pH, redox potential and water activity contribute to the minimisation of spoilage and prevention of growth of pathogens in cheese.

Salt affects the water activity of cheese as well as microbial growth, water activity and ripening rate.

Salt is often reported as salt-in-moisture (S/M) level in the curd, and the level influences the growth of the starter culture, other organism, cheese flavour and properties.

It is important to recognise that salt is not distributed evenly throughout the cured mass. Dry salt applied to the surface of milled cured required time to diffuse throughout the curd mass, hence micro-organisms in the curd will continue to grow. A salt gradient exists from the surface to the centre of cheeses where salt has been applied to the surface.

With 4% salt, many pathogenic micro-organisms will not grow, with the exception of *S. aureus* and *L. monocytogenes*.

S. aureus can grow in the presence of 6.5% of sodium chloride and *L. monocytogenes* can grow in the presence of 10% sodium chloride (Cogan, 2003). However, near these maximum levels, growth would be slow and require optimal conditions for other parameters.

1.2.3.4 Effect of water activity/moisture on microflora

The water activity is lowered during cheese ripening as the cheese loses moisture (whey) and as added salt binds free moisture and makes it unavailable for bacterial growth. The ability of bacteria to grow or survive is largely dependent on available moisture (in combination with other factors such as pH and temperature). Cheeses with relatively high a_w may readily support the growth of pathogenic bacteria compared to a low moisture cheese *i.e.* a_w less that 0.92 will inhibit the growth of bacterial pathogens, with the exception of *S. aureus*.

1.2.3.5 Effect of maturation/ripening on microflora

In hard cheeses, the combined effects of pH, salt, moisture and storage temperature come into play during ripening, inhibiting pathogen growth and promoting die off. Other than slow changes in pH and salt concentration, ripening/maturation leads to reduction in moisture content of the cheese (Sutherland, 2003). During the ripening/maturation process, pathogens in the cheese generally die off because of the low pH and low moisture content (a_w<0.96) combined with the relatively long ripening period at a low temperature. The physical-chemical characteristics of the cheese will also influence the decline of pathogens during this time. Long storage or ripening of cheese under controlled temperatures is likely to reduce any microbiological populations present.

Significant growth of pathogenic micro-organisms may occur with the ripening of soft cheeses because of their relatively high moisture (a_w varies from 0.97-0.99), high pH and the often high ripening temperature (Cogan, 2003). Hard cheeses, by virtue of their low moisture content and long maturation periods, are unlikely to support the survival and proliferation of microbial pathogens.

1.2.3.6 Post-process contamination:

Recontamination of cheese by pathogens can occur at all stages post-pasteurisation. Cheese products that are on-processed such as cut, cubed, shredded and grated cheeses are more susceptible to post-process contamination and significant numbers of *L. monocytogenes* have been found on these types of products. The introduction of *L. monocytogenes* into these products follows inadequately cleaned and sanitised shredding/grating equipment.

In particular the dominance of *L. monocytogenes* on soft cheeses can be attributed to the psychrotrophic nature of this pathogen, its tolerance of reduced water activity, tolerance to salt, and its ability to grow well at ripening temperatures (10-12ºC), once the pH has increased significantly above 5. In addition *L. monocytogenes* will continue to grow during retail storage (5ºC).

L. monocytogenes is ubiquitous in nature and can easily become established in processing plants. Its psychrotrophic nature allows it to colonise and grow where conditions tend to be wet and cool with areas of pooled water or liquid, including condensation on walls, ceilings and equipment surfaces; drains and floor puddles, condensate collected in refrigeration units and compressed air lines.

Specifically, brining tanks used in cheese making could be expected to be vectors for recontamination, depending upon the salt concentration, as *L. monocytogenes* is tolerant to salt.

1.3 Dried milk powders

1.3.1 Description

Whole milk, skim milk, whey, buttermilk, cheese and cream may be dried into powders by the application of heat. The fluid is initially concentrated by evaporation, then spray dried to form a powder. A description of the process of manufacturing dried milk powders is diagrammatically illustrated in Figure 3.

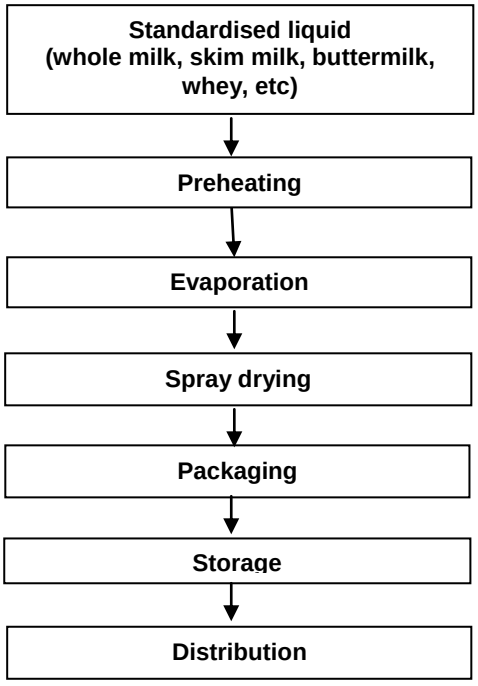

Figure 3: Manufacturing process for dried milk powders

1.3.2 Microbial pathogens of major concern

Microbial pathogens of major concern in dried milk powders include *Salmonella, L, monocytogenes, B. cereus, Cl. perfringens, S. aureus* and more recently *Enterobacter sakazakii.* While these organisms will not grow in powders, they may remain viable for long periods of time and resume growth when the powder is reconstituted and stored at favourable temperatures.

Surveys conducted overseas have shown the presence of *B. cereus* and *E. sakazakii* in dried milk powders, while Australian surveys have detected *Salmonella* spp. and *S. aureus* (Appendix 3).

The microflora of dried milk powders depend on many factors including the number and type of bacteria present in the raw milk or milk by-product, preheating temperatures, operating conditions of the evaporator and dryer, and plant hygiene. High numbers of micro-organisms in the raw milk may result in high numbers in the milk powder. For example, raw milk counts in excess of 10^5 cfu/ml have resulted in counts in the powder of more than 10^4 cfu/g (Varnam and Hall, 1994).

The decline in numbers as a result of exposure to heat, is offset by the removal of water and the concentration of bacteria in the powder. Post-processing contamination is a major factor impacting on contamination of milk powders, as the raw material is often subjected to lethal temperatures, which eliminate vegetative cells of pathogens.

Dried milk powders have been implicated in a number of food-borne disease outbreaks (Appendix 2) involving *Salmonella* and *Cl. perfringens* (ICMSF, 1998; Anonymous, 1982). In an outbreak due to consumption of milk powder contaminated with *S. aureus* (ICMSF, 1998), it was considered likely that illness was a result of preformed *S. aureus* enterotoxin surviving the heating process. Illness has also been attributed to *S. aureus* contamination and abuse of reconstituted non-fat dried milk (El Dairouty, 1989). More recently a large outbreak of illness from *S. aureus* in Japan caused more than 13,000 cases and was due to preformed staphylococcal enterotoxin in the milk powder. This was traced back to poor hygienic and manufacturing practices during processing of liquid milk in particular the storage conditions (Asae et al., 2003).

Outbreaks demonstrate that failures in preventive systems, such as presence of water allowing multiplication or the presence of zones difficult to maintain and to clean (isolation from a drying tower), were the origin of contamination (ICMSF, 1998). In other cases illness has been due to contamination and abuse or reconstituted products. *S. aureus*, if present, may grow and produce enterotoxin when dry milk powder is rehydrated, if it is subject to time/temperature abuse (Umoh et al., 1985; El Dairouty, 1989). *Cl. perfringens* and *B. cereus* are able to produce spores that can survive pasteurisation and survive the manufacture of powdered milk production. They represent a problem when powdered milks are reconstituted and stored for prolonged periods at incorrect temperatures. Most *B. cereus* strains isolated from dairy products are able to grow and produce toxins below 10°C (Institute of Environmental Science & Research Limited, 1995).

Although there have been no outbreaks of listeriosis linked to dry dairy products, the persistence of *Listeria* spp. in the dairy plant environment and the association of listeriosis with other dairy products indicate the potential for *Listeria* contamination of dry dairy products (ICMSF, 1998). There is evidence that *L. monocytogenes* can survive a typical spray-drying process in the manufacture of dried milk powders (Doyle et al., 1985). Although dried milk powders will not support microbial growth due to their low water activity, *L. monocytogenes* is one of the few food-borne pathogens that can grow at refrigeration temperatures and, if present in the dried milk powders, it could possibly multiply when made up and stored in the refrigerator for a long period.

1.3.3 Effect of dried milk powder processing on the growth and survival of microbial pathogens

Before milk is dried, it is submitted to preliminary treatments such as separation, standardization, and concentration. Milk is usually concentrated as a preliminary step in the production of milk powders by evaporation. Milk is usually held at 0-4°C prior to evaporation and consequently requires pre-heating that can vary from <70 - 135°C for 15-30 seconds (TetraPak, 2003) before it can be concentrated/evaporated. Water is progressively removed from the milk to effect the concentration of milk solids. Modern evaporators are predominantly falling film evaporators in which the liquid to be evaporated passes as a film down the inner surface of a vertical steel tube through which heat is transferred from steam applied to the outer surface of the tube.

To minimise damage to heat-sensitive components in milk, evaporation takes place under vacuum with approximate temperatures beginning at 70°C and reaching approximately 45°C at the last stages of evaporation (Gekas et al., 2003; personal communication, 2005).

The concentrate from the evaporation process is then dried. There are two types of drying treatments, spray drying or roller drying. Roller drying is sometimes referred to as drum-drying. Spray drying is now overwhelmingly used in the manufacture of dried milks because concentrated milk does not contact the steam-heated rotating rollers that adversely affect heat sensitive components of milk, especially proteins and lactose (Caric et al., 2003).

The drying temperature varies between 130ºC and 150°C for roller drying and 180-240°C for spray drying (Caric et al., 2003). In the latter situation, the residence time of concentrated milk in the spray-drying chamber is less than 30 seconds. The extent of microbial destruction during drying depends on the types of micro-organisms present and on the drying temperature of the exit air in spray-drying or on the drum temperature and retention time for roller-drying.

Skim milk is subjected to different heating processes prior to drying, depending on the intended use of the product. High heat skim milk powder is usually pre-heated using direct steam injection to temperatures of approximately 115-120°C for 3 minutes. For use in the baking industry, a high temperature preheat (5 minutes at 95ºC) is usual. However, low heat treatments are applied to skim milk powder (15 seconds at 72ºC) which is to be used in cheese making and standardisation of liquid milk.

Vegetative cells of bacteria including those of the family Enterobacteriaceae have been shown to survive drying process in manufacturing milk powder (Daemen et al., 1982). Therefore, milk for drying must be given a heat treatment equal to or greater than pasteurisation prior to drying and then contamination must be avoided after the drying process.

Subsequent processing steps such as cooling, intermediate storage, instantising, blending and packaging may also influence the microbiological quality by increasing the risk of contamination from the production line or the environment. The presence of food pathogens in a properly pasteurised and dried milk powder is indicative of post-process contamination.

During storage of dried milk powder, surviving organisms slowly die, but spore-formers, being the most resistant, retain viability for long periods of time (ICMSF, 1998).

Since the water activity of dried milk powders is too low to permit microbial growth, the occasional microbiological problems with instant milk powder are largely associated with handling during reconstitution. Micro-organisms present in milk powder, or those introduced at reconstitution, may proliferate in case of temperature abuse or mishandling of the reconstituted milk (ICMSF, 1998).

Outbreaks due to *Salmonella* usually share a common factor, the accumulation of contaminated dust and powder deposits in the factory environment, which are eventually, transferred to the product by mechanical fault. The most common hazard reported is the accumulation of powder deposits in the drier insulation, which having become contaminated by environmental salmonellae, gains access to the product via stress cracks in the inner skin of the dryer.

The second most important hazard is due to contaminated air and may occur during the secondary drier stages, transport of powder to silos, or during filling and packing operations (Early, 1998).

The effect of processing on pathogens in dried milk powders is described in Table 4.

Table 4: Effect of processing for dried milk powders on selected pathogens

Pathogen	Effect of processing
Salmonella	*Salmonella* should not be present in dried milk powders as it is destroyed by heat treatment. *Salmonella* may enter powders from foci of contamination within the plant where organisms are able to persist and multiply over extended periods. Contamination can originate from parts of the powder handling system and cracks in the dryer walls and powder storage silos.
Staphylococcus aureus	*S. aureus* is destroyed in the heat process. Organisms present are a result of post-processing contamination or due to preformed toxins surviving the heat-processing step (ICMSF, 1998). Growth and enterotoxin production by *S. aureus* occurs either in raw milk before heat treatment or in the concentrated milk before drying. Staphylococcal toxins in dried milk powder indicate unhygienic ingredients or unacceptable processing conditions.
Listeria monocytogenes	*L. monocytogenes* has been reported to survive a typical spray-drying process (ICMSF, 1998), however, it will not survive heat treatment prior to spray drying. Gradual die-off has been reported for dried milk product after the drying process (ICMSF, 1998), but there is a risk of post-heat treatment contamination as *Listeria* is ubiquitous.
Bacillus cereus	Vegetative cells of *B. cereus* are destroyed in the heat process used to produce dried milk powder, but spores can survive the processing. Generally low numbers of *B. cereus* and/or its spores do not cause problems unless growth is permitted to occur (Doyle, 1989). Spore germination is greatly reduced by unfavourable conditions such as low temperatures, however psychrotrophic strains can grow at 4-5ºC (AIFST, 1997). Growth and toxin production can be prevented by storing reconstituted products at temperatures below 4ºC (AIFST, 1997).
Clostridium perfringens	Although *Cl. perfringens* spores can survive heat-treatment processing, the temperature range for the growth of *Cl. perfringens* is 15-50ºC. At cold temperatures of 0-10ºC, vegetative cells die rapidly (AIFST, 1997).
Enterobacter sakazakii *(infant formulae)*	*E. sakazakii* will not survive pasteurisation and temperatures of 70ºC or above should provide virtually instantaneous inactivation (Codex). Recontamination of powdered infant formulae during handling and filling processes is a risk. Other sources of recontamination are the ingredients added to the formulation. *E. sakazakii* cannot grow in a dry substrate, but it can survive a long period of time and is potential hazard when the powder is reconstituted.

1.4 Infant formulae

1.4.1 Description

Powdered infant formula belongs to a special sub-set of powdered milks. These products are formulated to be as similar to human milk as is possible then concentrated and spray dried. In some cases, specific heat-labile ingredients are added after drying. Typically, infant formulae contain milk, or soy proteins, or protein hydrolysates together with those forms of fat, carbohydrate, vitamins, and minerals that are bioavailable to the infant.

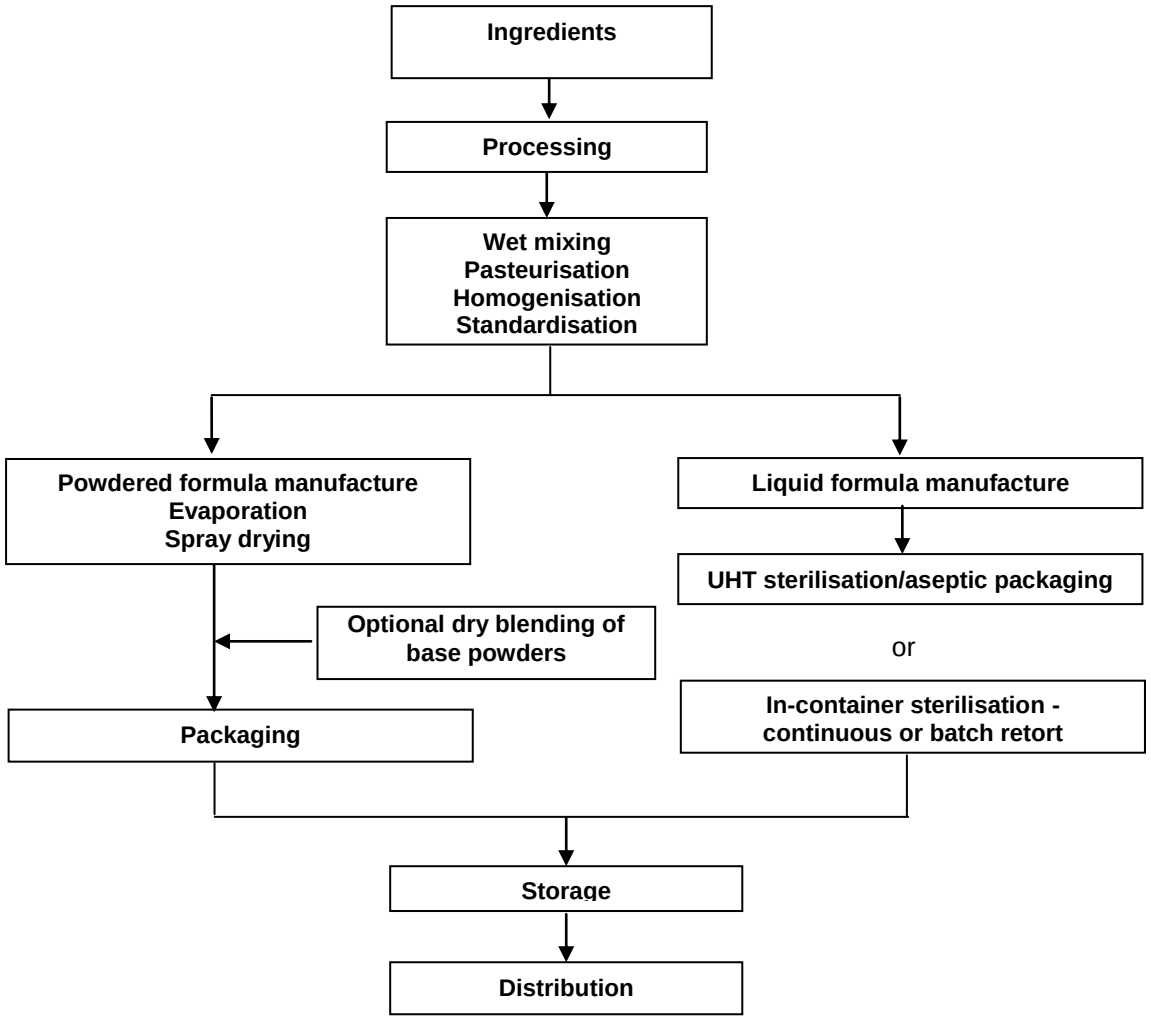

Figure 4: Manufacturing process for infant formulae

1.4.2 Microbial pathogens of major concern

Microbial pathogens of concern with powdered infant formulae are the same as those for dried milk powders, including *Salmonella*, *L. monocytogenes*, *B. cereus*, *Cl. perfringens*, *S. aureus* and more recently *Enterobacter sakazakii*. However, control over the microbiological status of these products is essential because of the vulnerable status of infants.

Surveys of infant formula overseas have indicated the presence of *B. cereus* and *E. sakazakii* (Appendix 3).

Several outbreaks have been associated with infant formulae (Appendix 2). While *Salmonella* is rarely found in surveys of powdered infant formula, low-level contamination of powdered infant formula with *Salmonella* has been epidemiologically and microbiologically associated with infections in infants (Picket and Agate, 1967, Rowe et al., 1987, CDC, 1993, Usera et al., 1996, Threlfall et al., 1998, Olsen et al 2001, Bornemann et al, 2002). .

Illness has also been attributed to *S. aureus* contamination and to abuse of reconstituted infant powdered milk (Umoh et al, 1985).

More recently a growing number of reports has linked *E. sakazakii* infection in infants to powdered infant formula (Biering et al., 1989,=; Simmons et al., 1989; Van aAcker et al., 2001; CDDC, 2002) In several investigations outbreaks of *E. sakazakii* infection has occurred among neonates in neonatal intensive care units. Mortality rates from *E. sakazakii* infection have been reported to be as high as 50% or more, but this figure has declined to <20% in recent years (Codex).

In Australia, an outbreak involving *Salmonella Bredeney* was traced to contamination of powdered milk-based infant formulae (Forsyth JR et al, 2003).

Liquid, ready-to-feed infant formulae are commercially sterile, generally do not support the growth of micro-organisms and are shelf stable.

1.4.3 Effect of infant formulae processing on the growth and survival of microbial pathogens

The manufacture of infant formulae involves the blending of water-soluble proteins, carbohydrates, vitamins and minerals with vegetable oils to achieve a homogenous solution, followed by sufficient heat treatment or dehydration to provide microbiological safety. The final steps in the technology used for infant formula manufacture has seen little change in the past 20 years, as powder manufacture, typically via spray-drying, or heat sterilization (ultra-high heat or retort sterilisation) of liquids are the processes of choice (O'Callagahan and Wallingford, 2002). Development of rewet agglomeration (instantisation) processes in the art of spray-drying has contributed to improved reconstitution ability of infant formula powders. Dry blending of prepared base powders provides flexibility for the manufacture of market-specific formulations, which may include, among others, heat-sensitive components, such as starches, flavours, probiotics or bioactive proteins.

In the dry blending process, the ingredients are received from suppliers in a dehydrated powdered form and are mixed together to achieve a uniform blend of the macro and micronutrients necessary for a complete infant formula product. This process does not involve the use of water in the manufacturing process, and therefore the processing line can be kept dry for long periods of time. In a dry environment, pathogens are denied the water needed to support growth, thereby reducing the possibility of these organisms becoming established in the plant environment in sufficient numbers to cause further product contamination. However, the microbiological quality of a dry-blended product is largely determined by the microbiological quality of the constituent dry ingredients as there is no heat treatment to destroy bacteria in the final product. Thus, if one or more ingredients in a dry-blended product are contaminated with pathogens these bacteria are likely to be present in the finished product.

The wet-mixing-spray drying process involves blending of ingredients, homogenisation, pasteurisation and spray drying to produce a powdered product. The ingredients either in liquid or powdered form are typically mixed with water to form a liquid mix which is dried to a powder in large spray driers. Prior to drying the liquid mix is pasteurised (71.6°C for 15 seconds or 74.4°C for 25 seconds for products containing starches or thickeners or at higher temperatures such as 105-125ºC for at least 5 seconds) (Codex). The severity of the pasteurisation process varies among manufacturers, but is always sufficient to destroy *Salmonella* and *E. sakazakii* and vegetative cells of pathogens such as *S. aureus* and *B. cereus*.

The liquid is homogenised then concentrated by passing through an evaporator or pumped directly to the spray dryer. Prior to spray drying, the product is pre-heated (approximately 82ºC) and passed through a high-pressure pump to the spray dryer nozzles. The inlet air temperatures of spray towers are normally between 150ºC and 220ºC (Becker *et al.*, 1994). Due to the cooking of the rapidly evaporating water, the inner temperature of the sprayed particles only reach 40-50ºC (Kessler , 1988) or up to 70ºC (Eschamann, 1970).

Enterobacteriaceae are ubiquitous in the processing environment. *E. sakazakii* is relatively resistant to osmotic and dry stress compared with other members of the Enterobacteriaceae family (Breeuwer *et al.*, 2003). The survival of *E. sakazakii* at elevated temperatures (45°C) and its capacity for growth up to 47°C, illustrate that in warm and dry environments, such as in the vicinity of drying equipment this bacterium has a competitive advantage compared with other members of the Enterobacteriaceae. Condensation in the drying and filling areas can lead to an increase in the normally very low numbers of *E. sakazakii* n that environment.

After spray drying the product may be agglomerated to increase the particle size and improve its solubility. Accidental microbiological contamination can occur during the agglomeration process. The finished powder is sifted and packaged. Airborne *E. sakazakii* can re-contaminate the powder during the handling and filling processes.

Bactofugation is used by some producers and removes bacteria, especially spores (up to 95%), from milk by high speed centrifugation.

The spray drying process requires processing equipment to be regularly wet cleaned, therefore providing a moist environment for bacterial growth in the plant environment. If not controlled, these bacteria can be a source of product contamination. *B. cereus* spores and *E. sakazakii* bacteria are able to adhere to several types of surfaces and hence it is difficult to remove them from equipment during cleaning. These spores also possess appendages and/or pili that are, at least in part, involved with adhesion (Doyle *et al.*, 1997).

During storage of infant formula, surviving organisms slowly die, but spore-formers, being the most resistant, retain viability for long periods of time (ICMSF, 1998).

The effect of processing on pathogens for infant formulae is the same as that for dried milk powders as described in Table 4.

1.4.4 Effect of preparation of infant formulae and possibility of growth of microbial pathogens after reconstitution

Recontamination of infant formulae with *E. sakazakii*, may take place during preparation or reconstitution of infant formulae due to poorly cleaned baby bottles and poorly maintained equipment at home and hospitals (Codex doc).

Incorrect storage and temperature abuse of reconstituted infant formulae may lead to multiplication of pathogens such as *E. sakazakii*, *B. cereus*, and *S. aureus* if present.

1.5 Concentrated milk products

1.5.1 Description

Concentrated milk products have reduced water content and include evaporated milks and sweetened condensed milks. Sweetened condensed milk is characterised by its high sugar content, which varies from 61-64% calculated as sucrose/(sucrose + water) in the product (Nieuwenhuijse, 2003b). Unlike evaporated milk, which is preserved by heat treatment (UHT treatment or sterilisation), sweetened condensed milk is preserved by its sugar content. Figure 4 shows the principal processing steps involved in the manufacture of concentrated milks.

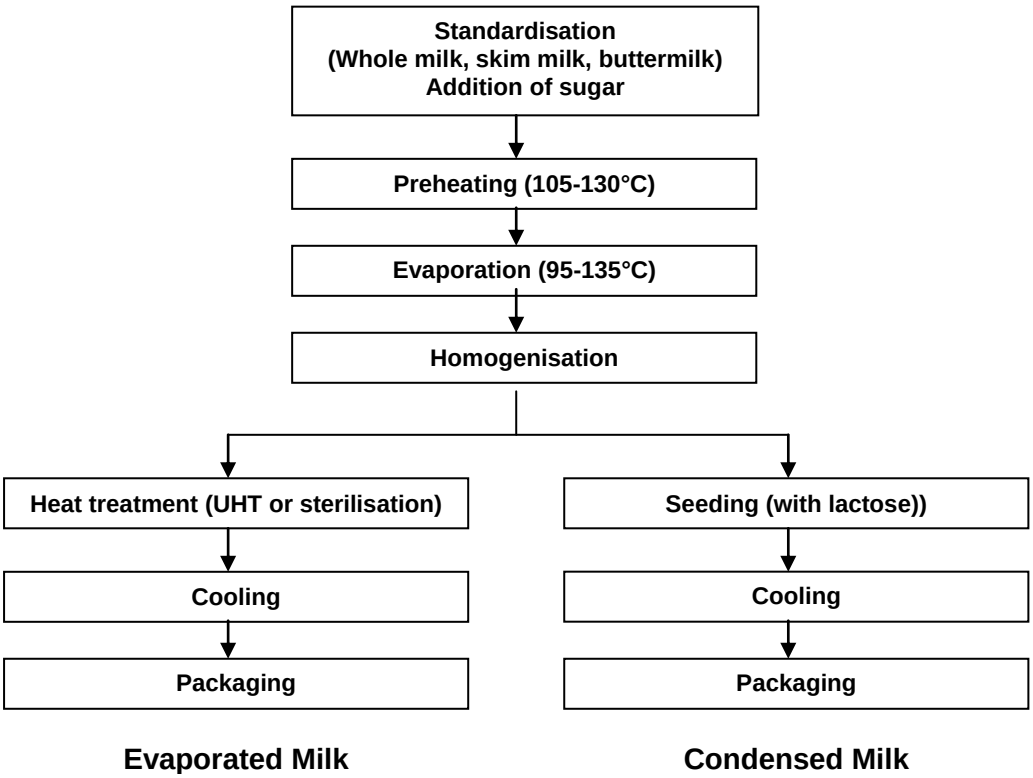

Figure 5: Indicative steps in the manufacture of evaporated milk and sweetened condensed milk

1.5.2 Microbial pathogens of major concern

No reported cases of food-borne disease outbreak have been attributed to the consumption of sweetened condensed milk or evaporated milk (ICMSF, 1998). These products generally do not support the growth of micro-organisms and are shelf stable.

The main microbiological concern with evaporated milk is primarily non-pathogenic thermophilic spore-forming bacteria such as *Bacillus stearothermophilus*, which spoil the product (Nieuwenhuijse, 2003a). The main concern for sweetened condensed milks is *S. aureus*.

1.5.3 Effect of processing of concentrated milk on the growth and survival of microbial pathogens

Both evaporated milk and sweetened condensed milk have had a considerable portion of the water removed through an evaporation process. For evaporated milk, the milk is initially preheated to temperatures between 110-130°C for 1-3 minutes (Nieuwenhuijse, 2003a) then concentrated in a multistage falling film evaporator at 50-95°C. Milk is homogenised, heat treated and cooled before being packed into cans. Alternatively the product is homogenised and canned, then heat treated.

For sweetened condensed milk, milk is preheated to temperatures are between 105-120°C for 15-60 seconds. Preheating applied in producing sweetened condensed milk is aimed at inactivating all enzymes, osmophilic yeasts, micrococci and moulds, and to regulate viscosity. Sweetened condensed milk is concentrated also in a multistage falling film evaporator at 35-50°C. Sugar can either be dissolved in cold milk before preheating, or added as syrup after preheating or at the end of evaporation. Following evaporation, the concentrate milk is homogenised and then seeded with lactose to prevent the formation of large crystals. Sweetened condensed milk does not get subjected to a heat treatment process such as UHT or sterilisation that is applied to evaporated milk and is not commercially sterile (Nieuwenhuijse, 2003b).

The preheating treatment of evaporated milk and sweetened condensed milk through a continuous flow heating is considered to be equal to or better than the process of pasteurisation and destroys the vegetative forms of microbial pathogens, but not bacterial spores. Subsequent heat treatment, either UHT or sterilisation after homogenisation and stabilisation in the manufacture of evaporated milk destroys any remaining micro-organisms, including spores, and leads to a product of commercial sterility. In the case of sweetened condensed milk, pathogenic or spoilage micro-organisms are unlikely to proliferate because of its high sugar content and thus low water activity (Nieuwenhuijse, 2003a; Nieuwenhuijse, 2003b).

Although sweetened condensed milk is not a sterile product, the low water activity (between 0.83-.85) makes it unlikely to support the growth of pathogenic bacteria. Likewise, spores of *Clostridium* and *Bacillus* spp. present in sweetened condensed milk will also not be able to grow (Nieuwenhuijse, 2003b). The exception is *S. aureus*, which can grow at a water activity of around 0.85. However vegetative cells of *S. aureus* will not survive the pre-heat treatment given to sweetened condensed milk, and growth and toxin production of any spores is severely limited because of the anaerobic environment of sweetened condensed milk (ICMSF, 1998).

1.6 Butter and butter products

1.6.1 Description

Butter is produced from cream by churning or an equivalent process. Butter spreads are based on vegetable fats, a blend of vegetable and butter fat, or butterfat alone (light butter). The main steps in the manufacturing butter are illustrated in Figure 5.

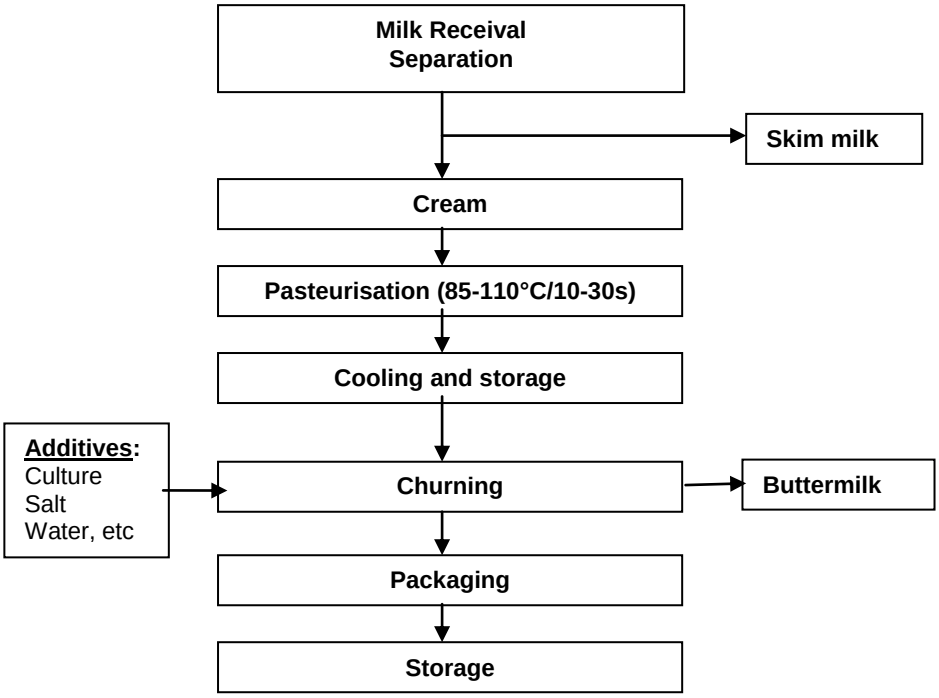

Figure 6: Indicative Manufacturing process for butter

1.6.2 Microbial pathogens of major concern

While butter represents a dairy product of low risk to public health there have been incidents of food-borne illness attributed to this product. Staphylococcal food poisoning has been traced to whipped butter in the United States, although temperature abuse was a contributory factor (ICMSF, 1998; Varnam and Sutherland, 1994).

There have also been two outbreaks of listeriosis linked to the consumption of butter. *L. monocytogenes* was isolated from several points in a production facility packaging small butter packages in an outbreak in Finland in 1998-1999 (Eurosurveillance, 1999). More recently a cluster of listeriosis cases occurred in England implicating butter (CDR Weekly, 2004). Mishandling may have been a contributing factor in this outbreak.

In addition in the US, there have been several recalls issued for *L. monocytogenes* contaminated butter (Ryser and Marth, 1999).

1.6.3 Effect of butter processing on the growth and survival of microbial pathogens

The process of butter manufacture begins with the separation of milk into cream and skim milk. The cream is typically pasteurised at 85°C for 15 seconds under vacuum (ICMSF, 1998). Pasteurisation destroys vegetative micro-organisms, especially pathogens, but will not eradicate bacterial spore-formers and some of the more heat-resistant vegetative spoilage flora (ICMSF, 1998). Preformed *S. aureus* enterotoxin resulting from poor sanitary conditions in the pre-pasteurisation stage may carry over to butter.

The pasteurised cream is then churned, a process where fat distributed in the aqueous phase of cream is converted into a substrate where water is dispersed through the butterfat.

Additives such as flavour concentrates and seasonings such as garlic or herbs may be added during this stage.

During the butter making process, micro-organisms are retained in the aqueous phase, the buttermilk, which is drained from the butter. The butter continues to be worked mechanically to create the right physical properties, with water being dispersed into minute droplets generally less than 10 µm in diameter in the fat matrix (ICMSF, 1998).

The microbiological stability of butter depends on its moisture content; the physical distribution of the aqueous phase and its nutrient content, and the presence, in the aqueous phase, of inhibitors (ICMSF, 1998; Varnam and Sutherland, 1994). Typically butter contains around 16 % moisture, and 2 % salt in salted butter. The extent of microbial growth is severely restricted by the very tiny physical size of the water droplets and extensive multiplication is not possible and will start to die off (Varnam and Sutherland, 1994; ICMSF, 1998). Salt is also an important inhibitor in some butters and at 2 % (will equal 12.5% in the aqueous phase) will be strongly inhibitory to most micro-organisms (Frede, 2003; Varnam and Sutherland, 1994).

Unsalted or low- salt butter is much more likely to support the growth of spoilage micro-organisms than salted butter as it presents a more favourable substrate for bacteria survival. Low fat spreads have a higher moisture content which requires that the droplet distribution be adequately spread throughout the product. Ideally, the moisture droplets should be in the range of 1µ - 10µm to reduce spoilage and ensure product safety. Any increase in the size of moisture droplets provides greater opportunity for microbial growth which places a greater reliance on any microbial inhibitors that may be present.

Butter does not appear to be a good growth medium for *L. monocytogenes,* as salt added during manufacture and distributed in the water phase is at or close to the limit for growth at refrigeration temperatures. However, growth has been demonstrated experimentally in butter during storage and it appears that *L. monocytogenes* favours the water rather than the lipid phase during butter making (Ryser and Marth, 1999). This is supported by the outbreaks and recalls that have been associated with *L. monocytogenes* and butter.

Anhydrous milk fat (AMF) is a purified form of butterfat made directly from cream or butter by centrifugal separation. The product has exceeding low moisture levels (0.1 – 0.3 %) and will not support the growth of bacteria. It is mixed with skim milk powder in the reconstitution of liquid milk. AMF may be further separated into various fat fractions, based on melting point, by fractional crystallisation. These products can be used in combination with skimmed milk powder to produce various milk products, and are used in the baking, confectionery, ice-cream and chocolate industries.

1.7 Ice-cream

1.7.1 Description

Ice-cream is a frozen aerated emulsion made from cream or milk products or both, and other food components. Manufacture of ice-cream involves the preparation of an ingredient mix comprising milk fat, milk solids, sweetener, water and other ingredients which are pasteurised and homogenised, aged, then whipped to incorporated air while being frozen. The final product is then packaging and hardened during frozen storage prior to distribution (Goff, 2003).

Other types of ice cream are available in many forms, flavours and packages. Different products prepared both from edible fats and milk or milk products; include gelati, soft serve, stick ice creams and confections, *etc.*

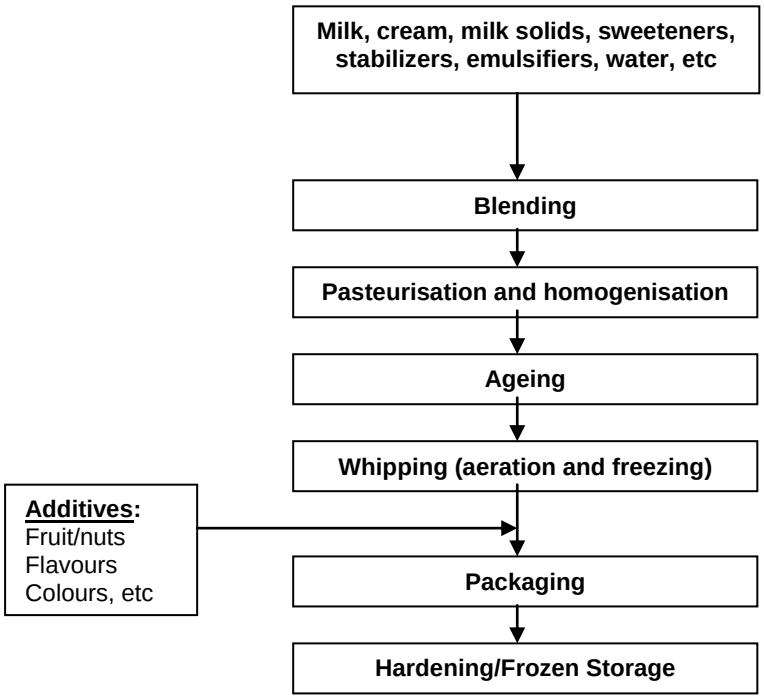

Figure 7: Manufacturing process for ice-cream

1.7.2 Microbial pathogens of major concern

While pathogenic bacteria will not grow in ice-cream, some pathogens, if present, may survive long periods of frozen storage. Therefore, any pathogens present in ice-cream as a result of post-process contamination may pose a potential hazard to consumers.

The major microbial pathogens of concern associated with ice-cream are *L. monocytogenes, Salmonella* spp. and *S. aureus*, (ICMSF, 1998). Reported outbreaks of food-borne illness attributed to ice-cream have typically involved home-made ice-cream where raw milk and/or raw egg were used and the heat treatment was inadequate (Taylor et al., 1984).

Outbreaks involving consumption of commercially manufactured ice-cream have been found to be the result of post-processing contamination. In the 1994 outbreak of *S. enteritidis* food-borne illness, a transport tanker previously used to transport unpasteurised raw egg was used to transport ice-cream mix that was not subsequently repasteurised (Hennessy et al., 1996).

Several recalls of ice-cream due to contamination of *L. monocytogenes* have occurred in the US since 1985. However no direct link to listeriosis has been documented. In Australia, chocolate-coated ice-creams were recalled in 1995 because of *L. monocytogenes* contamination.

An outbreak of *S. Oranienburg* associated with gelati was reported in South Australia in 1998. Contamination of the gelati most likely resulted from equipment contaminated with the *Salmonella* (Milazzo *et al.*, 1998).

Gelati differs from ice-cream in that it has a very low (dairy) fat content (varying from 1.4 - 8%). Milk based gelati also has less air incorporated (approximately 35-40%) compared to ice cream which is approximately 50%.

Aeromonas spp. and *Br. abortus* have been detected in surveys of ice cream and ice cream products overseas, while *L. monocytogenes* has been detected in surveys of ice cream in Australia (Appendix 3).

1.7.3 Effect of ice-cream processing on the growth and survival of microbial pathogens

A wide range of ingredients are blended to prepare the liquid ice-cream mix. This mix is then subjected to a heat treatment process to reduce bacterial numbers, and specifically to destroy pathogenic organisms (Goff, 2003). Time and temperature of processing is typically greater than that for pasteurisation because of the high fat and high solids content of ice-cream mixes.

The heat treated mix is then homogenised to enhance the body and texture of the frozen product by reducing the size of fat globules in order to prevent the fat from churning during the freezing process. Flavours may be added before or after pasteurisation and homogenisation. However, colours, fruits, confectionery, chocolate and nuts are generally added after pasteurisation, and these may represent a source of contamination of the final products.

The pasteurised mix is cooled to 4°C or lower and aged to allow physical changes to occur. After aging, the ice-cream mix is frozen, unless it is to be used for soft-serve ice-cream. Hardened ice-cream is frozen in a two step process. The first step involves partial freezing of the mix to -5° to -8°C while the air is beaten into the mix. The partially frozen mix is packaged and immediately placed in a hardening room or freezing tunnel where it is frozen to -25° to -30°C (ICMSF, 1998).

The microbiological quality of ice-cream depends upon the interaction of factors such as the:

- microbiological status of the ingredients and additives;
- processing conditions (*e.g.* heat treatment) which the mix has been exposed to during the manufacture of ice-cream; and
- hygienic control and cleaning of the manufacturing equipment (Robinson, 1985) and the hygienic status of packaging materials.

Ingredients used in the manufacture of ice-cream must be of a high microbiological standard. Milk, cream, skim milk and skim milk concentrate should have been heat treated, must be kept under refrigeration, and used promptly to ensure satisfactory quality. The main organisms present after heat treatment will be spore-forming bacilli, although there may be some psychrotrophic organisms surviving if the initial population was high, together with some micrococci and other thermoduric bacteria. Normally, none of these groups constitute a health hazard (Robinson, 1985).

Butter and butter oil (anhydrous milk fat) are made from heat treated cream under carefully controlled conditions, and should be of good microbiological quality.

Granulated sugar, glucose syrup solids and dextrose should be almost free of contaminating organisms. Similarly, sugar syrups should also be virtually sterile.

Emulsifying and stabilising agents could prove a hazard unless purchased from a reputable supplier and kept under good storage conditions (Robinson, 1985).

Other ingredients that are added to ice-cream or used as coatings may be added after heat-treatment of the mix and may introduce potential hazards. These ingredients include fruits (canned, fresh, or frozen and usually in concentrated sugar syrups), nuts, chocolate, pieces of toffee and biscuit, colours and flavours and may contribute significant contamination (ICMSF, 1998). Careful control of these ingredients is essential (Robinson 1985) although they are often difficult to decontaminate.

Heat treatments applied to ice-cream mixes are frequently more severe than pasteurisation requirements for liquid milk. As a result, vegetative cells are normally destroyed, with spores usually the only survivors. From survey data on industry pasteurisation practices in Australia HTST treatment of ice-cream mixes were in the range of 78-85°C for 13-45 seconds[37].

Where pathogens are present in ice-cream they may survive for many months. Ice-cream is stored frozen from the time of manufacture to the time of consumption. The low temperature of frozen ice-cream completely prevents microbial growth (ICMSF, 1998) but pathogens may survive.

Soft-serve ice-cream presents a special case as the mix must be transported to retail soft-serve outlets where it is stored until soft-frozen and dispensed to consumers. Contamination and temperature abuse of the mix may easily occur, plus procedures for the cleaning and sanitation of the freezer and associated equipment often are inadequate. Soft serve ice-cream is usually drawn from the freezer at about -6°C to -7°C (ICMSF, 1998).

The microbiological safety of ice-cream is ensured by eliminating vegetative pathogens by pasteurisation and the prevention of recontamination at all stages until the point of sale; control of the microbiological status of ingredients; and the prevention of microbial growth before freezing (Varnam and Sutherland, 1994). Pathogens are unable to grow in ice-cream/confection when stored at correct temperatures.

1.8 Cultured and fermented milk products

1.8.1 Description

Yoghurt and fermented milk products are prepared by fermentation of milk or milk products using specific micro-organisms that reduce the pH and coagulate milk proteins. Yoghurt is characterised by fermentation with thermoduric *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* with or without other lactic acid producing bacteria. Fermented milk products include yoghurt, cultured buttermilk, cream (sour cream), and acidophilus milk (Surono et al., 2003).

Figure 8 shows the basic steps involved in manufacture of stirred-style yoghurt. Manufacturing processes for other fermented milk products vary from product to product, but the common steps are fermentation of pasteurised milk with or without addition of flavour substances.

[37] Pasteurisation times and temperatures are from the ***Scientific Evaluation of Pasteurisation and Alternative Processes for Pathogen Reduction in Milk and Milk Products Report***

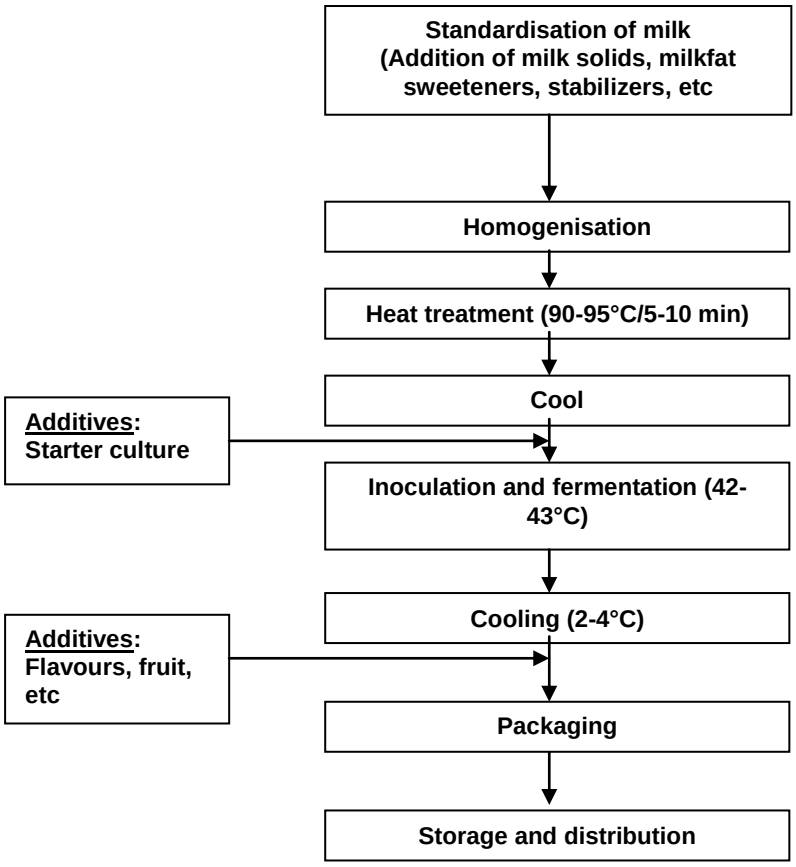

Figure 8: Indicative manufacturing process for stirred-style yoghurt

Products range from traditional yoghurts, to drinkable yoghurts, kefir, yoghurt cups and yoghurt tubes.

1.8.2 Microbial pathogens of major concern

Fermented products are rarely associated with food-borne disease as their pH is too low and the lactic acid concentration too high to permit growth of vegetative pathogens and death of non-growing cells is likely to be rapid (Varnam and Sutherland, 1994). However, consumption of yoghurt containing large numbers of yeasts can lead to digestive disturbances.

The limited outbreaks of food-borne illness that have been reported typically have involved *S. aureus, Cl. botulinum* and *E. coli* 0157:H7 (ICMSF, 1998; O'Mahoney et al., 1990; Morgean et al., 1993).

Slow growth by the starter culture provides an opportunity for growth of pathogens that contaminate the milk or ingredients, for example, staphylococcal toxin may accumulate in the ingredients where too much sugar inhibited the growth of starters but not the growth of *S. aureus*, resulting in illness (Mocquot et al., 1970). In another yoghurt outbreak, under processing of canned hazel-nut puree used to flavour the yoghurt caused growth and toxigenesis of *C. botulinum* spores in the puree. In addition the sugar in the ingredients was replaced by aspartame, leading to an increase in water activity to a level allowing growth of the pathogen (O'Mahony et al., 1990).

From a number of microbiological surveys of cultured and fermented milk products identified in the literature, only one reported the positive identification of a pathogen (*Y. enterocolitica* in fermented cow's milk) (Appendix 3).

1.8.3 Effect of cultured and fermented milk processing on the growth and survival of microbial pathogens

Fermented milks can be subdivided into three groups: lactic bacteria, lactic yeast and lactic mould fermentations, which are based on the metabolism of the respective groups of micro-organisms. All products are the result of fermentation of lactose into mainly lactic acid. Yoghurt is the dominant fermented milk product on the retail market.

Sour cream (or cultured cream) is manufactured using *Streptococcus lactis* as a starter culture. The process for making sour cream is largely equivalent to that of other fermented products. Cream is standardised, homogenised, heat treated for 5 minutes at 90°C, cooled, inoculated and packaged. The final pH value of a freshly produced sour cream is about 4.5 (Hoffmann, 2002).

Fermented milks based on probiotic strains are processed in a similar manner to yoghurt. The most commonly used strains include *Bifodobacterium* spp., *Lb. acidophilus* and *Lb. casei*. A specific starter medium is used to grow the fastidious organisms.

The initial steps involved in the manufacture of fermented milks are the same as those applied to milk (*i.e.* homogenisation and heat treatment). Most of the manufacturing process for cultured and fermented milk products involves a fortification step where different solids are added to the milk. Skim milk powder or whey protein concentrates are most widely used, although alternative protein sources are used for supplementation *e.g.* ultrafiltration retentate.

Heat treatments of blended mix prior to the addition of starter culture vary from HTST pasteurisation to a full UHT process. After cooling to the desired optimal fermentation temperature, starter cultures are added.

Sweeteners, colouring and flavouring are usually added after pasteurisation, but may be added pre- or post-fermentation. Fruit and nuts added to yoghurt are usually supplied as heat-treated purees in large cans or bulk containers for direct connection to the yoghurt handling line. Additions to stirred yoghurt[38] are made after fermentation, but in the case of set yoghurt[39] a layer of fruit in a viscous gel is placed in the container before addition of the inoculated ingredient mix.

The ingredient mix is fermented until the desired pH is reached, typically around a maximum pH of 4.5. Growth of the inoculated organisms and further acidification are minimised by cooling. The time taken to reach the required pH varies depending on whether a short or long set regime is used in manufacture. Most modern large-scale production uses the 'short-set' method, in which starter culture is added at 2%, permitting the fermentation to be completed within four hours at an incubation temperature of 42-43°C.

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[38] With **stirred yoghurt**, the background flavour of yoghurt is usually modified by the addition of fruit/flavours and sugar (Robinson, 2003), the fermentation is usually carried out in bulk, and the curd formed as a result of fermentation is stirred and cooled before being packaged into retail cartons.

[39] **Set yoghurt** is fermented in its retail cartons and the yoghurt formed as a result of fermentation has a firm, gel-like structure together with a clean, mildly acidic and slightly aromatic flavour (Robinson, 2003).

However, a small amount of yoghurt is still made using a long-set process, in which starter culture is added at 0.5% and the fermentation continues for 14-15 hours at 30°C (Varnam et al., 1994).

Acidophilus milk is obtained after slow fermentation with *Lb. acidophilus*. Due to the poor competitiveness of the starter, a high heat treatment or even UHT is required to eliminate spore-forming micro-organisms. The milk is cooled and inoculated with the starter and incubated for up to 24 hours.

Other fermented milks including traditional products such as Kefir and Koumiss, which are based on a combined fermentation of lactic acid bacteria and yeasts and are characterised by the presence of ethanol up to 2% and CO_2. A similar product is the Finnish yoghurt Viili that is made from the combination of mould and lactic acid bacteria. Concentrated or strained fermented milk products such as Ymer (Denmark), Skyr (Iceland) and Labneh (Lebanon) are popular in their respective regions. The whey in these products is drained off after fermentation.

Yeasts can become a problem in fermented milk products. The high moisture content of these products can allow excessive yeast growth causing gas production. Yeasts are not inhibited by the acidic conditions found in fermented dairy products (Stanly, 1998).

Pasteurisation is sufficient to kill vegetative cells including *Salmonella* and *Campylobacter* and the rapid development of the starter cultures is sufficient to inhibit outgrowth and development of spore-formers (ICMSF, 1998). The low pH, the presence of lactic acid and other organic acids as well as, in certain cases, inhibitory compounds such as bacteriocins generate an unfavourable environment for pathogenic organisms(ICMSF, 1998). However, if slow-fermenting strains are used, the outgrowth of spore-formers can occur and sterilisation or UHT treatment of the milk is necessary (ICMSF, 1998).

Fermented milks are stored at refrigeration temperatures after production, during distribution and during storage in the home. Low temperature storage minimises growth of surviving microbes and those present due to possible post-process contamination (Robinson, 2003).

Production of high-quality fermented products relies on application of GMP and reliable HACCP programs. Milk must be pasteurised and the fruits and flavours added after pasteurisation must be of high quality and free from vegetative pathogenic bacteria.

1.9 Dairy desserts

1.9.1 Description

In recent years there has been rapid proliferation in the range of dairy-based desserts available in the marketplace. These are typically branded, ready-to-eat products that are sold through retail outlets such as from supermarket cabinets with products ranging from medium to long shelf-life. These products often include probiotic bacteria, fibre, vitamins, minerals, and include flavours and colours that appeal to children and adults.

Dairy-based desserts include acidified and non-acidified products. Examples of these types of products include custards, crèmes and mousses, crème fraiche, puddings and sachet desserts.

Difficulties with differentiation are increasingly common with a blurring of the lines which differentiate yoghurt (fermented) products, from crème desserts, and products containing probiotics.

Dairy-based desserts can be based on fresh milk (skim or full-fat), milk powder (skim or whole) or on milk protein concentrates. Flavours, colours and sweeteners may be added, along with a wide variety of hydrocolloid thickening agents to improve texture, of which, starches and carrageenan are most common. Other additives used include emulsifiers and binding agents.

A typical flow sheet for the manufacture of these products includes the following processing operations:

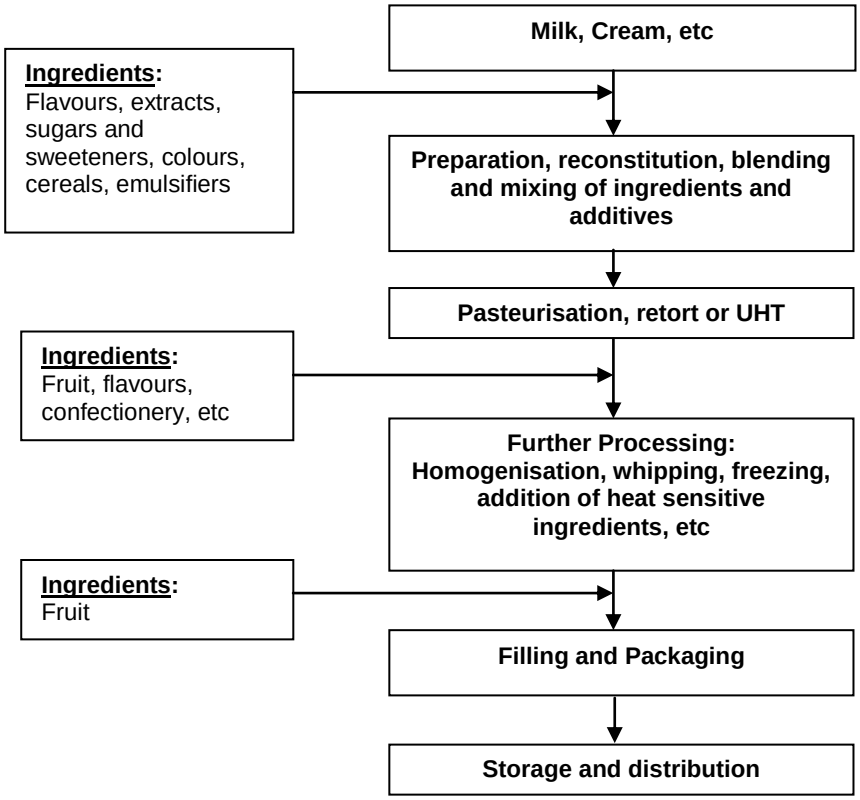

Figure 9: Major steps in the manufacture of dairy-based desserts

1.9.2 Microflora of major concern

The microbiological profile of these products is extremely varied, reflecting the nature of the ingredients incorporated into these products and variations in the preservation and processing operations employed in their manufacture.

Published microbiological data on these types of products is limited. Survey data typically indicates these products conform to national regulations (Pable-Busto, 2000) and are of acceptable microbiological quality (Rodriguez *et al*., 1994).

In determining the potential pathogens associated with these products, the typical microflora associated with milk and creams are combined with microflora originating from ingredients that vary from fruit to flavourings. Of particular concern is the survival of spores from *B. cereus* in the milk or presence their in ingredients such as thickeners.

1.9.3 Effect of processing on the growth and survival of microbial pathogens

The manufacturing process for dairy desserts involves heat and mechanical treatments.

The manufacture of ready to eat dairy desserts involves three basic steps, mixing of the ingredients into a homogenous fluid, heat treatment of the fluid and filling of the product into containers. Mousse-type products also require an aerations step before filling into containers.

Three basic types of heat treatment are used in the production of dairy desserts including pasteurisation, retort sterilisation, or UHT. Pasteurisation temperature/time combinations will vary depending upon the solids content, but must be sufficient to achieve product safety. Risks are also influenced by whether the product is hot or cold-filled. Shelf-life varies from about 3-28 days at 7ºC and may be shorter if cold filling is used.

Retort sterilisation is used for canned custards, while UHT treatments are used for long life creamy desserts. The time/temperature combination for UHT products is approximately 140ºC for 3 seconds, and the product usually undergoes preheating before the UHT treatment is applied. Product is generally packed aseptically with either hot (around 70ºC) or cold (<7ºC) filling.

Dry-mix sachet dairy desserts generally consist of milk powder, starch texturiser (generally carragenan), sugar, flavour and colour and are manufactured simply by dry blending.

The major risk in chilled dairy desserts is that they will become contaminated with pathogens which could grow during the products shelf-life. Components of this type of product such as cream and custards, are by formulation (pH and water activity) and method of manufacture (i.e. exposed to the factory environment) high risk. Custard and cream rely on proper heat treatment to eliminate pathogens that may be present in the raw materials used. Where these products are heat treated, non-sporeforming vegetative cells will be destroyed whereas, spores of *B. cereus*, may surive and become activated. The rapid cooling of products that have a heating step will help prevent growth of these spores. Another major public health concern can arise from post-pasteurisation contamination, particularly from heat labile ingredients and during filling and packaging. Points in the process where product can become re-contaminated is during assembly of the final product. Items such as roasted nuts added as decorative toppings to desserts can also be a route of contamination.

In addition, great care must be taken to avoid the addition of psychrotrophic bacteria such as *L. monocytogenes* which may grow during prolonged refrigerated storage. Another concern relates to spores of psychrotrophic *B. cereus* that may survive pasteurisation and grow and elaborate toxin during the extended storage of some types of dairy desserts (Beattie and Williams, 2002).

A risk to the consumer from these products if contaminated, is if they are temperature abused and consumed at or beyond end of normal shelf-life.

With the trend toward extended shelf-life dairy based desserts marketed in the chill chain, aseptic or ultra clean fillers are used, however in Australia UHT and hot-fill is more commonly used. These products comprise multilayed mousse type desserts that are heat processed at conditions between traditional pasteurisation and UHT conditions for ambient stable products. Where these products are UHT treated, vegetative cells and spores of pathogens are destroyed, and the product is typically shelf stable for extended periods of time.

1.10 Dairy-based dips

1.10.1 Description

As with dairy desserts there has been an increase in the number of dairy-based dips in the marketplace. These products are very diverse and typically ready-to-eat commodities and are sold from cabinets in retail outlets.

Dairy-based dips range from processed cheese-type products and starch-thickened bases flavoured with cheese solids to sour cream or yoghurt based and flavoured dips. A wide range of condiments can be added to the dairy dip base including herbs and spices, dehydrated vegetables and flavouring agents. These products range from medium to long shelf-life.

A typical flow sheet for the manufacture of these products includes the following processing operations:

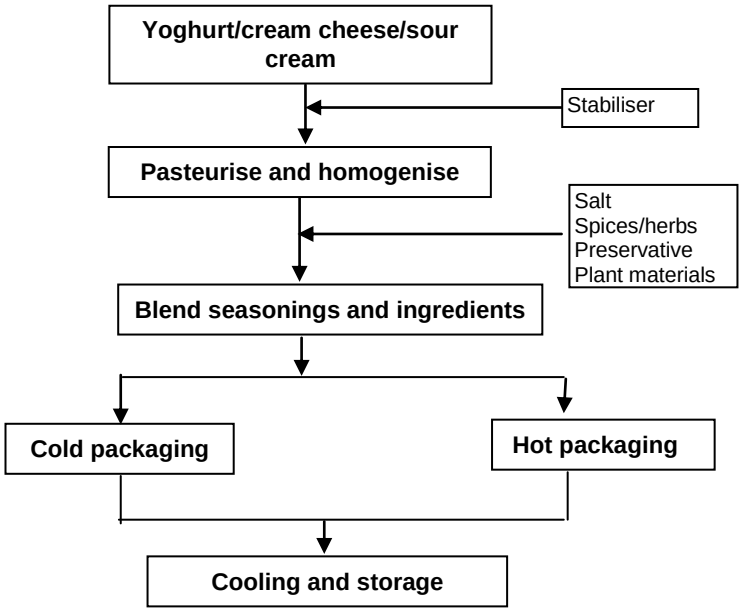

Figure 10: Major steps in the manufacture of dairy-based dips

1.10.2 Microflora of major concern

The microbiological profile of these types of products is extremely varied, reflecting the nature of the various components incorporated into these products and variations in the preservation and processing operations employed in their manufacture. Cold filling is frequently practiced, and careful management is essential to avoid contamination. This is

especially important where heat labile ingredients are added to the product after a terminal heat process.

Published microbiological data on these types of products is limited. Survey data typically indicates these products conform to national regulations (Pable-Busto, 2000) and are of acceptable microbiological quality (Rodriguez *et al*., 1994).

In determining the potential pathogens associated with these products, the typical microflora associated with milk and creams are combined with microflora originating from ingredients that vary from vegetables and fruit to flavourings, herbs and spices. Where heat labile ingredients are added after a heat treatment steps, great care must be taken to avoid the addition of psychrotrophic bacteria such as *L. monocytogenes*.

1.10.3 Effect of processing on the growth and survival of microbial pathogens

Sour cream-based dips can either be produced using a traditional culturing of a cream base, or by direct acidification. Both processes drops the pH to a range of 4.5 – 4.75. Heat treatments and homogenisation are applied to the sour cream. At this pH range, growth of vegetative pathogens is unlikely. In addition the presence of lactic acid is inhibitory to vegetative pathogens. Other additives such as potassium sorbate are also often used as a preservative which imparts an antimicrobial effect. Stabilisers are also added to cultured cream to prolong shelf life.

Cultured products can be heated and packaged (hot-fill) at high temperatures to obtain and extended shelf-life (up to 120 days), or cold-filled resulting in a much shorter shelf-life (e.g. 45 days).

The pH in yoghurt based dips is ranges from 4.1 to 4.5, and thus is also inhibitory to vegetative pathogens.

Some cheeses-based dips have the right combination of solids, salt and pH to inhibit bacterial growth, creating a shelf-stable product. Other shelf-stable cheese dips typically undergo a heat process, such as retorting.

Where these products are pasteurised, non-sporeforming vegetative cells will be destroyed, and the major public health concern arises as a result of post-pasteurisation contamination, introduced in heat labile ingredients and during filling and packaging operations.

1.11 Casein, whey products and other functional milk derivatives

1.11.1 Description

An increasing awareness of the nutritional and health benefits of dairy products has driven the development of markets for a wide array of functional and nutritional ingredients derived from milk. Improvements in fractionation technologies have allowed the manufacture of these on a commercial basis from surplus milk and other dairy by-products.

The production of functional milk derivatives is summarised in Figure 11. The process typically uses pasteurised milk as a starting material. Separation of milk into cream and skim milk leads to processes for enrichment of components derived from the fat- and protein-enriched fractions, respectively.

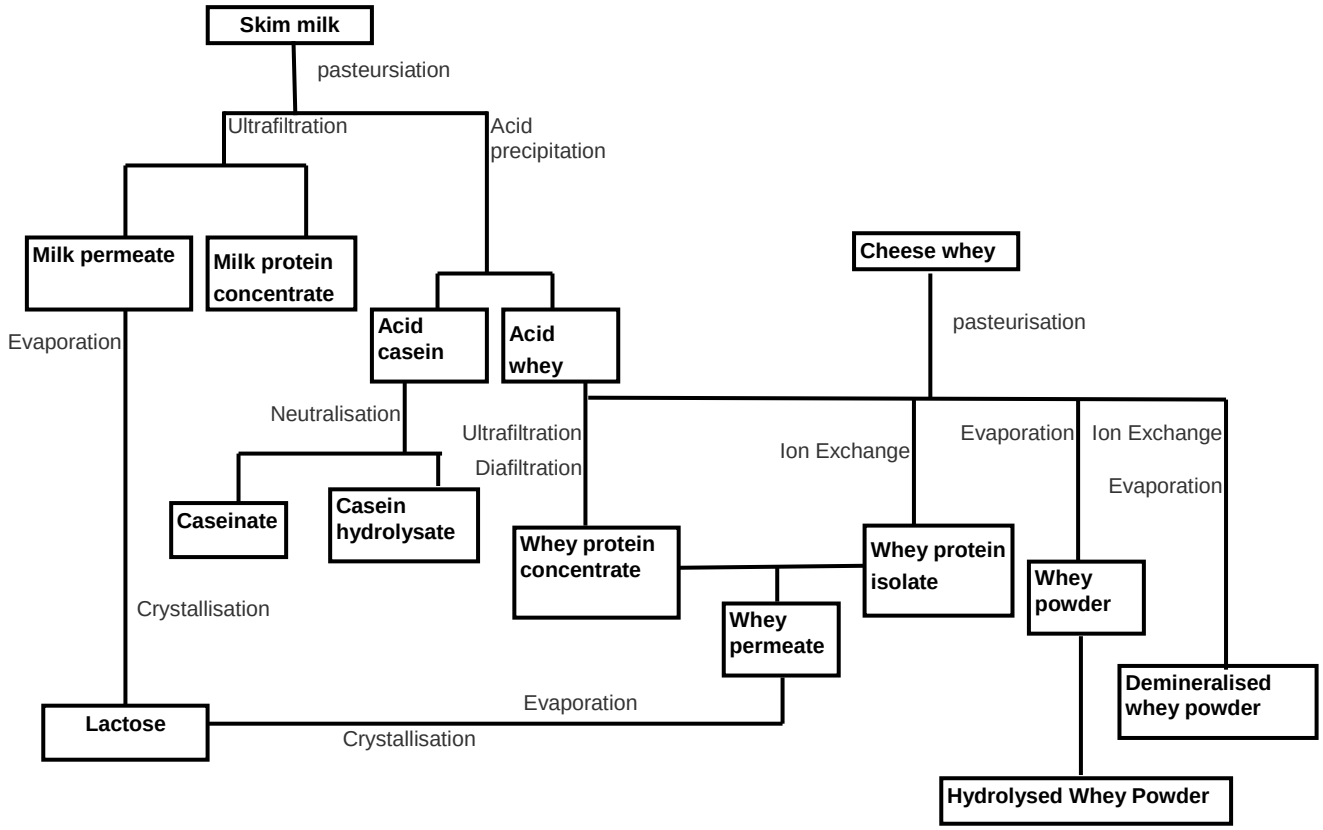

Figure 11: Indicative steps in the manufacture of casein, whey products and functional milk derivatives

For production of casein and caseinates, a low heat treatment is used (up to 1 minute at 85ºC).

Ultrafiltration of skim milk is used to produce milk protein concentrate (MPC), a casein and whey protein-enriched fraction used in dietetic and clinical nutritional supplements such as infant formula, adult medical foods, weight management products, liquid nutritional beverages, cheese products, cultured foods, powdered dietary supplements and sports nutrition products.

Aside from a wide variety of non-food and industrial uses (*e.g.* in adhesives, paints, textile fabrics, paper coatings, plastics, toothpastes, cosmetics, pharmaceuticals, nutritional and personal care products) casein and caseinates are used as functional ingredients in many food products. Edible acid casein is highly nutritious, low in fat and cholesterol, and flavourful, and is used in whiteners, infant formulas and processed cheeses. Ammonium caseinate is used in bakery products, while calcium caseinate is used as a nutrient supplement in creamed cottage cheese, powdered diet supplements, nutritional beverages, processed cheese, and frozen desserts. Potassium caseinate is used in frozen custard, ice-cream, ice milk, and fruit sherbets, and sodium caseinate is used as an emulsifier in coffee whiteners, cottage cheese, cream liqueurs, yoghurt, processed cheeses, and some meat products. It is also used to improve the whipping properties of dessert whips. Hydrolysed casein may be used as a binder in canned tuna.

Whey is usually recovered as a by-product from cheese making, with small amounts produced as a by-product of casein production. A variety of processes are used to recover components from whey.

Lactose and lactose derivatives are recovered by crystallisation after evaporative concentration. Whey proteins can be recovered by ultrafiltration and ion exchange, and may be further processed by enzymatic hydrolysis.

Food grade whey powder is used in the manufacture of ice-cream, bakery products (cakes, biscuits), chocolate flavouring, infant formula, yoghurt, beverages and processed meat. It may also be used as an ingredient in animal feed and as a calf milk replacer. Whey protein fractions are increasingly used as functional or nutritional components in foods, *e.g.* in health foods for high-energy diets and in bakery and confectionary products (Horton 1995). Demineralised whey powder is used in infant formulae manufacture.

There are also various high value, small volume components derived from whey, including α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulins, lactoferrin and lactoperoxidase. These products require additional purification, but show promise as functional and nutritional food ingredients as well as having potential therapeutic uses (Horton, 1995).

A further by-product of whey fractionation are milk salts, recovered from whey protein ultrafiltrates and electrodialysates (Horton 1995; de Wit 2003). This mix of minerals may be found in bakery products, health drinks and as a table salt substitute.

AMF may be separated into various fat fractions, based on melting point, by fractional crystallisation. These derivatives can be used in combination with skimmed milk powder to produce various milk products, and are used in the baking, confectionery, ice-cream and chocolate industries.

1.11.2 Microflora of major concern

Pathogens of concern in the production of skim milk and skim milk powder include *Salmonella, L. monocytogenes, B. cereus, Cl. perfringens* and *E. sakazakii*. Casein and whey products produced from skim milk might contain spores of the bacilli and clostridia, and vegetative cells of other pathogens might survive extended periods in the dried products if present, although growth will not occur. A microbiological survey of dairy products conducted overseas detected *B. cereus* in whey powder (Appendix 3).

Products formed from severely temperature-abused milk might contain *Staphylococcus aureus* enterotoxin, which is exceptionally heat stable (ICMSF 1998), but this is unlikely to occur in a well-regulated processing environment.

Fat-enriched milk fractions *e.g.* AMF may protect pathogenic micro-organisms such as *E. coli, Salmonella* or *Listeria* if present, however this is unlikely given the low moisture content of the product.

1.11.3 Effect of processing on the growth and survival of microbial pathogens

Milk fats, casein and whey protein components are derived from milk or cream that has been heat-treated to at least the level required by pasteurisation standards (de Wit 2003) and will be free from vegetative cells of pathogenic micro-organisms, but not bacterial spores (ICMSF, 1998). Recontamination by pathogens occurs via post-processing contamination.

AMF is produced from pasteurised cream or from butter made from pasteurised cream. The final step in production, vacuum drying at around 90ºC (Munroe et al in Early 1998), is also lethal to surviving vegetative cells of pathogens. In addition, the low moisture content of the product ensures that outgrowth of pathogens will be limited and they will eventually die off in these products during storage (ICMSF 1998).

Membranes used in filtration and/or concentration steps during production of milk protein concentrate and whey proteins present hygiene problems due to the potential for concentrating pathogens and the vast surface area available for biofilm formation (Varnam and Sutherland 1994 - book). Modern plants tend to run membrane processing at lower temperatures to reduce bacterial growth and extend run times in ultrafiltration plants. Ultrafiltration during production of high protein whey powder is conducted at around 12-17ºC.

Some processes, such as lactose production, evaporation and reverse osmosis, use temperatures suitable for growth of mesophilic bacteria (Varnam and Sutherland, 1996).

1.12 Colostrum

1.12.1 Description

Bovine colostrum is the initial mammary secretion after the birth of a calf. It is produced for about 1-2 days (depleted usually within 4-5 days or 8-10 milkings), and provides the newborn animal with a concentrated source of factors that boost its immune status and support physical and physiological development (Marnila and Korhonen 2003).

Immediately post-partum, the colostrum obtained from cows is excluded from bulk milk collection and was normally fed to farm animals. Until recently, it has not been widely commercially exploited, although the high concentration of bioactive substances in colostrum have attracted increasing interest in the last few years because of their potential pharmaceutical and dietary uses (Marnila and Korhonen 2003). The sports food market is rapidly expanding, due to the perceived benefits of colostrum in providing an immune and performance boost to athletes (Sanders and Van Gammeren, 2001). The use of colostrum as passive immune protection for humans has been reviewed recently (Van Hooijdonk et al., 2000; Gill 2003).

Important biologically active substances contained in colostrums include immunoglobulins, leucocytes, lactoferrin, lysozyme, cytokines (interleukin (IL)-1β, IL-6. IL-10, tumour necrosis factor-α and granulocyte-, macrophage- and granulocyte/macrophage colony-stimulating factors) and other hormones / growth factors (*e.g.* insulin-like growth factors I and II). Some of the bioactive substances found in bovine colostrum provide specific (antibody) or non-specific (*e.g.* lactoferrin and lactoperoxidase) defences against infectious agents and foreign antigens.

1.12.2 Microflora of major concern

The microflora in powdered bovine colostrum will be similar to that in other milk powder products, and include *Salmonella, L. monocytogenes, B. cereus, Cl. perfringens* and *S. aureus*. Post-pasteurisation, colostrum may contain viable spores. Micro-organisms present in the dried product will also arise from post-processing contamination, and vegetative cells of pathogens might survive extended periods in the dried product, although growth will not occur.

The final microbiological quality of colostrum powder will be influenced by the microbial load of the colostrum after milking, processing and the maintenance of good hygiene post-processing. *S. aureus* if present in the raw colostrum may grow and produce enterotoxin if the colostrum is subjected to temperature abuse prior to pasteurisation. The persistence of *Listeria* spp. in the dairy plant environment and the association of listeriosis with other dairy products (ICMSF, 1998) indicates the potential for contamination of dry dairy products such as colostrum powder.

1.12.3 Effect of processing on the growth and survival of microbial pathogens

Typically, cows produce more colostrum than is required by their calves (Marnila and Korhonen 2003), and the excess is collected by milking and stored either frozen or refrigerated. Processing usually involves a fat separation stage before pasteurisation, the liquid is then concentrated (often by membrane technology) and either spray dried or freeze-dried to produce a free-flowing, pale yellow powder. The manufacturing process is similar to that used for the production of skim milk powder.

Because many of the active components in colostrum are extremely heat labile, processors face the challenge of minimising exposure to high temperatures, while ensuring sufficient heat to produce a safe product.

In Australia, bovine colostrum for human consumption is regulated by the Therapeutic Goods Administration (TGA), which produces a compositional guideline including microbiological specifications.

Bovine colostrum is treated by pasteurisation or an equivalent process before drying. Re-introduction of pathogens via post-processing contamination is also of concern, although the low moisture content of the product ensures that outgrowth will be limited and vegetative cells will eventually die off during storage (ICMSF 1998).

Both *Cl. perfringens* and *B. cereus* are able to produce spores that can survive pasteurisation and even ultra-high temperature processing (Institute of Environmental Science and Research Limited, 1995). If colostrum is inadequately stored when made up, the spores of *Cl. perfringens* and *B. cereus* can germinate and rapidly multiply, creating a potential health risk.

The toxin of *S. aureus* is heat stable and, if poor sanitary conditions allow the organisms to proliferate and produce toxin in the pre-pasteurisation stage, the toxin will carry over to the final product.

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APPENDIX 2

Epidemiological Information on Outbreaks of Food-borne Illness Associated with Dairy Products

2.1 OzFoodNet data: 1995-June 2004

Table 1: Outbreaks associated with milk and milk products in Australia (1995-December 2004)

State	Year	Setting food prepared	Ill	Hosp	Food Vehicle	Aetiology	Comments
	1977				Infant formula	*Salmonella* Bredeney	
SA	1997	Caterer	27		Cheese sauce	*Clostridium perfringens*	Infection likely due to poor food handling and preparation.
WA	1998	Camp	9		Unpasteurised milk	*Campylobacter*	Infection due to consumption of unpasteurised milk
SA	1998	Commercially manufactured food	111		Gelati	*Salmonella* Orianberg	Gelati made with pasteurised milk
SA	1999	Farm	12		Unpasteurised milk	*Salmonella* Typhimurium 44	Infection due to consumption of unpasteurised milk
SA	2000	Farm	12		Unpasteurised milk	*Campylobacter*	Infection due to consumption of unpasteurised milk
Vic	2000	Camp	21		Unpasteurised milk	*Campylobacter*	Infection due to consumption of unpasteurised milk
Vic	2001	Camp	12	0	Unpasteurised milk	Unknown	Relative risk was higher for those that had consumed unpasteurised milk
Qld	2001	Community	8	4	Unpasteurised milk	*Cryptosporidium*	Strong epidemiological evidence of association between consumption of unpasteurised milk and cryptosporidium.
Vic	2003	School	13	0	Unpasteurised milk	*Campylobacter*	The risk of illness was 3.7 times higher among people who had drunk any unpasteurised milk.
SA	2003	Camp	14	0	Unpasteurised milk	*Campylobacter*	Unpasteurised milk was supplied for drinks and cereal.

Table 2: Outbreaks associated with foods containing dairy products in Australia (January 2001-December 2004)

State	Year	Setting food prepared	Ill	Hosp	Food Vehicle	Aetiology	Comments
NSW	2000	Restaurant	41	2	Fried ice-cream	*Salmonella* Typhimurium 9	
SA	2001	Bakery	16	3	Custard Tarts	*Salmonella* Typhimurium 126	Unable to identify original source of infection
WA	2001	Restaurant	38	4	Ice cream and sponge	*Salmonella* Typhimurium 64	Food handler positive for STM 64.
Vic	2002	Take away	10	1	Cream filled cakes	*Salmonella* Typhimurium U290	Illness strongly associated with eating at bakery.
SA	2002	Bakery	22	7	Cream filled cakes	*Salmonella* Typhimurium 99	Piping bags in the bakery reused/used for sausage meat and cream.
NSW	2002	Take Away	29	4	Cream filled cakes	*Salmonella* Typhimurium 135a	14 of 22 primary cases had eaten cream-filled cake
SA	2003	Bakery	6	1	Cheesecake	*Salmonella* Typhimurium 4	
Qld	2004	Bakery	5	0	Custard Tarts	*Salmonella* Typhimurium 135a	Almond sauce suspected source of infection.
SA	2004	Home	5	1	Ice Cream	*Salmonella* Typhimurium 9	Raw egg used in ice cream
SA	2004	Bakery	13	0	Cream filled cakes	*Salmonella* Typhimurium 108	Epidemiological evidence suggested cream filled cakes as source of infection
NSW	2004	Institution	43	17	Custard	Unknown	
NSW	2004	Institution	43	10	Custard	*Salmonella* Typhimurium 135	

2.2 International data on dairy-related outbreaks of food-borne illness

There have been a number of reports of outbreaks of illness associated with consumption of dairy products. A literature search was undertaken to identify and outline outbreaks of food-borne illness attributed to dairy products internationally. The search looked at peer-reviewed literature, as well as other relevant literature such as government documents, reports, electronic citations and follow up of reference lists on documents.

The information from the search describes 163 outbreaks associated with dairy products. Outbreaks of illness associated with pasteurised dairy products are 22 with pasteurised milk (13.5%) (Table 1), 17 with cheese from pasteurised milk or pasteurisation not stated so assumed pasteurised (10.4%) (Table 3). Faults with the pasteurisation process or a post pasteurisation contamination has been identified or suspected as the source of infection in each case. These pasteurised or undefined products are a total of 39/163 outbreaks (23.9%).

Unpasteurised dairy products are the most common cause of dairy associated outbreaks of illness, 30 due to unpasteurised milk (18.4%) (Table 2), 18 unpasteurised cheese (11.0%) (Table 4), 13 unpasteurised non-bovine species (8.0%) (Table 10) this brings the total number of dairy outbreaks associated with unpasteurised products to 61/163 (37.4%).

Ice cream was responsible for 23 outbreaks (14.0%) (Table 7) in which 14 identified raw eggs as an ingredient, the eggs may be the source of infection. Butter was associated with 6 outbreaks (3.7%) (Table 5). Yoghurt and fermented products were associated with 2 outbreaks (1.2%) (Table 6). Dried milk products, which did not identify whether they were manufactured from pasteurised milk, were associated with 5 outbreaks (3.0%) (Table 8). Eight outbreaks of illness from foods where a dairy product was a component were identified (5.9%) (Table 11). Infant formula was associated with 19 outbreaks of illness (11.7%) (Table 9).

This epidemiological evidence supports the microbiological evidence that pasteurisation is an effective method of reducing the risk of human illness from dairy products.

Table 3: Outbreaks of illness associated with Pasteurised Liquid Milk

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	UK	114	Pasteurised milk	*E. coli* O157	On-farm pasteurisation.	(Goh *et al*., 2003)
2000	Japan	14000	Low fat milk	Staphylococcal enterotoxin (SE) (identified by PCR)	Caused by a bacteria contaminated valve at a milk packaging factory and ineffective temperature control	(Yamashita *et al*., 2003)
2000	USA	38	Pasteurised milk	*Salmonella* Typhimurium	Likely contaminated containers or milk contact surfaces after pasteurisation because of environmental conditions in plant	(Olsen and et al, 2004)
1998	UK	40	Pasteurised milk	*Salmonella*	Pasteurisation failure	(Brown, 1998)
1997	UK	50	Pasteurised Milk	Cryptosporidiosis	Faulty pasteuriser on farm	(Gellietlie *et al*., 1997)
1997	USA	54	Chocolate milk	*L. monocytogenes*	*L. monocytogenes* isolated from implicated chocolate milk, and from tank drain at dairy	(Dalton *et al*., 1997)
1996	UK	9	Raw/Pasteurised milk	*E. coli* O157 (VTEC 0157)	Unpasteurised milk from the farm was consumed also pasteurised milk with a faulty pasteuriser	(Clark *et al*., 1997)
1995	USA	10	Pasteurised milk	*Y. enterocolitica* O: 8	Post pasteurisation contamination of milk bottles	(Ackers *et al*., 2000)
1995	UK	110	Milk	*C. jejuni*	Inadequately pasteurised milk from a local dairy	(Fahey *et al*., 1995)
1994	USA	11	Pasteurised	*E. coli* serotype O104:H21	Inspection of dairy plant producing the implicated brand of milk revealed faecal coliform contamination of post pasteurisation equipment	(Moore *et al*., 1994)
1994	USA	4	Milk	*Bacillus cereus*	Hot chocolate consumed at work	(CDC 2002)
1994	USA	18	Milk	*E. coli* 0157:H7	Consumed in a number of private homes	(CDC 2002)
1992	USA	23	Milk	*C. jejuni*	Consumed on a farm	(CDC 2002)
1991	USA	37	Milk	*Salmonella* Typhimurium	Chocolate milk consumed on a farm	(CDC 2002)
1990	UK	32	Pasteurised milk	*C. jejuni*	Due to the consumption or handling of milk from bottles that had been attacked by birds.	(Southern *et al*., 1990)
1990	USA	19	Milk	Unknown	Consumed at a bowling alley	(CDC 2002)
1990	USA	7	Milk	Unknown	Consumed in a restaurant	(CDC 2002)
1987	USA	16000	Pasteurised milk	*Salmonella* Typhimurium	Pasteurisation equipment had been modified to facilitate the running off of raw milk	(Ryan *et al*., 1987)
1985	USA	1500	2% Pasteurised milk	*Salmonella* Typhimurium	2% pasteurised milk ("Blue Brook" brand) from one processing plant	(MMWR, 1985)
1985	USA	49 (14)	Pasteurised Milk	L*. monocytogenes*	At the plant where the milk was processed, inspections revealed no evidence of improper pasteurisation	(Fleming *et al*., 1985)
1984	USA	16	Pasteurised milk	*Salmonella* Typhimurium	Inadequately pasteurised milk	(MMWR, 1984b)
1983 - 1984	UK	32 O/B 714 cases (8)	27 Raw milk 2 pasteurised milk 1cheese 1cream 1 ice cream	22 Salmonellosis 7 *Campylobacter* 1 *S. aureus*, 1 *Y. enterocolitica* 1 *Streptococcus zooepidemicus*	There were eight deaths, all associated with the *S. zooepidemicus* outbreak	(Barrett, 1986)

Table 4: Outbreaks of illness associated with Unpasteurised Liquid Milk

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	USA	62	Raw milk	*Salmonella* Typhimurium	Unpasteurised milk at dairy/petting zoo	(Mazurek *et al*., 2004)
2003	USA	13	Raw Milk	*C. jejuni*	Unpasteurised milk	(Peterson, 2003)
2001	USA	75	Unpasteurised milk	*C. jejuni*	Unpasteurised milk obtained at a local dairy farm	(Harrington *et al*., 2001)
2001	Austria	2	Raw cows/ goats milk	*E. coli* O157	isolated from dairy cow and goat, raw milk	(Allerberger *et al*., 2001)
2000	Austria	38	Unpasteurised milk	*C. jejuni*	Unpasteurised milk distributed by a local dairy	(Lehner *et al*., 2000)
2000	Germany	31	Raw milk	*C. jejuni*	Consuming raw milk farm visit	(Thurm *et al*., 2000)
1998	Hungary	52	Raw Milk	*C. jejuni* and *C. coli*	Unpasteurised milk	(Kalman *et al*., 2000)
1996	UK	33	Unpasteurised Milk	*C. jejuni* resistotype 02	Educational farm visit, exposure to raw milk	(Evans *et al*., 1996)
1995	USA	3	Raw milk	*S*. Typhimurium, variate Copenhagen	Consumed in private home	(CDC 2002)
1993	USA	4	Raw milk	*E. coli* 0157:H7	Consumed in a nursing home	(CDC 2002)
1993	USA	6	Raw Milk	*E. coli* O157:H7	Commercially distributed Unpasteurised milk	(Keene *et al*., 1997)
1992	USA	50	Raw milk	*C. jejuni*	Consumed at church	(CDC 2002)
1992	USA	11	Raw milk	*Campylobacter* spp.	Consumed in private home	(CDC 2002)
1992	Australia	3	Raw milk	*Streptococcus zooepidermicus*	Unpasteurised milk from a house cow	(Francis *et al*., 1993)
1990	USA	13	Raw milk	*Campylobacter* spp.	Consumed at school	(CDC 2002)
1990	USA	5	Raw milk	Unknown	Consumed in private home	(CDC 2002)
1990	USA	42	Raw milk	*C. jejuni*	Consumed at a dairy	(CDC 2002)
1986	Austria	28 (5)	Raw milk	*L. monocytogenes*	Consumption of raw milk and biologically grown vegetables as possible source of infection	(Allerberger and Guggenbichler, 1989)
1985	USA	25	Raw Milk	*C. jejuni*	Unpasteurised milk	(Korlath *et al*., 1985)
1984	USA	23	Raw milk	not identified	Associated with drinking raw milk from local dairy	(MMWR, 1984a)
1984	Canada	9	Raw milk	*C. jejuni*	A raw milk dairy	(MMWR,1984)
1983	USA x 2 OB	31 26	Raw milk	*C. jejuni*	A raw milk dairy	(MMWR, 1983)
1983	USA	122	Raw Milk	not identifed	Associated with consumption of raw milk from a single dairy	(Osterholm *et al*., 1986)
1983	USA	? (1)	Raw Milk	*S*. Typhimurium	Unpasteurised milk	(Tacket *et al*., 1985)
1983	UK	130	Raw Milk	*S*. Typhimurium	Unpasteurised milk	(Shanson *et al*., 1983)
1982	USA	38	Raw Milk	*C. jejuni* and thermo-tolerant strain (*C. fetus* subsp *fetus*)	Unpasteurised milk	(Klein *et al*., 1986)
1981-83	USA	46 70 123(32)	Raw milk	*S*. Dublin		(Potter *et al*., 1983)
1981	USA	250	Raw Milk	*C. jejuni*	Unpasteurised milk	(Kornblatt *et al*., 1985)
1979	UK	700	Unpasteurised milk	*S*. Dublin	Milk which had not been subjected to heat treatment	(Small and Sharp, 1979)
1973-1992	USA	40 out-breaks	Raw Milk	Various	In states with legal raw milk	(Headrick *et al*., 1998)

Table 5: Outbreaks of illness associated with Cheese from Pasteurised Milk or unknown Pasteurised/Unpasteurised

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2001	France	45	Brie cheese	Salmonellosis serotype infantis	Milk and factory workers contaminated with Salmonella serotype infantis	(Simon *et al*., 2002)
1999	Canada	700	Cheese	*S*. Enteritidis	Lunch pack products	(CCDR, 1999)
1996	USA	8 (1)	Cheese Sauce	*Clostridium botulinum*	A commercial, canned cheese caused a botulism outbreak	(Townes *et al*., 1996)
1996	Italy	8	Mascarpone cheese	*Clostridium botulinum* type A	Beak in cold-chain at retail likely caused germination of C. botulinum spores contaminating the products	(Aureli *et al*., 2000)
1996	UK	84	Cheddar cheese	*S*. Goldcoast		(Health Protection Agency 1997)
1995	Switzerland	57 (16)	Soft cheese	Listeriosis	Consumption of a soft cheese	(Bula *et al*., 1995)
1995	USA	9	Cheese	*Clostridium perfringens*	Consumed in restaurant	(CDC 2002)
1994	USA	5	Goats cheese	Salmonella enteridis	Consumed in a private home	(CDC 2002)
1993	USA	12	Cheese slices	Unknown	Consumed at a picnic	(CDC 2002)
1991	USA	25	Shredded cheese	Unknown	Consumed in a restaurant	(CDC 2002)
1990	USA	15	Cheese	Hepatitis A	Consumed in a private home	(CDC 2002)
1990	USA	23	Cheese sauce	*S*. Braenderup	Consumed in restaurant	(CDC 2002)
1990	USA	12	Processed Cheese	*S*. Enteritidis	Consumed in hospital	(CDC 2002)
1989	USA	167	Contaminated cheese	*S*. Javiana and S. Oranienburg	Mozzarella cheese manufactured at a single cheese plant	(Hedberg *et al*., 1992)
1983	USA	45	French Brie cheese	*E. coli* O27:H20	Three clusters of gastrointestinal illness, after office parties	(MacDonald *et al*., 1985)
1982	Canada	?	Cheddar Cheese	S. Typhimurium		(D'Aoust, 1985)
1976	USA	28,000 to 36,000	Cheddar cheese	*S*. Heidelberg	Consumption of cheddar cheese from a single shipment of a single manufacturer	(Fontaine *et al*., 1980)

Table 6: Outbreaks of illness associated with Raw Milk Cheese

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	France	18	Raw goats milk cheese	*S*. Stourbridge	Cheese was made from the unpasteurised milk of a single herd of 260 goats	(Vaillant V, 2005)
2003	Sweden	15	Cheese	*Listeria monocytogenes*	On farm manufactured fresh cheese	(Carrique-Mas *et al*., 2003)
2002	Canada	17	Raw milk cheese	*Listeria monocytogenes*	Environmental contamination	(CCDR, 2003)
2002	Canada	13	Unpasteurised gouda cheese	*E. coli* O157:H7	Implicated cheese was found to be contaminated with E. coli O157:H7 104 days after production, despite having met regulated microbiological and aging requirements	(Honish *et al*., 2005)
2001	France	190	Cantel cheese	*S.* Enteritidis	Cheese made from raw milk	(Haeghebaert *et al*., 2003)
2001	France	25	Cantel cheese	*S*. Enteritidis	Cheese made from raw milk	(Haeghebaert *et al.,* 2003)
2000	USA	13 (5 still births)	Mexican style cheese	*Listeria monocytogenes*	Mexican-style cheese made from contaminated raw milk traced to 1 local dairy	(MacDonald *et al*., 2005)
1998	USA	55	Fresh cheese curds	*E. coli* O157:H7	Produced during manufacture of Cheddar cheese from unpasteurised milk and had been incorrectly labelled as pasteurised	(Durch *et al*., 2000)
1997	USA	54	Mexican-style soft cheese made with unpasteurised milk	*S*. Typhimurium DT104	Raw milk samples from nearby dairies yielded Salmonella Typhimurium DT104	(Villar *et al*., 1999)
1997	France	113	Raw milk soft cheese	*S*. Typhimurium	From a single processing plant	(de Valk *et al*., 2000)
1997	USA	31	Unpasteurised Mexican-style soft cheese	*S*. Typhimurium DT104	Fresh Mexican-style cheese from street vendors and from cheese samples and raw milk	(Cody *et al*., 1999)
1996	Spain	81	Raw cheese	*Brucella mellitensis*	Home-made cottage cheese	(Castell *et al*., 1996)
1995	France	20	Raw milk cheese	*Listeria monocytogenes*		(Goulet *et al*., 1995)
1995	France	25	Raw milk cheese	*S*. Dublin		(Vaillant *et al*., 1996)
1994	Canada	82	Unpasteurised soft cheese	*S*. Berta	Cheese was contaminated by chicken carcasses during production	(Ellis *et al*., 1998)
1994	Scotland	22	Raw milk Cheese	*E. coli* 0157		(Ammon, 1997)
1988	UK	155	Stilton Cheese	Suggestive of a staphylococcal illness	Stilton cheese, produced from unpasteurised cow's milk	(Maguire *et al*., 1991)
1973-1992	USA	58 deaths	Cheese	*Salmonella*, *Listeria*, and *E. coli* O157:H7	Manufacturing errors caused most illnesses, manufacturing cheese with raw or improperly pasteurized milk and post pasteurization contamination	(Altekruse *et al*., 1998)

Table 7: Outbreaks of illness associated with Butter and Butter products

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	UK	17	Butter	*Listeria monocytogenes*	Listeria isolated from a drain at the dairy & from butter but not from other dairy products from the dairy	(ACMS and Advisory Committee on the Microbiological Safety of Food, 2003)
1999	Finland	25(6)	Butter	*Listeria monocytogenes*		(Lunden *et al*., 2004)
1995	USA	29	Butter	Unknown	Consumed in Pre release centre	(CDC 2002)
1991	USA	15	Whipped butter blend	*Staphylococcus aureus*	Consumed in a Hotel	(CDC 2002)
1991	USA	8	Butter	Unknown	Consumed in restaurant	(CDC 2002)
1990	USA	40	Butter	Unknown	Consumed on educational summit	(CDC 2002)

Table 8: Outbreaks of illness associated with Yogurt and Fermented Milk

Year	Country	Cases	Product	Causative Agent	Cause	Reference
1991	UK	16	Yoghurt	*E. coli* O 157	Consumption of a locally produced live yoghurt	(Morgan *et al*., 1993)
1989	UK	27(1)	Hazelnut flavoured yoghurt	*Clostridium botulinum* type B toxin	Can of hazelnut conserve, opened and unopened cartons of hazelnut yoghurt	(O'Mahony *et al*., 1990)

Table 9: Outbreaks of illness associated with Ice cream

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2000	Italy	113	Home made ice cream and Italian pastry	*S. Eenteritidis	Not confirmed but contamination appeared to be due to raw eggs in ice cream	(Lopalco *et al*., 2000)
1998	Australia	111	Gelati	*S*. Oranienberg	Gelati made from pasteurised milk	(2004)
1998	USA	3	Ice cream	**S*. Enteritidis		(Vought and Tatini, 1998)
1995	USA	27	Home made ice cream	**S*. Enteritidis	Consumed at a church	(CDC 2002)
1994	USA	2150	Ice cream	**S*. Enteritidis	Illness associated with consumption of a specific brand of ice cream	(Dept. Human Services, 1994)
1994	USA	6	Home made ice cream	**S*. Enteritidis	Consumed at a private home	(CDC 2002)
1994	USA	186	Ice cream	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1994	USA	743	Ice cream	**S*. Enteritidis	Commercially produced ice cream	(CDC 2002)
1994	USA	5	Home made ice cream	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1993	USA	8	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1993	USA	12	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed in a hospital	(CDC 2002)
1992	USA	10	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a home	(CDC 2002)
1992	USA	15	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a social function	(CDC 2002)
1992	USA	9	Home made ice cream with raw eggs	**S*. Heidelberg	Consumed at a home	(CDC 2002)
1992	USA	31	Home made ice cream with raw eggs	**S*. Typhimurim	Consumed at a home	(CDC 2002)
1991	USA	22	Home made ice cream with cooked eggs	**S*. Enteritidis	Consumed at school	(CDC 2002)
1991	USA	11	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a Picnic	(CDC 2002)
1991	USA	25	Home made ice cream with raw eggs	**S*. Typhimurium	Consumed at a church	(CDC 2002)
1990	USA	30	Home made ice cream with raw eggs	**Salmonella* spp .	Consumed at a church	(CDC 2002)
1990	USA	2	Home made ice cream with raw eggs	**S*. Typhimurium	Consumed at a home	(CDC 2002)
1990	USA	9	Home made ice cream with raw eggs	**S*. Enteritidis	Consumed at a Picnic	(CDC 2002)
1990	USA	96	Home made ice cream with raw eggs	**S*. Heidelberg	Consumed at a home	(CDC 2002)
1982	USA	8 (1)	Ice cream	**S*. Typhimurium	S. Typhimurium isolated from all cases, leftover ice cream, and family's hens eggs used to prepare ice cream.	(Taylor *et al*., 1984)

NB. Salmonella infection has a strong association with eggs, ice cream is usually made with raw eggs therefore the outbreaks marked * are probably a result of the egg component of the ice cream rather than the dairy component.

Table 10: Outbreaks of illness associated with Dried Milk Products

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	France	49	Milk powder	*S*. Worthington	Consumption of contaminated powdered milk	(Institut de Veille Sanitaire, 2005)
1981	UK	77	Dried milk	*Clostridium perfringens*	Consumption of dried milk	(Anon, 1981)
1979	USA	?	Milk powder	*Salmonella*		(ICMSF, 1998)
1977	UK	?	Milk powder	*Bacillus cereus*	Suspected milk powder	(Pinegar and Buxton, 1977)
1964	USA	?	Milk powder	*Salmonella*		(ICMSF, 1998)

Table 11: Outbreaks of illness associated with Infant Formula

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	UK	1	Infant formula	*Clostridium botulinum*	C. botulinum type B isolated from opened infant formula milk pwd	(Brett *et al*., 2005)
2004	Korea	31	Infant formula	*S*. London	Highly likely source of the infection was infant formula	(Park *et al*., 2004)
2003	India	16	Infant formula	Enterotoxigenic *E. coli*	Contaminated by infected food handler	(Taneja *et al*., 2003)
2002	USA	?	Infant formula	*Enterococci sakazakii*		(Weir, 2002)
2002	Switzerland	11	Infant formula	*Serratia marcescens*	Cultures of milk from used milk bottles yielded *S. marcescens*	(Fleisch *et al*., 2002)
2002	USA	11	Infant formula	*S*. Saintpaul	Formula mixed by the hospital appears to have been the source of this Salmonella outbreak	(Bornemann *et al*., 2002)
2001	Belgium	12	Infant formula	*E. sakazakii*	E. sakazakii isolated from implicated prepared formula milk and from several unopened cans	(van Acker *et al*., 2001)
2001	USA	1(1)	Powdered infant milk formula	*E. sakazakii*	Infection associated with presence of organism in commercial powdered formula	(MMRW, 2002)
1999	Israel	?	Infant formula	*E. sakazakii*	Recovered from prepared formula and kitchen blender.	(Block *et al*., 2002)
1998	UK	17	Infant formula	*S*. Anatum	Formula-dried milk responsible for outbreak	(Threlfall *et al*., 1998)
1995	Spain	3	Infant formula	*S*. Virchow	Dried-milk formula was confirmed as the source of the infection	(Ruiz *et al*., 1995)
1994	Spain	48	Infant powdered milk	*S*. Virchow		(Usera *et al*., 1998)
1990	USA (2 outbreaks)	?	Infant formula	*E. sakazakii*	*E. sakazakii* from intrinsically contaminated dried infant formula was source of neonatal infection	(Clark *et al*., 1990)
1990	USA (2 outbreaks)	?	Infant formula	*E. sakazakii*	*E. sakazakii* from intrinsically contaminated dried infant formula source of neonatal infection	(Clark *et al.,* 1990)
1989	USA	4	Infant Formula	*E. sakazakii*	Infant formula contaminated during the manufacturing process	(Simmons *et al*., 1989)
1989	Iceland	3	Infant formula	*E. sakazakii*	Organism grown from several lots of the powdered-milk formula used in the hospital	(Biering *et al*., 1989)
1985	UK	?	Infant formula	*S*. Ealing	The source of infection was traced to the factory spray-drier	(Rowe *et al*., 1987)
1984	Chille	35	Infant formula	*Bacillus cereus*		(Cohen *et al*., 1984)
1977	Australia	17	Infant formula	*S*. Bredeney	Contamination of powdered infant formulae during manufacture	(Forsyth *et al*., 2003)

Table 12: Outbreaks of illness associated with Other Species

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2003	Spain	11	Raw goats cheese	*Brucella melitensis* serovar 3	Unpasteurised raw goat cheese produced in a farmhouse	(Mendez *et al*., 2003)
2001	Canada	5	Unpasteurised goats milk	*E. coli* 0157:H7	Source of implicated goat's milk was a co-operative farm	(McIntyre *et al*., 2001)
1999	Canada	?	Cheese from goats milk	*Coxiella burnetii*	Associated with contact with goat placenta, smoking tobacco	(Hatchette *et al*., 2001)
1995	Czech Republic	5	Raw goats milk	*E*. coli O157	Unpasteurised goat's milk from the same farm	(Bielaszewska *et al*., 1997)
1994	France	?	Raw goats milk cheese	*E. coli 0103*		(Ammon 1997)
1993	France	273 (1)	Unpasteurised goats milk cheese	*S*. Paratyphi B phage type 1 var 3	Brand A unpasteurised goats' milk cheese	(Desenclos *et al*., 1996)
1992	France	40	Raw goats milk	*Coxiella burnetii*	Persons who worked on farm and consumed unpasteurised milk products	(Fishbein and Raoult, 1992)
1991	USA	3	Raw goats milk	*C. jejuni*	Consumed on farm	(CDC 2002)
1988	Czech Republic	74	Non pasteurised sheep milk cheese	*C. jejuni/coli*	Cheese prepared from unpasteurised sheep's milk	(Kourilova and Kultan, 1990)
1988	England	1	Goats milk soft cheese	*Listeria*	Immunocompromised case	(Azadian *et al*., 1989)
1983	USA	6	Raw goats Milk	*C. jejuni*	Associated with dairy that produced raw goat's milk	(Harris *et al*., 1987)
1983	France	20	Ewe milk cheese	*S. aureus*	Made with raw sheep milk, shepherd asymptomatic carrier of S. aureus	(DeBuyser *et al*., 1985)
1975	Mexico	3	Raw cheese	*B. melitensis*	Mexican raw goats milk cheese	(Eckman, 1975)

Table 13: Outbreaks of illness associated with Mixed Food including a Dairy Product

Year	Country	Cases	Product	Causative Agent	Cause	Reference
2005	Israel	43	Cream cake	*Salmonella enterica* (Group D Salmonella)	Ingestion of cream cake at birthday party	(Hefer *et al*., 2005)
2002	Spain		Ravioli With cheese sauce	*Clostridium perfringens*	Ravioli With cheese sauce, consumed at a restaurant	(Sanz *et al*., 2002)
2001	UK	138	Cream, mints, or profiteroles	Small round structured virus (SRSV)	At a charity function	(Steel *et al*., 2001)
1999	UK	80	Ham, coleslaw, bread rolls, cheese and pineapple	Small round structured virus (SRSV)	Contamination appears to be due to poor food handling	(Fone *et al*., 1999)
1996	Mexico	83	Chilli rellenos ingredients included shelled eggs and cheese	*S*. Enteritidis phage type 4	Salmonella was isolated from the leftover cheese but the isolate was not serotyped	(Shane *et al*., 2002)
1995	UK	?	Kebabs with yogurt sauce	*S*. Typhimurium DT170	Raw kebab mince positive for *S*. Typhimurium DT170, yogurt stored under mince stained with blood	(Evans *et al*., 1999) S.
1990	Thailand	400	Eclairs	*Staphylococcus aureus* producing toxins A and C and Bacillus cereus	Eclairs which were prepared during the night before the dinner and kept at room temperature for at least 12 hours	(Thaikruea *et al*., 1995)
1981	USA		Macaroni Cheese	*Bacillus cereus*	Epidemiologic investigation incriminated macaroni and cheese as a cause of the illness and samples of this food contained large numbers of Bacillus cereus	(Holmes *et al*., 1981)

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APPENDIX 3

Occurrence of microbiological hazards associated with dairy products

3.1 Occurrence of microbiological hazards in Dairy products in Australia

The occurrence of microbiological hazards in Australian finished dairy products is extremely low, due largely to all products originating from pasteurised milk. Of dairy samples sent to the Melbourne Diagnostic Unit, and collated by the National Enteric Pathogen Surveillance Scheme (NEPSS) over a 20-year period (1983-2004), *Salmonella* has been isolated from a total of 1,156 samples. Although the data presented in Table 1 does not provide the prevalence of *Salmonella* spp. in Australian dairy products (only positive samples) it does give information on the range of *Salmonella* serovars associated with these products.

Surveys undertaken by the South Australian Dairy Authority confirm the high compliance of dairy products in Australia (Table 2). Samples are analysed for a number of microbiological and chemical contaminants. However, results are reported as either pass or fail, without identifying which specific test the product failed (Table 3). Testing undertaken by Dairy Food Safety Victoria on dairy products are summarised in Tables 4-6.

Table 1: *Salmonella* isolates from raw milk and milk products, NEPPS data 1983-2004.

Product	Organism	State of origin and no. of times isolated
Liquid raw cows milk	*S.* Agona	Vic 1
	S. Anatum	Vic 1
	S. Bovismorbificans 24	WA 24
	S. Dublin	Vic 12
	S. Kiambu	WA 24
	S. Mbandaka	WA 1
	S. Ohio	NSW 1
	S. Typhimurium 13	Vic 1
	S. Typhimurium 44	SA 6, Vic 1
	S. Typhimurium 135	Vic 2
	S. Typhimurium RDNC	NSW 1, Qld 1
	S. Zanzibar	Vic 4
Raw goats milk	*S.* Anatum	NSW 1, Qld 1
	S. Choleraesuis bv Kunzendorf Australia	WA 7
	S. Saintpaul	NSW 3
	S. subsp IIIb ser 61:l,v:z35	Qld 2
Dried milk powders*	*S.* Adelaide	Vic 2, WA 1
	S. Agona	Vic 79
	S. Anatum	Vic 125, Qld 8
	S. Anatum var 15+	Vic 14
	S. Bredeney	Vic 24
	S. Chester	Vic 1
	S. Derby	Vic 78, NSW 1
	S. Dublin	Vic 5, NSW 1
	S. Emmastad	Vic 4
	S. Havana	Vic 254, Qld 27, NSW 9, Tas 2
	S. Havana H2S negative	Vic 8
	S. Johannesburg	Vic 16
	S. Kottbus	Vic 1
	S. Mbandaka	Qld 1, Vic 1

Table 1: (cont.)

Product	Organism	State of origin and no. of times isolated
Dried milk powders*	*S.* Muenchen	Qld 1
(*Cont*)	*S.* Newport	Vic 15
	S. Ohio	Vic 59, NSW 1
	S. Ohio var 14+	NSW 2
	S. Oranienburg	Vic 6, Qld 4
	S. Orion	Qld 18, Vic 2
	S. Orion var 15+	NSW 1
	S. Sachsenwald subsp IV	Qld 1
	S. Senftenberg	Vic 6
	S. Senftenberg z27 phase	Qld 2
	S. Singapore	Qld 1
	S. Tennessee	Qld 14
	S. Typhimurium 6	Vic 1
	S. Typhimurium 44	Vic 1
	S. Typhimurium 135	Tas 2
	S. Typhimurium 170	NSW 4
	S. Typhimurium RDNC	Vic 1
	S. Zanzibar	Qld 1
	S. subsp I ser 4,12:d:-	Qld 1
Infant formula	*S.* Agona	NSW 1
	S. Anatum	Vic 1
	S. Bredeney	Vic 1
	S. Potsdam	Qld 1
Ice-cream	*S.* Anatum var 15+	Vic 1
Concentrated milk	*S.* Schwarzengrund	Tas 1
Whey powder	*S.* Agona	Vic 14
	S. Anatum	Vic 62, Tas 1
	S. Anatum var 15+	Vic 13
	S. Chester	Vic 1
	S. Give var 15+	Qld 2
	S. Havana	Vic 21
	S. Mbandaka	Vic 1
	S. Newport	Vic 5
	S. Senftenberg	Vic 7
	S. Wandsworth	Qld 1
Casein	*S.* Adelaide	Vic 24, NSW 3
	S. Agona	Qld 5, Vic 1
	S. Anatum	Vic 3
	S. Anatum var 15+	Vic 19
	S. Bareilly	NSW 1
	S. Bovismorbificans 14	WA 3
	S. Braenderup	Vic 4
	S. Derby	Vic 2
	S. Gaminara	Qld 2
	S. Havana	Vic 4
	S. Infantis	Vic 4
	S. Ohio	Vic 33, Qld 1
	S. Senftenberg	Vic 2, Qld 1
	S. Virchow (not typed)	Qld 4
	S. Virchow PT19	Qld 1, NSW 1
	S. Virchow PT19 var	Qld 3
	S. Virchow PT34	Qld 1

* dried milk powders include buttermilk, skim milk, full cream milk, unspecified powdered milks

Table 2: Dairy Authority of South Australia survey results

Product	Year	No of tests	Number failed (%)
Pasteurised milk	1998	22	1 (4.5)
	1999	26	0
	2000	26	0
	2001	26	0
	2002	29	0
	2003	44	3 (6.8)
	2004	59	8 (13.6)
	TOTAL	**232**	**12 (5.2)**
Cheese	1998	101	13 (12.9)
	1999	123	11 (8.9)
	2000	122	12 (9.8)
	2001	111	2 (1.8)
	2002	119	5 (4.2)
	2003	141	10 (7.1)
	2004	140	0
	TOTAL	**857**	**53 (6.2)**
Dip/Dessert	1998	ND	ND
	1999	ND	ND
	2000	7	0
	2001	13	1 (7.7)
	2002	25	0
	2003	27	0
	2004	33	0
	TOTAL	**105**	**1 (1.0)**
Yoghurt	1998	32	5 (15.6)
	1999	22	1 (3.3)
	2000	34	1 (2.9)
	2001	31	0
	2002	30	1 (3.3)
	2003	25	0
	2004	26	0
	TOTAL	**200**	**8 (4.0)**

Table 3: Tests conducted by the Dairy Authority of South Australia

Product	Test	Standard
Pasteurised milk	Standard Plate Count Coliforms Antimicrobial substances	<50,000 cfu/ml <1 cfu/ml <0.003 ug/ml
Cheese	Coliforms and *E. coli* Coagulase positive *S. aureus* *Listeria monocytogenes*	<10 cfu/ml <100 cfu/ml Not detected in 25g
Dip/Dessert*	Standard Plate Count Coliform Coagulase positive *S. aureus* *Listeria monocytogenes*	<50,000 cfu/ml <10 cfu/ml <100 cfu/ml Not detected in 25g
Yoghurt	Coliforms Yeasts Moulds	<10 cfu/ml <100 cfu/ml <100 cfu/ml

*Dip/Dessert includes Gelati, ice cream, cheese-based dip, yoghurt-based dip, cream.

Table 4: Summary of outcomes from Dairy Food Safety Victoria product testing program for 2004-05

Product	No. samples tested	Coliforms	*E. coli*	*L. monocytogenes*	*CP Staph*	*Salmonella*
High Moisture Cheese	102	41 (40%)	13 (13%)	4 (4%)	5 (%%)	0
Low Moisture Cheese	48	12 (25%)	4 (8%)	5 (10%)	0	0
Ice Cream	39	37 (95%)	0	0	0	0
Dips	14	5 (36%)	0	1 (7%)	0	0
Desserts	2	1 (50%	0	0	0	0

Table 5: Summary of outcomes from Dairy Food Safety Victoria product testing program for 2003-04

Product	No. samples tested	Coliforms	*E. coli*	*L. monocytogenes*	*CP Staph*	*Salmonella*
High Moisture Cheese	150	47 (31%)	14 (9%)	2 (2%)	2 (2%)	0
Low Moisture Cheese	57	16 (28%)	8 (14%)	1 (2%)	0	0
Ice Cream	59	31 (53%)	1 (2%)	0	0	0
Dips	22	5 (23%)	0	1 (5%)	0	0
Yoghurt	28	4 (14%)	0	0	0	0
Desserts	10	4 (40%)	0	0	0	0

Table 6: Summary of outcomes from Dairy Food Safety Victoria product testing program for 2002-03

Product	No. samples tested	Coliforms	*E. coli*	*L. monocytogenes*	*CP Staph*	*Salmonella*
High Moisture Cheese	129	52 (40%)	10 (8%)	2 (2%)	0	0
Low Moisture Cheese	80	23 (29%)	4 (5%)	2 (3%)	0	0
Ice Cream	50	19 (38%)	0	0	0	0
Dips	29	8 (28%)	6 (21%)	1 (3%)	0	0
Desserts	6	1 (17%)	0	0	0	0
Powder	33	4 (12%)	0	1 (3%)	0	0
Milk	19	0	0	0	0	0

3.1.1 Imported Foods Inspection Scheme testing results

The Imported Food Program, operated by the Australian Quarantine and Inspection Service (AQIS), tests a large number of samples of dairy products entering Australia each year. In the period 2002 to 2004, failures were recorded for imported cheeses for *E. coli*, and *L. monocytogenes* (Table 7). No failures were recorded in tests for *Salmonella* spp.

Table 7: Significant imported food testing failures for dairy products 2002-2004.

Product	Test	Number sampled	Number failed*
Cheese	*E. coli*	53	2 (3.8%)
	L. monocytogenes	15	0
	Salmonella spp.	2	0
Soft Cheese	*E. coli*	330	21 (6.4%)
	L. monocytogenes	894	21 (2.3%)
	Salmonella spp.	288	0

* All imported dairy products must meet the microbiological limits specified in Section 1.6.1 of the Code

3.1.2 Food recalls

FSANZ is responsible for the coordination and monitoring of food recalls in Australia. FSANZ collates and disseminates information on recalls in consultation with the senior food officers or their deputies in the States and Territories, and the product' s supplier such as the manufacturer or the importer.

There were 43 recalls for dairy products due to microbiological concerns for the period 1990 to 2005 (Table 8). There were a total of 716 food recalls notified to FSANZ during this period, thus recalls attributed to dairy products only represents 6% of the total number of recalls notified to FSANZ.

Table 8 Summary of Food recalls notified to FSANZ 1990 – August 2005

Product	Number of recalls
Milk	16
Cheese	7
Cream	6
Ice cream	4
Dips	3
Dairy desserts	2
Yoghurt	2
Milk powder	2
Custard	1

Table 9 Food recalls notified to FSANZ 1990 – August 2005

Product	Reason
Skim milk	*Lactobacillus* spp.
Various flavoured UHT milk	Coliforms
Ice cream party cakes	Suspected *Salmonella* contamination
Chocolate flavoured UHT milk	*B. cereus*
Chocolate coated ice cream sticks	*L. monocytogenes*
Milk	*L. monocytogenes*
Full cream milk powder (1 kg pack)	*Salmonella* anatum
Spring onion dip	*L. monocytogenes*
Smoked trout dip	*L. monocytogenes*
Ice cream and ice cream sticks	*E. coli*
Choc coated ice cream bars	*L. monocytogenes*
Gourmet dips (various flavours)	*L. monocytogenes*
Frozen unpasteurised goat milk, soft cheese and fetta	Unacceptable levels of micro-organisms
Thickened cream	*L. monocytogenes*
Cheese	*Staphylococcus*
Milk (plain and flavoured)	*L. monocytogenes*
Whole milk	*L*. monocytogenes

Table 9: (cont.)

Product	Reason
Cream	*E. coli*
Fetta made from goat milk	*E. coli*
Goat milk yoghurt	*E. coli*
Goat milk fetta cheese, yoghurt, pasteurised milk	*E. coli*
Ice cream (berry flavour)	*L. monocytogenes*
Flavoured milk	*L. monocytogenes*
Chocolate milk	*L. monocytogenes*
Yoghurt	*E. coli*
Cheese	*L. monocytogenes*
Cappuccino topping (skim milk powder (1 kg and 500g packs)	*Salmonella*
Parmesan and skim milk fetta wedges	*L. monocytogenes*
Chocolate mousse	*L. monocytogenes*
Various mousse and cheese cake products	*L. monocytogenes*
Ice cream cake	*E. coli*
Ice cream cake	*E. coli*
Custard	*Bacillus*
Whipping cream	*E. coli*
Chocolate flavoured UHT milk	*Bacillus*
Petit fromage	*E. coli*
UHT Banana flavoured milk	Spoilage
Whole milk and fresh cream	Pasteurisation fault
Flavoured milk	Spoilage
UHT milk	Premature spoilage – faulty packaging
UHT milk	Premature spoilage
UHT milk	Premature spoilage – faulty packaging

3.1.3 Data submitted by Industry to Dairy Australia (2005)

The following data was complied by Dairy Australia, and represents the larger processors of dairy products in Australia.

Table 10: Prevalence of *Bacillus* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Cheese	*B. cereus*	2004	31	0	Dairy Australia
Cheese	*Bacillus* spp.	2003/04	31	25.8	Dairy Australia
Cream and butter	*Bacillus* spp.	2003/04	65	0	Dairy Australia
Dried milk	*Bacillus* spp.	2003/04	250	2	Dairy Australia

Table 11: Prevalence of *Campylobacter* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Milk and milk products	*Campylobacter* spp.	2004	25	0	Dairy Australia

Table 12: Prevalence of *Escherichia coli* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Cream and cream products	*E. coli*	2004	1,672	0.54	Dairy Australia
Custard	*E. coli*	2004	58	0	Dairy Australia
Ice cream products	*E. coli*	2004	8,015	0	Dairy Australia
Milk and milk products	*E. coli*	2004	22,440	0	Dairy Australia
Yoghurt, fermented milk	*E. coli*	2004	260	0	Dairy Australia

Table 13: Prevalence of *Listeria* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Milk, cream, cream products, yoghurts	*Listeria* spp.	2004	748	0	Dairy Australia
Cream and butter	*Listeria* spp.	2003/04	4	0	Dairy Australia
Cheese	*Listeria* spp.	2003/04	1,957	0.05	Dairy Australia
Cheese	*Listeria* spp.	2004	184	0	Dairy Australia
Cheese	*Listeria* spp.	2004	253	0	Dairy Australia
Cheese	*Listeria* spp.	2004	12	0	Dairy Australia
Cheese	*Listeria* spp.	2004	31	0	Dairy Australia
Dried milk	*Listeria* spp.	2003/04	126	0	Dairy Australia
Ice cream products	*L. monocytogenes*	2004	4	0	Dairy Australia
Milk	*Listeria* spp.	2003/04	95	0	Dairy Australia
Milk	*L. monocytogenes*	2004	1,560	0	Dairy Australia

Table 14: Prevalence of *Salmonella* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Dried milk	*Salmonella* spp.	2003/04	100	1	Dairy Australia
Dried milk	*Salmonella* spp.	2003/04	1,658	0	Dairy Australia
Dried milk	*Salmonella* spp.	2004	1,797	0	Dairy Australia
Cream and cream products	*Salmonella* spp.	2004	200	2	Dairy Australia
Cream and butter	*Salmonella* spp.	2003/04	65	0	Dairy Australia
Cheese	*Salmonella* spp.	2003/04	691	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	40	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	40	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	31	0	Dairy Australia
Cheese	*Salmonella* spp.	2004	35	0	Dairy Australia
Milk powder	*Salmonella* spp.	2004	285	0	Dairy Australia
Milk powder	*Salmonella* spp.	2004	12	0	Dairy Australia
Ice cream products	*Salmonella* spp.	2004	4	0	Dairy Australia
Ice cream products	*Salmonella* spp.	2004	100	0	Dairy Australia
Milk and milk products	*Salmonella* spp.	2004	424	0	Dairy Australia
Yoghurt products	*Salmonella* spp.	2004	7	0	Dairy Australia
Dairy desserts	*Salmonella* spp.	2004	4	0	Dairy Australia

Table 15: Prevalence of *Staphylococcus aureus* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Dairy based dips	*S. aureus*	2003/04	50	0	Dairy Australia
Custard	*S. aureus*	2004	27	0	Dairy Australia
Cheese	*S. aureus*	2003/04	3,457	0	Dairy Australia
Cheese	*S. aureus*	2004	1,265	0	Dairy Australia
Cheese	*S. aureus*	2004	12	0	Dairy Australia
Cheese	*S. aureus*	2004	31	0	Dairy Australia
Cheese	*S. aureus*	2004	274	0	Dairy Australia
Cream and butter	*S. aureus*	2003/04	66	0	Dairy Australia
Dried milk	*S. aureus*	2003/04	696	0.29	Dairy Australia
Ice cream products	*S. aureus*	2004	4	0	Dairy Australia
Milk	*S. aureus*	2003/04	102	1.96	Dairy Australia
Milk	*S. aureus*	2004	0	0	Dairy Australia
Milk Powder	*S. aureus*	2004	285	0	Dairy Australia

Table 16: Prevalence of *Yersinia enterocolitica* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Milk and milk products	*Y. enterocolitica*	2004	25	0	Dairy Australia

3.1.4 Surveys from the scientific literature

Table 17: Prevalence of *Aeromonas* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Pasteurised cow's milk	*Aeromonas*	-	183	4	Kirov et al., 1993
Raw cow's milk	*Aeromonas*	-	72	60	Kirov et al., 1993
Raw cow's milk	*Aeromonas*	-	150	27	Ibrahim and Mac Rae, 1991

Table 18: Prevalence of *Listeria* spp. in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Raw cow's milk	*L. monocytogenes*	-	600	0	Anon, 2003
Cheese - soft	*L. monocytogenes*	-	437	3.4	Arnold and Coble, 1995
Ice cream and ice cream products	*L. monocytogenes*	-	166	13.8	Arnold and Coble, 1995
Pasteurised cow's milk	*L. monocytogenes*	-	33	0	Arnold and Coble, 1995
Raw goat's milk	*L. monocytogenes*	-	69	1.4	Arnold and Coble, 1995
Raw cow's milk	*L. monocytogenes*	-	150	0	Ibrahim and MacRae, 1991
Cheese	*L. monocytogenes*	-	255	2	Venables, 1989
Ice cream	*L. monocytogenes*	-	277	6	Venables, 1989

Table 19: Prevalence of *Yersinia enterocolitica* in dairy products in Australia

Sample type	Organisms Isolated	Sampling period	Samples	% Positive	Reference
Raw goat's milk	*Y. enterocolitica*	-	274	12.8	Hughes and Jensen, 1981
Pasteurised cow's milk	*Yersinia* spp.	-	551	2.0	Hughes, 1987

3.2 Occurrence of microbiological hazards in Dairy products overseas

The majority of published reports on the occurrence of microbiological hazards in dairy products are for raw milk. As these hazards are inactivated by pasteurisation, the prevalence of these organisms in finished product is extremely low.

The following tables provide a summary of the reported prevalence of microbiological hazards in dairy products overseas. It is difficult to directly compare results between individual studies due to differences in the number of type of samples analysed, the stage of production that samples were taken, and the methodology used to isolate and/or enumerate the organisms. In general, the reported prevalence of microbiological hazards in raw milk is highly variable and is dependant on local factors. With the exception of *Bacillus cereus* spores, pasteurisation effectively inactivates these hazards.

Table 20: Prevalence of *Aeromonas* spp.in dairy products

Sample type	**Organisms Isolated**	**Country**	**Samples**	**% Positive**	**Reference**
Raw cow's milk	*A. caviae, A. hydrophila, A. sobria*	Turkey	157	47.7	Yucel and Citak, 2003
Pasteurised cow's milk	*A. hydrophila*	Turkey	31	5	Yucel and Citak, 2003
Cheese - soft	*A. hydrophila*	Brazil	45	17.7	Araujo et al., 2002
Cheese – homemade minas frescal	*A. caviae, A. hydrophila, A. schubertii*	Brazil	160	51.2	Bulhoes and Junior, 2002
Raw cow's milk	*A. caviae, A. hydrophila, A. sobria*	Egypt	60	75	Ibrahim, 2001
Cheese - Domiati	*A. caviae, A. hydrophila, A. sobria*	Egypt	50	36	Effat at el., 2000
Cheese - Kareish	*A. caviae, A. hydrophila, A. sobria*	Egypt	50	58	Effat at el., 2000
Cheese - Ricotta	*A. caviae, A. hydrophila, A. sobria*	Italy	20	45	Villari et al., 2000
Cheese - Mascarpone	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Cheese - Mozzarella	*A. caviae*	Italy	20	5	Villari et al., 2000

Table 20: (cont.)

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Cheese - Fiordilatte	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Cheese - Treccia	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Ice cream	*A. caviae, A. hydrophila, A. sobria*	Italy	20	0	Villari et al., 2000
Raw cow's milk	*Aeromonas*	Greece	138	40.6	Melas at al., 1999
Raw ewe's milk	*Aeromonas*	Greece	57	35.1	Melas at al., 1999
Pasteurised cow's milk	*Aeromonas*	Greece	80	0	Melas at al., 1999
Cheese - Anthotyros	*Aeromonas*	Greece	39	10.3	Melas at al., 1999
Cheese - Manouri	*Aeromonas*	Greece	36	8.3	Melas at al., 1999
Cheese - Feta	*Aeromonas*	Greece	23	0	Melas at al., 1999
Raw cow's milk	*A. hydrophila*	Turkey	200	0.5	Uraz and Citak, 1998
Pasteurised cow's milk	*A. caviae, A. hydrophila, A. sobria*	Turkey	100	19	Sarimehmetoglu et al., 1998
Cheese - Villalón	*Aeromonas*	Spain	8	25	Santos et al., 1996
Raw cow's milk	*A. caviae, A. hydrophila, A. sobria*	Turkey	80	28.7	Akan et al., 1996
Raw cow's milk	*A. hydrophila*	Germany	200	14	Schweizer et al., 1995
Ice cream	*A. hydrophila*	India	42	7.1	Shankar et al., 1994
Raw cow's milk	*Aeromonas*	Spain	5	20	Pin et al., 1994
Cheese - unripened	*Aeromonas*	Spain	5	20	Pin et al., 1994
Cheese - soft	*Aeromonas*	England	43	2	Walker and Brooks, 1993
Milk and other dairy products	*Aeromonas*	England	97	2	Walker and Brooks, 1993
Pasteurised cow's milk	*Aeromonas*	Brazil	35	28.5	Freitas et al., 1993
Cheese - white	*Aeromonas*	Brazil	25	32	Freitas et al., 1993
Raw cow's milk	*Aeromonas*	Sweden	4	0	Krovacek et al., 1992
Cream - whipped	*Aeromonas*	Denmark	32	28	Knøchel and Jeppesen, 1990
Pasteurised cow's milk	*A. hydrophila*	China	248	0.4	Chen et al., 1988
Raw cow's milk	*A. hydrophila*	China	53	20	Chen et al., 1988
Milk and milk products	*A. hydrophila*	Canada	41	2.4%	Banerjee and Black, 1986

Table 21: Prevalence of *Bacillus cereus* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Infant formula - dried	*B. cereus*	Italy	30	16.7	Pizzin et al., 2003
Infant formula - liquid	*B. cereus*	Italy	20	5	Pizzin et al., 2003
Pasteurised cow's milk	*B. cereus*	Turkey	120	46.6	Ozdemir, 2003
Bulk cow's milk	*B. cereus*	Czech Republic	111	0	Schlegelova, 2002
Cheese - ricotta	*B. cereus*	Italy	50	0	Brindani et al., 2001
Milk powder	*B. cereus*	Germany	1,365	10.7	Hammer at al., 2001
Cheese - ricotta	*B. cereus*	Italy	50	0	Brindani et al., 2001
Raw cow's milk	*B. cereus*	Germany	149	8.1	Hahn et al., 1999
Raw cow's milk	*B. cereus*	Sweden	144	69.4	Christiansson et al., 1999
Cheese – Pichtogalo Chanion	*B. cereus*	Greece	62	14.5	Papageorgiou et al., 1998
Cheese – ricotta, raw cow's milk	*B. cereus*	Italy	32	6.25	Cosseddu et al., 1997
Infant formula - dried	*B. cereus*	UK	100	17	Rowan et al., 1997
Infant formula - prepared	*B. cereus*	UK	24	8.3	Rowan et al., 1997
Pasteurised cow's milk and cream	*B. cereus*	Denmark	458	56	Larsen and Jorgensen, 1997
Raw cow's milk	*B. cereus*	Denmark	115	25	Larsen and Jorgensen, 1997
Raw cow's milk	*B. cereus*	Canada	298	0.7	Odumeru et al., 1997
Dried milk powder	*B. cereus*	UK	45	53	Crielly et al., 1994
Infant formula - imported	*B. cereus*	Germany	92	52	Becker et al., 1994

Table 22: Incidence of *Bacillus cereus* in infant formula (Extracted from Becker *et al*., 1994)

Product	Positive/total samples examined	MPN/g		
		0.3-10	>10-100	>100
Infant formula (milk protein)	48/92 (52%)	37	10	1
Infant formula (soy protein)	11/16 (69%)	11	-	-
Follow-on formula	45/86 (52%)	35	7	3

Table 23: Incidence of *Bacillus cereus* in milk based infant food (extracted from Becker *et al*., 1994)

Country	Product	Samples	% positive	*B. cereus* /g	Authors
Romania	Milk products[a]	?	(25.8)	?	Ionescu and Ionescu, 1971
FRG	Infant Food	60	8 (13.3)	100-400	Könning, 1972
Korea	Dried milk[a]	3 Brands	?	1.5-100	Kwun et al., 1979
India	Infant food	10	9 (90.0)	200-2,000	Singh et al., 1980
Poland	Infant food	25	15 (60.0)	10-1000	Stec and Burzynska, 1980
USSR	Infant food	?	?	5-650	Kirilenko et al., 1983
FRG	Infant food	90	39 (43.3)	<1500	Döll, 1983
Egypt	Infant food	10	10 (100)	10-3,000	Helmy et al., 1984
FRG	Infant food Whey powder[a] Skim milk powder[a] Lactose[a]	140 10 6 50	54 (38.6) 6 (60.0) 4 (66.7) 0	3-460 3-100 3-100 0	Becker et al., 1984
Egypt	Infant food	30	24(80.0)	<500	Moustafa et al., 1984

[a] intended for infant feeding

Table 24: Prevalence of *Brucella* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Ice cream	*Br. abortus*	Turkey	217	6.25	Kuplulu and Sarimehmetoglu, 2004
Cheese – cow's milk	*Brucella* spp.	Turkey	35	0	Kasimoglu, 2002
Cheese – ewe's milk	*Brucella* spp.	Turkey	35	14.2	Kasimoglu, 2002
Raw cow's milk	*Br. melitensis*	Turkey	35	0	Kasimoglu, 2002
Cheese – ewe's and goat's milk	*Brucella* spp.	Italy	46	46	Tantillo et al., 2001
Cheese – mozzarella	*Brucella* spp.	Italy	150	0	Serpe et al., 2000
Cheese - ricotta	*Brucella* spp.	Italy	100	0	Serpe et al., 2000
Cheese – white, fresh	*Br. abortus and Br. melitensis*	Mexico	335	7.5	Acedo et al., 1997
Raw cow's milk	*Br. abortus and Br. melitensis*	Mexico	265	2.3	Acedo et al., 1997
Raw goat's milk	*Br. abortus and Br. melitensis*	Mexico	24	4.2	Acedo et al., 1997
Raw camel's milk	*Br. abortus*	Sudan	153	10.5	Obied et al., 1996
Raw cow's milk	*Br. abortus*	New Zealand	115	31	Blair, 1948

Table 25: Prevalence of *Campylobacter* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*Campylobacter*	UK	610	0.8	Food Standards Agency, 2003
Pasteurised cow's milk	*Campylobacter*	UK	1413	0	Food Standards Agency, 2003
Raw cow's milk	*Campylobacter*	Ireland	62	1.6	Whyte et al., 2004
Raw goat's milk	*Campylobacter*	Switzerland	344	0	Muehlherr et al., 2003
Raw ewe's milk	*Campylobacter*	Switzerland	63	0	Muehlherr et al., 2003
Raw cow's milk	*Campylobacter*	US	131	9.2	Jayarao and Henning, 2001
Raw cow's milk	*Campylobacter*	Turkey	211	8.1	Uraz and Yucel, 1999
Raw goat's milk	*Campylobacter*	UK	100	0	Little and De Louvois, 1999
Raw sheep's milk	*Campylobacter*	UK	26	0	Little and De Louvois, 1999
Raw cow's milk	*Campylobacter*	Canada	1,720	0.47	Steele et al., 1997
Bulk cow's milk	*Campylobacter*	Trinidad	177	0	Adesiyun et al., 1996
Raw cow's milk and other dairy products	*Campylobacter*	Switzerland	93	6.5 (PCR) 0(culture)	Wegmuller et al., 1993
Raw cow's milk	*Campylobacter*	US	292	12.3	Rohrbach et al., 1992
Raw cow's milk	*Campylobacter*	UK	-	6	Humphrey and Hart, 1988
Raw cow's milk	*C. jejuni*	Netherlands	904	4.5	Beumer et al., 1988
Raw goat's milk	*Campylobacter*	-	2,493	0.04	Roberts, 1985
Raw cow's milk	*C. jejuni*	US	195	1.5	Lovett et al., 1983
Raw cow's milk	*Campylobacter*	US	108	0.9	Doyle and Roman, 1982

Table 26: Prevalence of *Coxiella burnetii* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Bulk tank milk	*C. burnetii*	UK	373	21.2 (ELISA)	Paiba et al., 1999
Raw cow's milk	*C. burnetii*	Japan	62	33.9 (PCR-ELISA)	Muramatsu et al., 1997
Raw cow's milk	*C. burnetii*	Nigeria	169	24	Adesiyun et al., 1985
Raw cow's milk	*C. burnetii*	US	109	7.3	Enright et al., 1957

Table 27: Prevalence of *Enterobacter sakazakii* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Cheese product	*E. sakazakii*	UK	82	2.4	Iversen and Forsythe, 2004
Milk powder	*E. sakazakii*	UK	72	4.2	Iversen and Forsythe, 2004
Infant formula - dried	*E. sakazakii*	UK	62	62	Iversen and Forsythe, 2004
Infant formula	*E. sakazakii*	UK	58	13.8	Leuschner et al., 2004
Infant formula	*E. sakazakii*	Netherlands	40	2.5	Heuvelink et al., 2001
Milk powder	*E. sakazakii*	Netherlands	170	4.1	Heuvelink et al., 2001
Infant formula - cans	*E. sakazakii*	Canada	120	6.7	Nazarowec-White and Farber, 1997

Table 28: Prevalence of pathogenic *Escherichia coli* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Pasteurised bovine products	*E. coli* O157	Italy	657	0	Conedera et al., 2004
Raw bovine products	*E. coli* O157	Italy	811	0	Conedera et al., 2004
Pasteurised ovine products	*E. coli* O157	Italy	477	0	Conedera et al., 2004
Raw ovine products	*E. coli* O157	Italy	502	0	Conedera et al., 2004
Cheese – buffalo milk mozarella	*E. coli* O157	Italy	501	0	Conedera et al., 2004
Raw cow's milk	*E. coli* O157	Northern Ireland	420	2.14	McKee et al., 2003
Raw cow's milk	*E. coli*	US	77,172	3.1-6.7	Makovec and Ruegg, 2003
Raw goat's milk	STEC	Switzerland	344	16.3	Muehlherr et al., 2003
Raw ewe's milk	STEC	Switzerland	63	12.7	Muehlherr et al., 2003
Raw cow's milk (bulk tank)	*E. coli* O157:H7	US	268	0.75	Murinda et al., 2002
Raw goat's milk	*E. coli* O157:H7	Italy	60	1.7	Foschino et al., 2002
Raw cow's milk (bulk tank)	*E. coli* O157:H7	US	131	0	Jayarao and Henning, 2001
Raw cow's milk (bulk tank)	STEC	US	131	3.8	Jayarao and Henning, 2001
Raw cow's milk	*E. coli* O157	Scotland	500	0	Coia et al., 2001
Cheese- raw milk	*E. coli* O157	Scotland	739	0	Coia et al., 2001
Cheese	STEC	France	603	1	Pradel et al., 2000
Raw cow's milk	*E. coli* O157:H7	Italy	100	0	Massa et al., 1999
Raw ewe's milk	*E. coli* O157:H7	UK	26	0	Little and De Louvois, 1999
Raw goat's milk	*E. coli* O157:H7	UK	100	0	Little and De Louvois, 1999
Raw cow's milk	*E. coli* O157:H7	Netherlands	1,011	0	Heuvelink et al., 1998
Raw cow's milk	VTEC	Canada	1,720	0.87	Steele et al., 1997
Raw cow's milk	*E. coli* O157:H7	UK	329	0	Mechie et al., 1997
Raw cow's milk	*E. coli*	France	69	~80	Desmasures et al., 1997
Cheese – raw milk, soft	Toxigenic *E. coli*	Spain	221	1.4	Quinto and Cepeda, 1997
Cheese– pasteurised, soft	Toxigenic *E. coli*	Spain	75	0	Quinto and Cepeda, 1997
Raw cow's milk	*E. coli* O157:H7	US	115	10	Padhye and Doyle, 1991
Raw cow's milk	*E. coli* O157:H7	US	23	4.3	Wells et al., 1991

Table 29: Prevalence of *Listeria monocytogenes* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*L. monocytogenes*	UK	610	17	Food Standards Agency, 2003
Pasteurised cow's milk	*L. monocytogenes*	UK	1413	0	Food Standards Agency, 2003
Cheese, pasteurised ewe's milk, soft	*L. monocytogenes*	Portugal	63	46	Pintado et al., 2005
Raw cow's milk	*L. monocytogenes*	US	861	6.5	van Kessel et al., 2004
Raw cow's milk	*L. monocytogenes*	Brazil	6	16.7	Silva et al., 2003
Raw cow's milk	*L. monocytogenes*	US	474	4.9–7.0	Muraoka et al., 2003
Bulk raw cow's milk	*L. monocytogenes*	Sweden	294	1	Waak et al., 2002
Raw cow's milk	*L. monocytogenes*	US	131	4.6	Jayaroo and Henning, 2001
Raw cow's milk (farm milk filters)	*L. monocytogenes*	US	404	12.6	Hassan et al., 2000
Raw cow's milk	*L. monocytogenes*	Spain	774	3.62	Gaya et al., 1998
Raw cow's milk	*L. monocytogenes*	Canada	1,720	2.7	Steele et al., 1997
Raw cow's milk	*L. monocytogenes*	France	69	5.8	Desmasures et al., 1997
Bulk cow's milk	*L. monocytogenes*	Trinidad	177	1.1	Adesiyun et al., 1996
Raw cow's milk	*L. monocytogenes*	Scotland	160	15.6	Fenlon et al., 1995
Cheese - soft	*L. monocytogenes*	UK	251	0.4	MacGowan et al., 1994
Raw cow's milk	*L. monocytogenes*	US	292	4.1	Rorhbach et al., 1992
Raw cow's milk (bulk tank)	*L. monocytogenes*	Finland	134	2.9	Husu, 1990
Raw cow's milk	*L. monocytogenes*	Scotland	180	1.0 – 3.8	Fenlon and Wilson, 1989
Raw cow's milk	*L. monocytogenes*	Canada	445	1.3	Farber et al., 1988

See also "Quantitative Assessment of Relative Risk to Public Health and Safety from Food-borne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods" (2003)
http://www.foodsafety.gov/~dms/lmr2-toc.html

Table 30: Prevalence of *Mycobacterium* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	MAP	Ireland	389	13 (PCR) 0.3 (culture)	O'Reilly et al., 2004
Pasteurised cow's milk	MAP	Ireland	357	9.8 (PCR) 0 (culture)	O'Reilly et al., 2004
Raw goat's milk	MAP	Norway	340	7.1 (PCR) 0 (culture)	Djonne et al., 2003
Raw goat's milk	MAP	Switzerland	344	23	Muehlherr et al., 2003
Raw sheep's milk	MAP	Switzerland	63	24	Muehlherr et al., 2003
Pasteurised cow's milk	MAP	Canada	710	15 (PCR) 0 (culture)	Gao et al., 2002
Raw cow's milk	MAP	UK	244	7.8 (PCR) 1.6 (culture)	Grant et al., 2002
Pasteurised cow's milk	MAP	UK	567	12 (PCR) 1.8 (culture)	Grant et al., 2002
Pasteurised cow's milk	MAP	Ireland	77	0	O'Doherty et al., 2002

Table 30: (cont.)

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Pasteurised goat's milk	MAP	Ireland	9	0	O'Doherty et al., 2002
Raw sheep's and goat's milk	MAP	UK	104	1 (PCR) 0 (culture)	Grant et al., 2001
Raw cow's milk	*M.* spp.	Tanzania	805	3.9	Kazwala et al., 1998
Pasteurised cow's milk	MAP	UK	312	7 (PCR) 3.5 (culture)	Millar et al., 1996
Raw cow's milk	MAP	Pakistan	72	8.3	Sabir et al., 1993
Cow's milk	*Mycobacterium* spp.	Russia	127	25.9	Gertman et al., 1990
Pasteurised cow's milk	*Mycobacterium* spp.	Germany	290	2.1	Beerwerth, 1970
Raw cow's milk	*Mycobacterium* spp.	Germany	1,764	7.9	Beerwerth, 1970

Table 31: Prevalence of *Salmonella* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*Salmonella*	UK	610	0.3	Food Standards Agency, 200?
Pasteurised cow's milk	*Salmonella*	UK	1,413	0	Food Standards Agency, 200?
Raw cow's milk	*Salmonella*	US	861	2.6	Van Kessel et al., 2004
Raw goat's milk	*Salmonella*	Switzerland	344	0	Muehlherr *et al*., 2003
Raw ewe's milk	*Salmonella*	Switzerland	63	0	Muehlherr *et al.,* 2003
Raw goat's milk	*Salmonella*	Italy	60	0	Foschino et al., 2002
Raw milk (bulk tank)	*Salmonella*	US	268	2.2	Murinda et al., 2002
Raw milk (bulk tank)	*Salmonella*	US	131	6.1	Jayarao and Henning, 2001
Raw cow's milk	*Salmonella*	US	131	6.1	Jayarao and Henning, 2001
Raw cow's milk (farm milk filters)	*Salmonella*	US	404	1.5	Hassan et al., 2000
Raw goat's milk	*Salmonella*	UK	100	0	Little and De Louvois, 1999
Raw ewe's milk	*Salmonella*	UK	26	0	Little and De Louvois, 1999
Raw cow's milk	*Salmonella*	France	69	2.9	Desmasures et al., 1997
Raw cow's milk	*Salmonella*	Canada	1,720	0.17	Steele et al., 1997
Bulk cow's milk	*Salmonella*	Trinidad	177	1.7	Adesiyun et al., 1996
Raw cow's milk	*Salmonella*	England and Wales	1,673	0.36	O'Donnell, 1995
Raw milk	*Salmonella*	Switzerland	456	0	Bachmann and Spahr, 1995
Raw cow's milk	*Salmonella*	US	292	8.9	Rohrbach et al., 1992
Cheese – Turkish white	*Salmonella*	Turkey	38	0	Turantas et al., 1989
Raw cow's milk	*Salmonella*	US	678	4.7	McManus et al., 1987

Table 32: Prevalence of *Shigella* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Cheese - white pickled	*Shigella* spp.	Turkey	50	8	Sik et al., 2004
Cheese – raw milk (Palermitano)	*Shigella* spp.	Italy	12	0	Turtura and Grasselli, 2001
Frozen yoghurt	*Shigella* spp.	Spain	170	0	Lopez et al., 1997
Cheese – ripened, pasteurised milk	*Shigella* spp.	Spain	37	0	Massa-Calpe, 1996
Cheese – fresh, pasteurised milk	*Shigella* spp.	Spain	23	0	Massa-Calpe, 1996
Raw cow's milk	*Shigella* spp.	India	65	0	Singh et al., 1996
Flavoured ice cream	*Shigella* spp.	Canary Islands	150	0	Rodriguez-Alvarez et al., 1995
Cheese - farmouse	*Shigella* spp.	Ireland	25	0	Coveney et al., 1994
Cheese – non-farmhouse	*Shigella* spp.	Ireland	2	0	Coveney et al., 1994
Cheese - imported	*Shigella* spp.	Ireland	4	0	Coveney et al., 1994
Commercial curd	*Shigella* spp.	Spain	21	0	Jordano et al., 1987
Pasteurised cream	*Shigella* spp.	Spain	20	0	Jordano et al., 1987
Sheep's milk cream product (kishfa)	*Shigella* spp.	Iraq	90	2.2	Al-Rajab et al., 1986
Spoiled UHT milk	*Shigella* spp.	China	37	2.7	Lee, 1984
Market milk	*Shigella* spp.	Poland	135	0	Maciejska-Roczan and Burzynska, 1981
Raw buffalo's milk	*Shigella* spp.	India	240	0	Kumar et al., 1978

Table 33: Prevalence of *Staphylococcus aureus* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Bulk cow's milk	*S. aureus*	Norway	220	75	Jorgensen et al., 2005
Goat's milk	*S. aureus*	Norway	213	96.2	Jorgensen et al., 2005
Bulk cow's milk	*S. aureus*	US	118	60	Sato et al., 2004
Bulk cow's milk	*S. aureus*	Denmark	40	55	Sato et al., 2004
Raw cow's milk	*S. aureus*	Malaysia	930	>60	Fook-Yee-Chye et al., 2004
Raw cow's milk	*S. aureus*	US	77,172	9.7-17.7	Makovec and Ruegg, 2003
Raw ewe's milk	*S. aureus*	Switzerland	63	33.3	Muehlherr et al., 2003
Raw goat's milk	*S. aureus*	Switzerland	344	31.7	Muehlherr et al., 2003
Bulk cow's milk	*S. aureus*	Czech Republic	111	34.2	Schlegelova, 2002
Raw goat's milk	*S. aureus*	Italy	60	43	Foschino et al., 2002
Cheese – cottage	*S. aureus*	Slovakia	35	0	Belickova et al., 2001
Cheese - Ondava	*S. aureus*	Slovakia	29	0	Belickova et al., 2001
Cheese - ricotta	*S. aureus*	Italy	50	2	Brindani et al., 2001
Whole acidophilus milk	*S. aureus*	Slovakia	18	0	Belickova et al., 2001
Raw cow's milk	*S. aureus*	Canada	21	90.4	Tondo et al., 2000
Raw ewe's milk	*S. aureus*	UK	126	7	Little and De Louvois, 1999
Composite milk	*S. aureus*	Trinidad	287	97.6	Adesiyun et al., 1998
Raw cow's milk	*S. aureus*	Italy	794	34.3	Moretti et al., 1998
Cheese – ricotta, raw cow's milk	*S. aureus*	Italy	32	0	Cosseddu et al., 1997
Raw cow's milk	*S. aureus*	France	69	62	Desmasures et al., 1997
Cheese – pasteurised milk, ripened	*S. aureus*	Spain	37	2.7	Massa-Calpe, 1996
Cheese – Minas Frescal	*S. aureus*	Brazil	18	22.2	Gomes and Galla, 1995
Raw cow's milk	*S. aureus*	Brazil	19	57.9	Gomes and Gallo, 1995
Raw cow's milk	*S. aureus*	Denmark	4,645	10.2	Aarestrup et al., 1995
Raw cow's milk	*S. aureus*	Trinidad	287	100	Adesiyun et al., 1995
Raw goat's milk	*S. aureus*	UK	2,493	4	Roberts, 1985

Table 34: Prevalence of *Streptococcus* spp. in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Raw cow's milk	*S. agalactie*	US	77,172	3.0-8.1	Makovec and Ruegg, 2003
Raw milk	*Streptococcus* spp.	Venezuela	200	9.5	Faria-Reyes et al., 2002

Table 35: Prevalence of *Yersinia enterocolitica* in dairy products

Sample type	Organisms Isolated	Country	Samples	% Positive	Reference
Pasteurised cow's milk	Y. enterocolitica	Iran	40	0	Soltan-Dallal et al., 2004
Raw cow's milk	Y. enterocolitica	Iran	310	1.6	Soltan-Dallal et al., 2004
Raw cow's milk	Y. enterocolitica	US	131	6.1	Jayaroo and Henning, 2001
Raw cow's milk	Y. enterocolitica	Turkey	211	3.8	Uraz and Yucel, 1999
Raw cow's milk	Y. enterocolitica	France	69.	36	Desmasures et al., 1997
Bulk cow's milk	*Y. enterocolitica*	Trinidad	177	1.1	Adesiyun et al., 1996
Fermented cow's milk	Y. enterocolitica	Morocco	63	6.3	Hamama et al., 1992
Raw cow's milk	Y. enterocolitica	Morocco	30	30	Hamama et al., 1992
Cheese – raw milk	Y. enterocolitica	Morocco	94	4	Hamama et al., 1992
Pasteurised cow's milk	Y. enterocolitica	Russia	120	35.8	Kuznetsof and Bagriantsev, 1992
Raw cow's milk	Y. enterocolitica	US	292	15.1	Rohrbach et al., 1992
Raw cow's milk	*Yersinia* spp.	Ireland	589	39	Rea et al., 1992
Pasteurised buffalo's milk	Y. enterocolitica	India	60	0	Toora et al., 1989
Raw buffalo's milk	Y. enterocolitica	India	207	24.1	Toora et al., 1989
Raw bulk cow's milk	Yersinia *spp.*	Northern Ireland	150	22.7	Walker and Gilmour, 1986
Farm bottled raw cow's milk	Yersinia *spp.*	Northern Ireland	20	25	Walker and Gilmour, 1986
Creamery pasteurised cow's milk	Yersinia *spp.*	Northern Ireland	100	6	Walker and Gilmour, 1986
Farm pasteurised cow's milk	Yersinia *spp.*	Northern Ireland	50	8	Walker and Gilmour, 1986
Raw cow's milk	Y. enterocolitica	Bulgaria	286	11.9	Pavlov, 1985
Raw goat's milk	Y. enterocolitica	UK	2,493	0.08	Roberts, 1985
Raw cow's milk and raw cow's milk products	Y. enterocolitica	Japan	374	3.2	Fukushima et al., 1984
Raw cow's milk	Y. enterocolitica	Nigeria	319	4.4	Umoh et al., 1984
Raw cow's milk	Y. enterocolitica	France	75	81.4	Vidon and Delmas, 1981

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APPENDIX 4

Consumption figures of dairy products for Australian consumers

4.1 Milk And Cream Consumption Data For Australia

Table 1: Australian average daily consumption of milk and milk products by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming milk (% of no. surveyed)		Average amount of milk consumed per day (g)	% of total food consumed
Male	2 - 3	170	150	(88)	457	20.3
Male	4 - 7	416	338	(81)	412	15.5
Male	8 - 11	385	321	(83)	419	13.5
Male	12 - 15	349	277	(79)	495	12.4
Male	16 - 18	215	163	(76)	536	10.6
Male	19 - 24	485	382	(79)	417	7.7
Male	25 - 44	2140	1795	(84)	314	6.2
Male	45 - 64	1554	1312	(84)	273	5.7
Male	65+	902	794	(88)	249	6.5
Female	2 - 3	213	193	(91)	420	20.9
Female	4 - 7	383	317	(83)	316	13.4
Female	8 - 11	354	273	(77)	352	11.9
Female	12 - 15	304	212	(70)	349	9.4
Female	16 - 18	218	145	(66)	298	7.1
Female	19 - 24	575	460	(80)	269	6.8
Female	25 - 44	2385	2048	(86)	234	6.0
Female	45 - 64	1752	1513	(86)	227	5.9
Female	65+	1058	907	(86)	215	6.3

Table 2: Australian average daily consumption of goat milk by gender and age (National Nutrition Survey, 1995).

Gender	Age	No. consumers surveyed	No. consuming goat milk (% of no. surveyed)		Average amount of goat milk consumed per day (g)	% of total food consumed
Male	2 - 3	170	1	(0.59)	371	0.110
Male	4 - 7	416	-		-	-
Male	8 - 11	385	-		-	-
Male	12 - 15	349	-		-	-
Male	16 - 18	215	-		-	-
Male	19 - 24	485	-		-	-
Male	25 - 44	2140	-		-	-
Male	45 - 64	1554	1	(0.06)	526	0.008
Male	65+	902	-		-	-
Female	2 - 3	213	-		-	-
Female	4 - 7	383	-		-	-
Female	8 - 11	354	-		-	-
Female	12 - 15	304	1	(0.33)	258	0.033
Female	16 - 18	218	-		-	-
Female	19- 24	575	-		-	-
Female	25 - 44	2385	3	(0.13)	337	0.013
Female	45 - 64	1752	3	(0.17)	120	0.006
Female	65+	1058	2	(0.19)	128	0.008

Table 3: Australian average daily consumption of cream and cream products by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cream (% of no. surveyed)		Average amount of cream eaten per day (g)	% of total food consumed
Male	2 - 3	170	4	(2.3)	15.77	0.02
Male	4 - 7	416	8	(1.9)	11.39	0.01
Male	8 - 11	385	13	(3.4)	24.33	0.03
Male	12 - 15	349	18	(5.2)	32.08	0.05
Male	16 - 18	215	8	(3.7)	19.95	0.02
Male	19 - 24	485	38	(7.8)	46.07	0.08
Male	25 - 44	2140	155	(7.2)	47.87	0.08
Male	45 - 64	1554	137	(8.8)	39.13	0.09
Male	65+	902	88	(9.8)	37.35	0.11
Female	2 - 3	213	9	(4.2)	20.09	0.05
Female	4 - 7	383	16	(4.2)	16.39	0.04
Female	8 - 11	354	22	(6.2)	35.35	0.10
Female	12 - 15	304	14	(4.6)	20.92	0.04
Female	16 - 18	218	20	(9.2)	32.36	0.11
Female	19 - 24	575	40	(7.0)	35.01	0.08
Female	25 - 44	2385	207	(8.7)	33.32	0.09
Female	45 - 64	1752	159	(9.1)	28.46	0.08
Female	65+	1058	107	(10.1)	27.92	0.10

4.2 Cheese Consumption Data For Australia

Table 4: Australian average daily consumption of cheese (for all cheeses) by gender and age (National Nutrition Survey, 1995).

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	67	(39.41)	26.36	0.5240
Male	4-7	416	173	(41.59)	28.06	0.5406
Male	8-11	385	126	(32.73)	36.06	0.4549
Male	12-15	349	125	(35.82)	45.11	0.5092
Male	16-18	215	97	(45.12)	45.68	0.5351
Male	19-24	485	190	(39.17)	49.62	0.4551
Male	25-44	2140	957	(44.72)	43.51	0.4554
Male	45-64	1554	618	(39.77)	36.79	0.3646
Male	65+	902	339	(37.58)	26.87	0.3005
Female	2-3	213	86	(40.38)	25.87	0.5736
Female	4-7	383	141	(36.82)	27.18	0.5123
Female	8-11	354	152	(42.94)	29.44	0.5535
Female	12-15	304	115	(37.83)	30.51	0.4450
Female	16-18	218	95	(43.58)	34.94	0.5414
Female	19-24	575	236	(41.04)	36.69	0.4770
Female	25-44	2385	1027	(43.06)	32.28	0.4141
Female	45-64	1752	761	(43.44)	31.83	0.4144
Female	65+	1058	411	(38.85)	24.82	0.3283

Table 5: Australian average daily consumption of very hard cheese by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	2	(1.17)	4.25	0.0025
Male	4-7	416	8	(1.92)	4.55	0.0040
Male	8-11	385	7	(1.82)	2.51	0.0017
Male	12-15	349	2	(0.58)	5.95	0.0011
Male	16-18	215	6	(2.79)	11.50	0.0083
Male	19-24	485	11	(2.27)	32.26	0.0171
Male	25-44	2140	57	(2.66)	10.67	0.0066
Male	45-64	1554	32	(2.06)	13.93	0.0071
Male	65+	902	13	(1.44)	9.72	0.0042
Female	2-3	213	1	(0.47)	1.70	0.0004
Female	4-7	383	7	(1.82)	8.30	0.0078
Female	8-11	354	9	(2.54)	5.01	0.0056
Female	12-15	304	9	(2.96)	1.78	0.0019
Female	16-18	218	8	(3.67)	6.80	0.0089
Female	19-24	575	11	(1.91)	7.38	0.0045
Female	25-44	2385	79	(3.31)	7.96	0.0078
Female	45-64	1752	41	(2.34)	9.86	0.0069
Female	65+	1058	14	(1.32)	8.42	0.0038

Table 6: Australian average daily consumption of soft cheese by gender and age (National Nutrition Survey, 1995).

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)		Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	3	(1.76)	17.43	0.0155
Male	4-7	416	9	(2.16)	23.01	0.0231
Male	8-11	385	13	(3.38)	30.49	0.0397
Male	12-15	349	8	(2.29)	48.84	0.3528
Male	16-18	215	3	(1.39)	68.33	0.0248
Male	19-24	485	23	(4.74)	28.48	0.0316
Male	25-44	2140	106	(4.95)	43.20	0.0501
Male	45-64	1554	75	(4.83)	39.05	0.0470
Male	65+	902	32	(3.55)	38.12	0.0402
Female	2-3	213	6	(2.82)	10.24	0.0158
Female	4-7	383	8	(2.09)	13.84	0.0148
Female	8-11	354	14	(3.95)	32.49	0.0562
Female	12-15	304	14	(4.60)	29.97	0.5323
Female	16-18	218	13	(5.96)	27.44	0.0582
Female	19-24	575	36	(6.26)	42.07	0.0834
Female	25-44	2385	158	(6.62)	33.61	0.6634
Female	45-64	1752	144	(8.22)	36.15	0.0891
Female	65+	1058	63	(5.95)	31.65	0.0642

Table 7: Australian average daily consumption of semi-soft cheese by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)	Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	-		
Male	4-7	416	2 (0.48)	16.20	0.0036
Male	8-11	385	1 (0.26)	8.40	0.008
Male	12-15	349	2 (0.57)	31.66	0.0057
Male	16-18	215	2 (0.93)	55.55	0.0134
Male	19-24	485	4 (0.83)	11.24	0.0022
Male	25-44	2140	41 (1.92)	41.81	0.0187
Male	45-64	1554	34 (2.19)	23.06	0.0126
Male	65+	902	21 (2.33)	22.23	0.0154
Female	2-3	213	-		
Female	4-7	383	2 (0.52)	28.80	0.0077
Female	8-11	354	2 (0.56)	16.35	0.0040
Female	12-15	304	1 (0.33)	14.40	0.0018
Female	16-18	218	3 (1.38)	52.30	0.0256
Female	19-24	575	6 (1.04)	28.70	0.0095
Female	25-44	2385	43 (1.80)	25.15	0.0135
Female	45-64	1752	38 (2.17)	27.72	0.0180
Female	65+	1058	24 (2.27)	17.48	0.0135

Table 8: Australian average daily consumption of processed cheese by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming cheese (% of no. surveyed)	Average amount of cheese consumed per day (g)	% total food consumed
Male	2-3	170	34 (20)	27.43	0.2767
Male	4-7	416	81 (19.47)	27.12	0.2446
Male	8-11	385	39 (10.13)	30.53	0.1192
Male	12-15	349	29 (8.31)	39.68	0.1039
Male	16-18	215	20 (9.30)	54.70	0.1321
Male	19-24	485	27 (5.57)	39.80	0.0519
Male	25-44	2140	187 (8.74)	35.79	0.0732
Male	45-64	1554	107 (6.88)	28.72	0.0493
Male	65+	902	56 (6.21)	25.70	0.0475
Female	2-3	213	40 (18.78)	27.57	0.2843
Female	4-7	383	62 (16.19)	25.02	0.2081
Female	8-11	354	41 (11.58)	23.59	0.1196
Female	12-15	304	38 (12.50)	22.37	0.1078
Female	16-18	218	24 (11.10)	27.40	0.1073
Female	19-24	575	47 (8.17)	26.56	0.0688
Female	25-44	2385	199 (8.34)	26.87	0.0669
Female	45-64	1752	174 (9.93)	24.80	0.0738
Female	65+	1058	104 (9.83)	22.09	0.0740

4.3 Dried Milk Consumption Data For Australia

Table 9: Australian average daily consumption of dried milk powder by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming dried milk powder (% of no. surveyed)	Average amount of dried milk powder consumed per day (g)	% of total food consumed
Male	2 - 3	170	-	-	-
Male	4 - 7	416	2 (0.48)	13.74	0.0031
Male	8 - 11	385	2 (0.52)	7.15	0.0014
Male	12 - 15	349	3 (0.86)	33.33	0.0090
Male	16 - 18	215	-	-	-
Male	19 - 24	485	3 (0.62)	9.47	0.0014
Male	25 - 44	2140	11 (0.51)	17.92	0.0022
Male	45 - 64	1554	26 (1.67)	16.34	0.0068
Male	65+	902	23 (2.55)	14.41	0.0109
Female	2 - 3	213	-	-	-
Female	4 - 7	383	2 (0.52)	40.74	0.0109
Female	8 - 11	354	2 (0.56)	10.95	0.0027
Female	12 - 15	304	2 (0.66)	7.35	0.0019
Female	16 - 18	218	-	-	-
Female	19- 24	575	1 (0.17)	2.00	0.0001
Female	25 - 44	2385	22 (0.92)	17.07	0.0047
Female	45 - 64	1752	36 (2.05)	20.09	0.0124
Female	65+	1058	40 (3.78)	21.98	0.0283

4.4 Condensed And Evaporated Milk Consumption Data For Australia

Table 10: Australian average daily consumption of condensed & evaporated milk by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming condensed milk (% of no. surveyed)	Average amount of condensed milk consumed per day (g)	% of total food consumed
Male	2 - 3	170	-	-	-
Male	4 - 7	416	-	-	-
Male	8 - 11	385	-	-	-
Male	12 - 15	349	2 (0.57)	107.25	0.0193
Male	16 - 18	215	-	-	-
Male	19 - 24	485	3 (0.62)	120.82	0.0175
Male	25 - 44	2140	16 (0.75)	24.54	0.0043
Male	45 - 64	1554	29 (1.87)	37.67	0.0175
Male	65+	902	16 (1.77)	38.93	0.0205
Female	2 - 3	213	-	-	-
Female	4 - 7	383	2 (0.52)	10.56	0.00282
Female	8 - 11	354	-	-	-
Female	12 - 15	304	1 (0.33)	102.38	0.0129
Female	16 - 18	218	1 (0.46)	3.25	0.0005
Female	19- 24	575	2 (0.34)	104.00	0.0115
Female	25 - 44	2385	11 (0.46)	36.40	0.0182
Female	45 - 64	1752	17 (0.97)	62.68	0.0124
Female	65+	1058	22 (2.08)	41.92	0.0297

4.5 Butter Consumption Data For Australia

Table 11: Australian average daily consumption of butter by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming butter (% of no. surveyed)		Average amount of butter consumed per day (g)	% of total food consumed
Male	2 - 3	170	16	(9)	7	0.03
Male	4 - 7	416	37	(9)	11	0.04
Male	8 - 11	385	41	(11)	11	0.04
Male	12 - 15	349	29	(8)	13	0.03
Male	16 - 18	215	17	(8)	20	0.04
Male	19 - 24	485	62	(13)	17	0.05
Male	25 - 44	2140	319	(15)	18	0.06
Male	45 - 64	1554	229	(15)	19	0.07
Male	65+	902	160	(18)	19	0.1
Female	2 - 3	213	23	(11)	7	0.04
Female	4 - 7	383	50	(13)	8	0.05
Female	8 - 11	354	44	(12)	10	0.06
Female	12 - 15	304	34	(11)	9	0.04
Female	16 - 18	218	24	(11)	10	0.04
Female	19- 24	575	70	(12)	11	0.04
Female	25 - 44	2385	385	(16)	11	0.05
Female	45 - 64	1752	271	(16)	13	0.06
Female	65+	1058	171	(16)	15	0.08

4.6 Ice Cream Consumption Data For Australia

Table 12: Australian average daily consumption of ice cream by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming ice cream (% of no. surveyed)		Average amount of ice cream consumed per day (g)	% of total food consumed
Male	2 - 3	170	24	(14.1)	80.6	0.57
Male	4 - 7	416	106	(25.5)	94.8	1.12
Male	8 - 11	385	93	(24.2)	139.6	1.30
Male	12 - 15	349	81	(23.2)	178.2	1.30
Male	16 - 18	215	45	(20.9)	216.7	1.78
Male	19 - 24	485	68	(14.0)	150.9	0.50
Male	25 - 44	2140	283	(13.2)	131.6	0.41
Male	45 - 64	1554	248	(16.0)	118.6	0.47
Male	65+	902	157	(17.4)	83.9	0.43
Female	2 - 3	213	39	(18.3)	59.2	0.59
Female	4 - 7	383	81	(21.1)	89.0	0.96
Female	8 - 11	354	81	(22.9)	115.8	1.16
Female	12 - 15	304	83	(27.3)	122.2	1.29
Female	16 - 18	218	26	(11.9)	121.1	0.51
Female	19- 24	575	75	(13.0)	98.4	0.41
Female	25 - 44	2385	263	(11.0)	88.4	0.29
Female	45 - 64	1752	209	(11.9)	72.4	0.26
Female	65+	1058	133	(12.6)	64.2	0.27

4.7 Yoghurt Consumption Data For Australia

Table 13: Australian average daily consumption of yoghurt and cultured milk products by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming yoghurt (% of no. surveyed)		Average amount of yoghurt consumed per day (g)	% of total food consumed
Male	2 - 3	170	22	(12.9)	154	1.00
Male	4 - 7	416	41	(9.6)	132	0.60
Male	8 - 11	385	24	(6.2)	192	0.46
Male	12 - 15	349	19	(5.4)	257	0.44
Male	16 - 18	215	15	(7.0)	243	0.44
Male	19 - 24	485	26	(5.4)	170	0.21
Male	25 - 44	2140	132	(6.2)	182	0.26
Male	45 - 64	1554	109	(7.0)	170	0.30
Male	65+	902	72	(8.0)	146	0.35
Female	2 - 3	213	23	(10.8)	150	0.89
Female	4 - 7	383	41	(10.7)	154	0.84
Female	8 - 11	354	22	(6.2)	179	0.49
Female	12 - 15	304	26	(8.6)	237	0.78
Female	16 - 18	218	17	(7.8)	200	0.55
Female	19- 24	575	43	(7.5)	168	0.40
Female	25 - 44	2385	250	(10.5)	162	0.51
Female	45 - 64	1752	238	(13.6)	155	0.63
Female	65+	1058	136	(12.9)	137	0.60

4.8 Dairy Based Dips Consumption Data For Australia

Table 14: Australian mean daily consumption of dairy dips* by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming dairy dips (% of number surveyed)		Mean amount of dairy dips consumed per day (g)
Male	2 - 3	170	1	(0.6)	20
Male	4 - 7	416	5	(1.2)	38
Male	8 - 11	385	6	(1.6)	64
Male	12 - 15	349	4	(1.1)	91
Male	16 - 18	215	3	(1.4)	52
Male	19 - 24	485	7	(1.4)	67
Male	25 - 44	2140	44	(2.1)	54
Male	45 - 64	1554	16	(1.0)	36
Male	65+	902	5	(0.6)	33
Female	2 - 3	213	4	(1.9)	13
Female	4 - 7	383	2	(0.5)	25
Female	8 - 11	354	4	(1.1)	35
Female	12 - 15	304	1	(0.3)	43
Female	16 - 18	218	6	(2.8)	46
Female	19 - 24	575	12	(2.1)	58
Female	25 - 44	2385	54	(2.3)	49
Female	45 - 64	1752	27	(1.5)	26
Female	65+	1058	5	(0.5)	24

* Includes yoghurt, cream cheese, and sour cream based dips, including eggplant and guacamole.

4.9 Dairy Based Dessert Consumption Data For Australia

Table 15: Australian mean daily consumption of dairy desserts* by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming dairy desserts (% of number surveyed)		Mean amount of dairy desserts consumed per day (g)
Male	2 - 3	170	15	(8.8)	105
Male	4 - 7	416	36	(8.7)	153
Male	8 - 11	385	24	(6.2)	180
Male	12 - 15	349	17	(4.9)	195
Male	16 - 18	215	14	(6.5)	162
Male	19 - 24	485	15	(3.1)	182
Male	25 - 44	2140	69	(3.2)	152
Male	45 - 64	1554	74	(4.8)	161
Male	65+	902	59	(6.5)	159
Female	2 - 3	213	15	(7.0)	103
Female	4 - 7	383	27	(7.0)	157
Female	8 - 11	354	23	(6.5)	168
Female	12 - 15	304	11	(3.6)	113
Female	16 - 18	218	6	(2.8)	148
Female	19 - 24	575	17	(3.0)	148
Female	25 - 44	2385	81	(3.4)	122
Female	45 - 64	1752	78	(4.5)	126
Female	65+	1058	71	(6.7)	133

* Includes custard, junket, dairy dessert, blancmange, flummery, mousse, cheesecake and creamed rice.

4.10 Whey Consumption Data For Australia

Table 16: Australian mean daily consumption of whey powder by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming whey powder (% of number surveyed)		Mean amount of whey powder consumed per day (g)
Male	2 - 3	170	0		-
Male	4 - 7	416	1	(0.2)	24
Male	8 - 11	385	0		-
Male	12 - 15	349	1	(0.3)	68
Male	16 - 18	215	0		-
Male	19 - 24	485	1	(0.2)	6
Male	25 - 44	2140	0		-
Male	45 - 64	1554	1	(0.1)	3
Male	65+	902	0		-
Female	2 - 3	213	0		-
Female	4 - 7	383	0		-
Female	8 - 11	354	0		-
Female	12 - 15	304	0		-
Female	16 - 18	218	0		-
Female	19 - 24	575	0		-
Female	25 - 44	2385	0		-
Female	45 - 64	1752	2	(0.1)	25
Female	65+	1058	0		-

Table 17: Australian mean daily consumption of whey based frozen dessert by gender and age (National Nutrition Survey, 1995)

Gender	Age	No. consumers surveyed	No. consuming whey-based frozen dessert (% of number surveyed)		Mean amount of whey-based frozen dessert consumed per day (g)
Male	2 - 3	170	0		-
Male	4 - 7	416	0		-
Male	8 - 11	385	0		-
Male	12 - 15	349	0		-
Male	16 - 18	215	0		-
Male	19 - 24	485	0		-
Male	25 - 44	2140	0		-
Male	45 - 64	1554	0		-
Male	65+	902	0		-
Female	2 - 3	213	0		-
Female	4 - 7	383	0		-
Female	8 - 11	354	0		-
Female	12 - 15	304	0		-
Female	16 - 18	218	0		-
Female	19 - 24	575	1	(0.2)	133
Female	25 - 44	2385	0		-
Female	45 - 64	1752	2	(0.1)	154
Female	65+	1058	0		-

APPENDIX 5

Hazard identification / hazard characterisation of pathogens

5.1 *Aeromonas* spp.

Aeromonas spp. are ubiquitous and occur worldwide, but are most frequently isolated from treated and untreated water and animals associated with water such as fish and shellfish. They may be pathogenic to amphibians, reptiles and fish. Although not yet definitively proven, there is epidemiological and clinical evidence that implicates aeromonads as causes of food-borne illness in humans.

Aeromonas hydrophila[40] is a gram-negative, facultatively anaerobic, non-spore forming rod-shaped bacterium that is present in all freshwater environments and in estuarine environments. It is also found in a wide range of foods, including seafood products and shellfish, raw foods of animal origin (for example, poultry, ground meat, raw milk), and raw vegetables and salads (Kirov, 2003).

5.1.1 Growth characteristics

Aeromonads are psychrotrophic and grow rapidly at refrigeration temperatures. Temperature range for growth is 2–45°C, with an optimum range between 28°C and 35°C (ICMSF, 1996). The organism is heat-sensitive, being easily destroyed by pasteurisation and cooking (Kirov, 2003).

Growth is optimal in the presence of 1–2 per cent NaCl (a_w = 0.991–0.986) and has been found to be inhibited completely at a NaCl concentration of 6.0 per cent (a_w = 0.96) or pH 5.5 (ICMSF, 1996). Optimal pH for growth is in the range 6.5-7.5 although high pH, up to 8.8, can be tolerated (Kirov, 2003).

5.1.2 Pathology of illness

Aeromonas spp. cause a broad spectrum of infections in humans, usually in immunocompromised patients. While identified as waterborne pathogens, *Aeromonas* spp. have not been definitively implicated as a significant cause of food-borne illness. *A. hydrophila* may cause gastroenteritis in healthy individuals or septicaemia in individuals with impaired immune systems or various malignancies. Two distinct types of gastroenteritis have been associated with *A. hydrophila*: a cholera-like illness with a watery (rice water) diarrhoea; and a dysenteric illness characterised by loose stools containing blood and mucus.

Symptoms associated with *Aeromonas*-related gastroenteritis include diarrhoea, abdominal pain, nausea, chills and headache, dysentery-like illness and colitis. Symptoms usually occur within 24–48 hours of exposure and generally last from one to 7 days (Kirov, 2003). On rare occasions, the dysentery-like syndrome is severe and may last for several weeks (Anon 2003).

[40] While many authors use the name of *A. hydrophila* as a general term to include *A. sobria* and *A. caviae* as well as the main species of *A. hydrophila* (ICMSF, 1996), in this document, *A. hydrophila* refers to this species only, unless otherwise indicated.

5.1.3 Mode of transmission

Water is considered the main source of human aeromonad infections, with food-borne illness still not firmly established for this genus (Kirov, 2003).

5.1.4 Incidence of illness

To date, there have been only a few incidents implicating *A. hydrophila* in food-borne illness. Most cases of illness attributed to *A. hydrophila* have been sporadic, rather than associated with large outbreaks. A summary of known incidents is given in Table 1.

Table 1: Food-borne illness associated with *Aeromonas* species.

Location	No. of people involved	Suspect food	Reference
Russia	'mass' poisoning	Fish (pre-frozen)	(Kalina, 1997)
Hungary	'several cases	Soups, starchy broths	(Janossy and Tarjan, 1980)
Nigeria	1	Edible land snails (pre-frozen)	(Agbonlahor *et al*., 1982)
USA	472	Oysters	(Abeyta *et al*., 1986)
USA	7	Oysters	(Abeyta *et al*., 1986)
USA	29	unknown (school lunch)	(Kobayashi and Ohnaka, 1989)
Japan	4	Seafood (sashimi)	(Kobayashi and Ohnaka 1989)
Scotland	>20	Cooked prawns	(Todd *et al*., 1989)
England	3	Oysters	(Todd *et al.,* 1989)
England	14	Cooked prawns	(Todd *et al.,* 1989)
England	2	Cooked prawns	(Todd *et al.,* 1989)
Switzerland	1	Shrimp cocktail	(Altwegg *et al*., 1991)
USA	2	Egg salad	(Bottone, 1993)
Sweden	24	shrimps, smoked sausage, liver pate, ham	(Krovacek *et al*., 1995)
Norway	3	Raw fermented fish	(Granum *et al*., 1998)
France	10	Dried fish sauce	(Hansman *et al*., 2000)

Source: (Kirov, 2003).

Suspect foods have been principally prawns and oysters, or other foods consumed with little or no cooking. In only one case, which was linked to ready to eat shrimp cocktail, has the isolate from the suspect food and from the diarrhoeal faeces been shown to be the same by ribotyping (Kirov, 2003). Most recently, reported *Aeromonas*-associated outbreaks have occurred in Sweden, Norway and France (Krovacek *et al*., 1995; Granum *et al.,* 1998; Hansman *et al.,* 2000). They are, however, still insufficiently documented to definitively established *Aeromonas* spp. as the causative agents.

5.1.5 Occurrence in foods

Aeromonas spp. are ubiquitous throughout the environment (particularly fresh and marine waters) and have been isolated from a variety of foods, including vegetables, raw and pasteurised milk and dairy products, meat and seafood (Chen *et al*., 1988; Knochel and Jeppesen, 1990; Ibrahim and Macrae, 1991; Krovacek *et al*., 1992; Shin *et al*., 1993; Craven and Macauley, 1993; Walker and Brooks, 1993; Kirov *et al*., 1993b; Freitas *et al*., 1993b; Shankar *et al*., 1994; Pin *et al*., 1994; Schweizer *et al*., 1995; Santos *et al*., 1996; Penchev *et al*., 1996; Akan *et al*., 1996; Zahran and AlSaleh, 1997; Lindberg, 1997; Eneroth *et al*., 1998; Uraz and Citak, 1998; Borrell *et al*., 1998; Sarimehmetoglu *et al*., 1998; Mauro *et al*., 1999; Melas *et al*., 1999; Villari *et al*., 2000; Neyts *et al*., 2000; Effat *et al*., 2000; Ibrahim, 2001;

Bulhoes and Junior, 2002; Araujo *et al*., 2002; Grassi *et al*., 2002; Birkenhauer and Oliver, 2002; Aly and Abo-Al-Yazeed, 2003; Yucel and Citak, 2003; Gran *et al*., 2003; Alisarli, 2003).

1.1.6 Virulence and infectivity

Illness caused by aeromonads is thought to be mediated partly by production of several virulence factors including elastases, lipases, lipopolysaccharides, adhesins, flagellae and cytolytic enterotoxins (Wadstrom and Ljungh, 1991; Kirov and Brodribb, 1993; Freitas *et al*., 1993a; Kirov *et al*., 1993a; Kirov *et al.,* 1993b; Krovacek *et al.,* 1995; Handfield *et al*., 1996; Granum *et al.,* 1998; Chopra and Houston, 1999; Kingombe *et al*., 1999; Grassi *et al.,* 2002; Sen and Rodgers, 2004). Some virulence factors are optimally expressed at lower temperatures (Kirov, 2003).

5.1.7 Dose Response

The infectious dose of *A. hydrophila* is unknown. It is possible that illness can result from a low dose, as scuba divers who have ingested small amounts of water have become ill, and *A. hydrophila* has been isolated from their stools (Anon 2003). However, it is likely that illness requires $>10^6$ cfu, based on one human trial (Morgan *et al*., 1985) and limited data from suspected food-borne incidents (Kirov, 2003).

5.1.8 Host Factors

All people are believed to be susceptible to *Aeromonas*-related gastroenteritis, although it is most frequently observed in very young children. People with impaired immune systems or underlying malignancy are considered more susceptible to infection, which often results in more severe clinical symptoms compared with the general population (Anon 2003).

5.1.9 Food Matrix

Aeromonads are unlikely to grow at refrigerated storage temperatures in foods with pH below 6.0 and water phase salt higher than about 3.0-3.5% (*w/w*) (Kirov, 2003). Organic acids, nitrite and polyphosphates reduce the growth rate of aeromonads in foods (Palumbo and Buchanan, 1988; Kirov, 2003).

5.1.10 References

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5.2 *Bacillus cereus*

The genus *Bacillus* encompasses a great diversity of species and strains. *B. cereus* is a Gram-positive, facultatively aerobic spore-forming bacteria whose cells are large rods and are motile by means of peritrichous flagella. *B. cereus* is widely distributed in the environment, and is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments.

5.2.1 Growth characteristics

Strains of *B. cereus* vary widely in their growth and survival characteristics. Psychrotrophic strains are able to grow at 4 – 5ºC but not at 30 – 35ºC, whereas mesophilic strains grow between 15ºC and 50 or 55ºC. The optimum growth temperature ranges from 30 – 40ºC. The minimum pH at which growth will occur is 5.0, maximum 8.8 and optimum 6.0-7.0 (ICMSF, 1996). The minimum water activity for survival and growth for *B. cereus* is 0.93 (ICMSF, 1996). The maximum salt concentration tolerated by *B. cereus* is 7% at pH 6-7 and 30-35ºC (Jenson and Moir, 2003).

Growth is optimal in the presence of oxygen but can occur under anaerobic conditions. Toxin production is reduced under anaerobic conditions (ESR, 2001).

Vegetative cells are relatively sensitive to environmental stress such as heat, chemicals, preservatives and radiation. However, *B. cereus* spores are more resistant due to their metabolic dormancy and tough physical nature (Jenson and Moir, 2003). Spores are more resistant to dry heat than moist heat. Spores can survive for long periods in dried foods.

The heat resistance of *B. cereus* spores has been reported as $D_{85ºC}$ = 33.8-106 min in phosphate buffer; $D_{95ºC}$ = 1.5-36.2 min in distilled water and 1.8-19.1 min in milk (ICMSF, 1996). Thus, there is considerable strain variability, with D-values for spores of some *B. cereus* strains up to 15 to 20 times greater than for the more heat sensitive strains (ICMSF, 1996).

Preservatives such as 0.26% sorbic acid at pH 5.5, and 0.39% potassium sorbate at pH 6.6 can inhibit growth. Nisin is inhibitory to *B. cereus*. Other antimicrobials which have an effect on *B. cereus* include benzoate, ethylenediaminetetraacetic acid (EDTA) and polyphosphates (Jenson and Moir, 2003).

Spores are more resistant to radiation than vegetative cells (Farkas, 1994).

5.2.2 Pathology of illness

There are two types of *B. cereus*-mediated intoxication. The two forms of illness are caused by significantly different toxins; diarrhoeal toxins (enterotoxins) and emetic toxins.

Diarrhoeagenic enterotoxins are formed in the small intestine following consumption of a large number of cells, which then results in illness. These toxins are heat labile, being inactivated in 5 minutes at 56ºC (but not 45ºC for 30 min). Four enterotoxins have been identified and characterised; two three-component enterotoxins (haemolysin BL and non-haemolytic); enterotoxin T; and a cytotoxin. The toxins are unstable at pH values outside the range 4 to 11 and sensitive to proteolytic enzymes (Jenson and Moir, 2003). Toxin activity is reduced after 1 to 2 days at 32ºC, one week at 4ºC and several weeks at –20ºC (Andersson, 1995).

The emetic toxin is preformed during growth in foods, survives the gut environment and causes illness. It has been identified as a small ring form peptide of 1.2 kDa, called cereulide (Hui et al., 2001), and is thought to be an enzymatically synthesised peptide (Granum and Lund, 1997). The emetic toxin is extremely resistant to heat and can survive 90 minutes at 126ºC (ESR, 2001). It as also very resistant to pH and proteolysis, but is not antigenic.

The incubation period for the diarrhoeal type of food poisoning is usually 10-13 hours post ingestion, although incubation periods from 8 –16 hours have been reported. Gastroenteritis is usually mild, with abdominal cramps, profuse watery diarrhoea, rectal spasms and moderate nausea, usually without vomiting. Recovery typically occurs within 24 hours.

Illness caused by the ingestion of emetic toxin generally has a shorter incubation period. Acute nausea and vomiting often occur 1-5 hours post ingestion, with recovery within 12 - 24 hours. Diarrhoeal symptoms are not normally associated with the emetic illness.

Neither form of illness is considered life-threatening to normal healthy individuals, with very few fatal cases have being reported (Jenson and Moir, 2003). Humans may vary in their susceptibility to *B. cereus* illness. Since most strains of *B. cereus* have the potential to produce toxins, the severity of illness is dependent on the quantity of toxins produced (Notermans and Batt, 1998).

In a small number of cases, both types of symptoms (diarrhoeal and vomiting) have been recorded, and this is probably due to the production of both types of toxin.

Characteristics of the two types of illness caused by *B. cereus* are summarised in Table 1.

Table 1: Characteristics of the two types of illness caused by *B. cereus* (Granum and Lund, 1997)

	Diarrhoeal syndrome	**Emetic syndrome**
Infective dose	10^5-10^7 (total)	10^5-10^8 (cells/g)
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein	Cyclic peptide
Incubation period	8-16 h (occasionally >24 h)	0.5-5 h
Duration of illness	12-24 h (occasionally several days)	6-24 h
Symptoms	Abdominal pain, watery diarrhoea and occasionally nausea	Nausea, vomiting and malaise (sometimes followed by diarrhoea, due to additional enterotoxin production?)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces and milk/milk products	Fried and cooked rice, pasta, pastry and noodles

B. cereus has also been associated with non-food-borne non-gastrointestinal infections such as ocular and wound infections; bacteraemia; central nervous system infections; respiratory tract infections; and endocarditis.

Individuals who are immunocompromised, either by illness or medication, are more susceptible to illness caused by this organism (Hui et al., 2001).

5.2.3 Mode of transmission

The enterotoxin (diarrhoeal syndrome) form of *B. cereus* poisonings is caused by the ingestion of a large number of cells and the subsequent production of the toxin in the small intestine.

The emetic syndrome of *B. cereus* food poisoning occurs after the ingestion of food in which the organism has grown and formed toxin(s). Most documented reports of *B. cereus* intoxication from this toxin have involved a cereal, or cereal- or spice-containing product as the food vehicle (ICMSF, 1996).

5.2.4 Incidence of illness

B. cereus food poisoning is not considered a reportable illness in most countries and therefore incidence data is limited (Granum and Lund, 1997). However, France, Germany and the USA report less than 0.1 cases per 10,000,000 population per annum whereas Finland, Scotland, England/Wales, Hungary and Cuba all report more than 4.0 cases per 10,000,000 per annum (Jenson and Moir, 2003).

Within Australia, during the years 1977-1984, *B. cereus* was associated with 39% of food-borne illness incidents investigated in New South Wales, and this was mostly associated with fried rice (Jenson and Moir, 2003). In the period 1995 – 2000 there were 2 identified food-borne outbreaks (total of 28 cases) due to *B. cereus* in Australia (Dalton et al., 2004). However, there may be significant under reporting of *B. cereus* illness due to the generally mild, short duration, self-limiting symptoms, in addition to it being infrequently tested for in routine laboratory analyses of stool samples.

Outbreaks of emetic-type illness have resulted from consumption of rice products or starchy foods (such as potato or pasta) that have been cooled slowly and stored incorrectly. Fried or cooked rice has been implicated in approximately 95% of cases with emetic symptoms and only a small proportion of cases have been attributed to the consumption of other foods such as crumpets, vanilla slices, cream and pasta (Kramer & Gilbert 1989; Lee, 1988).

A wide range of foods have been associated with the diarrhoeal syndrome, including meat-based dishes, soups, vegetables, puddings and sauces (Kramer & Gilbert 1989).

Powdered milk used in the preparation of vanilla slices, a milk-gelatine dessert and macaroni cheese was indicated as the source of the *B. cereus* contamination contributing to outbreaks involving these foods (Holmes, 1981; Pinegar & Buxton, 1977; Anon, 1977).

A food-borne outbreak involving 35 neonates was linked to *B. cereus* in powdered milk in Chile (Cohen et al., 1984). Levels of *B. cereus* detected in the powder ranged between 50-200 spores/g. However, analysis of preparation methods revealed a certain degree of time and temperature abuse. No further cases were detected following changes to preparation systems of infant formula.

5.2.5 Occurrence in foods

B. cereus is distributed widely in the environment and hence foods are often contaminated, particularly raw foods of plant origin. Cereal products are often a source, but numbers are rarely high (Jenson and Moir, 2003). Rice is a well recognised source, with most samples containing low levels of the organism (Jenson and Moir, 2003). Spices are also frequently contaminated with *B. cereus* (Jenson and Moir, 2003).

A survey by Nygren (1962) of the incidence of *B. cereus* in food materials revealed that 52% of 1546 food ingredients, 44% of 1911 cream and dessert dishes and 52% of 431 meat and vegetable products were contaminated, illustrating its widespread distribution. A study of milk and dairy products showed contamination rates of 9-48% and UHT-treated milk was contaminated in approximately 50% of samples (ICMSF, 1996).

The available data indicates that under normal circumstances, *B. cereus* is found in food at concentrations $<10^3$/g and mostly $<10^2$/g (ICMFS, 1996).

The presence of *B. cereus* in processed foods results from contamination of raw materials and the subsequent spore resistance to heat treatment processes during manufacture.

5.2.6 Virulence and infectivity of B. cereus

The pathogenic mechanism for the emetic toxin has been elucidated. The emetic toxin is a dodecadepsipeptide named cereulide, and causes vacuole formation in Hep-2 cells and emesis.

The pathogenic mechanism for the diarrhoeal form of illness has not been clearly elucidated although it is known that at least four different enterotoxins are involved (Jenson and Moir, 2003).

One of these enterotoxins, Haemolysin BL (HBL), consists of three protein components (L2, L1, and B), and causes the destruction of red blood cells. The second enterotoxin, non-haemolytic enterotoxin (NHE), also consists of 3 protein moieties (B, L1 and L2) and all components are needed for maximum cytotoxicity. Both HBL and NHE have been responsible for outbreaks of diarrhoeal food poisoning. The third enterotoxin, Enterotoxin T, consists of a single protein that is cytotoxin positive in the mouse ileal loop assay and possesses vascular permeability activity but does not appear to be involved in food poisoning (Hui et al., 2001). The role of enterotoxin T is unclear (Jenson and Moir, 2003).

Lund et al., (2000) recently identified the fourth enterotoxin which is a single cytotoxin protein (CytK). CytK is necrotic and haemolytic. This toxin was implicated in a severe food poisoning outbreak in France resulting in three deaths (Lund et al., 2000).

Since diarrhoeal enterotoxins are unstable and are inactivated by low pH and digestive enzymes, any preformed toxins should be destroyed during passage through the stomach and not likely to cause illness (Notermans and Batt, 1998; Granum and Lund, 1997).

Other potential virulence factors associated with diarrhoeal illness that have been identified include sphingomyelinase, phosphatidylinositol- and phosphatidylcholine-specific phospholipases and haemolysins I and II (Jenson and Moir, 2003).

The involvement of intestinal receptor site(s) for the tripartite enterotoxins in diarrhoeal symptoms has not been fully elucidated. It has been postulated that the enterotoxins disrupt the membrane of epithelial cells (Notermans and Batt, 1998). The mechanisms for cereulide synthesis is also unclear, but data suggest the peptide is enzymatically produced (Hui et al., 2001).

5.2.7 Dose response

Kramer and Gilbert (1989) have summarised a large number of outbreaks caused by *B. cereus*. The concentration of *B. cereus* in foods implicated in diarrhoeal illness ranged from $1.2 \times 10^3 - 10^8$ cfu/g. It has also been reported that 10% of outbreaks have been associated with food containing less than 10^5 cfu/g (Kramer and Gilbert, 1989).

Becker et al. (1994) indicated that concentrations of *B. cereus* of 10^3 to 10^5/g can result in illness in infants or aged and infirm individuals, although such illness was rare

Granum and Lund (1997) reported that concentrations ranging from 200 to 10^9/g (or /ml) of *B. cereus* have been reported in foods implicated in food poisoning, giving total infective doses ranging from about 5×10^4 to 10^{11}organsims. Partly due to the large differences in the amount and type of enterotoxin produced by different strains, the total infective dose seems to vary between about 10^5 and 10^8 viable cells or spores. Thus, Granum and Lund (1997) suggest an average serving of food containing more than 10^3 *B. cereus*/g cannot be considered completely safe for consumption.

Rowan et al., (1997) and Notermans and Batt (1998) also suggest that the infectious dose for *B. cereus* may vary from about 1×10^5 to 1×10^8 viable cells or spores. Notermans and Batt (1998) also suggest food servings containing greater than 1×10^4 *B. cereus*/g may not be safe for consumption.

From the available data it is estimated that the minimum total infectious dose is 10^5 viable cells or spores.

5.2.8 Immune status

All people are believed to be susceptible to *B. cereus* food poisoning. *B. cereus* has the potential to cause mild food-poisoning which does not, as a rule, last more than 12-24 hours. However, some individuals, especially young children, are particularly susceptible and may be more severely affected (ICMSF, 1996). Infants, therefore, may be susceptible to illness from a lower infectious dose, but there is no available data to support this.

5.2.9 Food matrix

The impact of the food matrix on the heat resistance of spores has been investigated. *B. cereus* spores are moderately heat resistant, however resistance is increased in high-fat and oily foods (e.g. for soybean oil, the $D_{121^oC} = 30$ min) and in foods with lower water activity (Jenson and Moir, 2003).

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5.3 *Brucella* spp.

There are six species in the genus *Brucella*, these being *B. abortus* with eight biovars; *B. melitensis* with three biovars; *B. suis* with five biovars and *B. ovis*, *B. neotomae* and *B. canis* (Corbel 1984).

Brucellae are non-motile, short, Gram-negative coccoid to rod-shaped cells. Brucellae grow aerobically, but many strains of *B. abortus* and *B. ovis* require an atmosphere of increased carbon dioxide tension (5-10%) for primary isolation. They are catalase-negative and usually also oxidase negative (ICMSF 1996). *Brucella* species are pathogenic for humans and a wide range of animals. Brucellae are located intracellularly in infected animals (Tantillo *et al.* 2001).

Brucella abortus causes bovine brucellosis, a highly contagious disease. The dominant feature is late-term abortion and infertility in cattle. The disease is also a serious zoonosis, causing undulant fever in humans.

Until recently, bovine brucellosis was present throughout the world. However, a number of countries have now succeeded in eradicating this disease. These include Australia, Canada, Israel, Japan, Austria, Switzerland, Denmark, Finland, Norway, Sweden and New Zealand.

Australia has been free of bovine brucellosis since 1989. It was prevalent throughout Australia by the 1920s, particularly in dairy herds where it was a source of major economic loss and public health concern. Various regional control schemes were in operation from the 1930s, and a nationally coordinated brucellosis eradication program commenced in 1970 as a component of the brucellosis and tuberculosis eradication campaign (BTEC). Freedom from bovine brucellosis freedom was achieved progressively - Tasmania in 1975; Western Australia in 1985; the Australian Capital Territory, New South Wales, Victoria and South Australia in 1988; and Queensland and the Northern Territory in July 1989. Australia officially declared its freedom from bovine brucellosis to the Office International des Epizooties (OIE) in August 1989. There have been no recurrences of the disease since 1989 (Animal Health Australia, 2005a).

Brucella melitensis is a major cause of brucellosis in sheep and goats. The disease affects mainly adult female animals, causing abortion and udder infection. It is also a serious zoonosis and is more pathogenic to man than *Brucella abortus*.

B. melitensis infection has never been reported in sheep or goats in Australia. However, overseas travellers occasionally arrive in Australia suffering from *B. melitensis* infection, and they may travel widely in Australia. Since the organism is excreted in the urine of infected humans, infection of sheep and goats from this source is possible, although highly unlikely (Animal Health Australia, 2005b).

5.3.1 Growth and survival characteristics

Temperature
Whilst the optimum temperature for growth on artificial media is 37ºC, Brucellae can grow at temperatures between 20 and 42ºC (ICMSF 1996). There is some discrepancy in what time and temperatures are adequate to kill the bacteria.

Encev (1965) found that heat treatment at 60ºC for 30 minutes or 70ºC for more than one minute was sufficient to kill *B. suis* (4×10^8 CFU/mL in glycerol-dextrose broth). However, a time/temperature combination of 75 minutes at 85ºC was necessary to kill all 40 tested strains of *B. abortus* 1, 2 and 3 in studies conducted by (Swarm *et al.* 1981).

Survival of *Brucella* in milk and milk products declines with increasing storage temperature. Brucellae at a concentration of 8×10^9 CFU/mL survived for 800 days at –40ºC, but were eliminated within 2 days at 25ºC (Kuzdas and Morse 1954). An increase in storage temperature from 2-4ºC to 18-22ºC reduced survival time by approximately 50% for *Brucella* in Egyptian white cheese of the Domiati and Tallaga variety (Salem *et al.* 1977). In addition, high fat content of products may have a protective effect. *B. abortus* survived in higher fat cheese types for 3-4 times longer than lower fat content cheeses held at the same temperature of between 2 and 4ºC (Salem *et al*. 1977).

ICMSF reports that most published data is presented as lethal or non-lethal time/temperature combinations, with the result that D- and z-values are not quoted. One reference (Stumbo 1973) reports Brucellae in milk as having a D-value at 65.5ºC of 0.10-0.20 minutes and a z-value between 4.4 and 5.6ºC. Kronenwett *et al.* (1954) experimentally determined the z-values for eight strains of *B. abortus* in milk with the range being 4.3 to 4.8 ºC.

Water activity
A NaCl content of >3% for *B. suis* or 4% for *B. melitensis* will prevent growth of *Brucella* spp on liver agar (Lerche *et al.* 1960).

The survival rate of *Brucella* appears to decrease with increased sodium chloride in milk products (ICMSF 1996 Table 2). A survival time of 6 months was reported for salted butter (2.3% NaCl), whereas in unsalted butter Brucellae remained viable for 13 months (ICMSF 1996). However, Brucellae may resist high salt concentrations at lower temperatures. A survival time of 45 days was reported for *Brucella* in a sheep cheese brine containing 27% salt and stored at a temperature of between 11 and 14ºC (ICMSF 1996).

Water content
There is a positive correlation between the survival of *B. abortus* in different cheeses and the water content of the cheeses (Kästli and Hausch 1957). Brucellae survived six days in hard cheese (Emmentaler and Gruyer; water content of 35-36%), 15 days in Tilsit cheese (water content of 39-41%), 20 days in ‘quarterfat’ round cheese (water content 41-45%) and 57 days in soft cheeses (Munster and Camembert; water content 50%).

pH
The optimum pH for growth in artificial media for all *Brucella* spp. ranges between 6.6 and 7.4 at 37ºC (Gerhardt 1958; Corbel and Morgan 1982). The upper growth limit is between pH 8.4 (Zobell and Meyer 1932) and 8.7. Huddleson (1954) reported a lower growth limit of between 5.8 and 6.8, whilst Lerche and Entel (1959) reported a lower growth limit of between pH 4.1 and 4.5.

Kästli and Binz (1948) recorded a lower limit of pH of 5.3 for growth of *Brucella* in sterilised milk held at a temperature of 37ºC. As pH falls, the survival time of Brucellae in milk and milk products decreases (Lerche 1931; Kästli and Binz 1948). When stored at 38ºC and a pH of 5.0 survival of *B. abortus* is less than 24hr (Kästli and Binz 1948).

5.3.2 Pathology of illness

Brucellosis is a significant public health problem in endemic areas such as the Mediterranean region, western Asia, parts of Africa, the Indian subcontinent and Latin America (Kasimoğlu 2002).

The signs and symptoms of food-borne illness associated with *B. abortus*, *B. melitensis* and *B. suis* include fever, chills, sweating, weakness, headache, muscle and joint pain, diarrhoea and bloody stools during the acute phase (CDC website). The incubation period ranges from seven to 21 days, with the duration of illness being in the order of weeks. Raw milk, goat cheese made from unpasteurised milk and contaminated meats are the foods most commonly associated with food-borne transmission. The laboratory testing utilised is blood culture and positive serology. Treatment is usually with a combination of tetracyclines, streptomycin and sulphonamides/trimethoprim (ICMSF 1996).

5.3.3 Mode of transmission

Zoonotic transmission from infected animals to humans may be either via direct or indirect transmission (Kasimoğlu 2002). Direct transmission occurs via close contact with infected animals and involves the respiratory, conjunctival and cutaneous routes. Airborne transmission of brucellea is often associated with occupational exposure to infected animals. Indirect transmission to humans is generally food-borne, and is often associated with consumption of raw milk and raw milk products.

5.3.4 Incidence of illness

There have been several outbreaks of brucellosis in humans in various parts of the world. An outbreak in 1991 in Italy of brucellosis in humans involving a total of 60 cases was traced to consumption of fresh ewe's milk cheese, particularly ricotta. The majority of the cases (46) were from the town of Termoli. Patients of all ages were involved in the outbreak. A veterinary survey conducted at the same time indicated a high incidence of brucellosis in local sheep flocks (Montanaro *et al.* 1992).

Unpasteurised raw goat milk cheese contaminated with *B. melitensis* was implicated in an outbreak in Spain during 2002 which resulted in 11 cases of brucellosis (Méndez et al., 2003). Eckman (1975) also reported an outbreak of brucellosis in the US associated with consumption of unpasteurised raw goat milk cheese obtained from Mexico which contained *B. melitensis*.

A case of *Brucella* meningitis in Mexico in 1987 was due to consumption of milk and cheese made from unpasteurised goats milk. *B. melitensis* was identified in the blood and cerebrospinal fluid cultures from the patient (Challoner, Riley and Larsen 1990).

In Germany during 1995 there were 34 reported cases of brucellosis. consumption of raw milk cheese was implicated as the possible source of the infection in 14 of the cases (Rasch *et al.* 1997).

5.3.5 Occurrence in foods

Brucella is most commonly transmitted via raw milk or raw milk products, such as cheeses (Kasimoglu 2002). Ewes milk has been found to be a more significant source of *Brucella* spp than cows milk.

In a survey of 217 ice cream samples collected from small-scale producers in Turkey, *B. abortus* was isolated in 5 (6.25%) of the vanilla flavoured ice cream samples (Kuplulu and Sarimehmetoglu 2004). The levels of contamination ranged from 1.1×10^2 to 2.3×10^3 MPN/g.

The incidence of *Brucella* spp. was investigated in 289 samples of raw milk (265 from cows' milk and 24 of goats' milk), and in 335 samples of soft, white fresh cheese made in the Cajeme Sonora municipality of Mexico. Seven samples of milk and 25 of cheese samples were positive for *Brucella* spp. The species in the milk and cheese samples were identified as *B. abortus* and *B. melitensis* (Acedo, Diaz and Leon 1997).

A survey of 46 cheese samples produced from goats' and ewes' milk to detect *Brucella* spp. resulted in 46% of the samples being positive, in particular those cheeses made from ewe's milk (Tantillo *et al.* 2001).

Prevalence of animals infected with Brucella
53% of representative herd milk was found to react positively to the *Brucella* Milk Ring Test (BMRT) and 17% of herd milk provided to the Johannesburg, South Africa was found to contain viable *B. abortus* (Van Den Heever, Katz and Te Brugge 1982).

In a survey of 763 Sudanese camels from 400 herds, 16 of the 153 samples tested contained *Brucella abortus* agglutinating antibodies (Obied, Bagadi and Mukhtar 1996).

A survey of 6472 cows found that 397 were positive for *B. abortus.* In one herd alone, 30% of the cows with positive blood serum excreted *Brucella* in their milk (Ebadi, Ardalan and Zoughi 1981).

5.3.6 Virulence and infectivity

Brucella can infect and multiply in both phagocytic and nonphagocytic cells (Sarinas and hitkara, 2003). The exact mechanism of *Brucella* pathogenesis is not fully understood, with no specific cell components specifically promoting cell adhesion and invasion being characterised (Corbel, 1997).

5.3.7 Dose response

There are no quantitative data on the infective dose (ICMSF1996). Precise information is lacking on the minimal effective oral dose of *Brucella* spp., but it is estimated that inhalation of 10-100 bacteria is sufficient to cause disease in humans (Kasimoglu 2002).

5.3.8 Host factors

Individuals who are generally at greater risk of infection and/or risk of developing more severe outcomes from exposure to *Brucella* include the very young, the elderly, pregnant women and the immunucompromised (organ transplant patients, cancer patients, AIDS patients).

5.3.9 Food matrix

Brucella spp. are unlikely to multiply in food kept under hygienic conditions, and are controlled most effectively by eliminating infected subjects from the animal stock. Pasteurisation or sterilisation of milk pre-market is sufficient to prevent milk-borne brucellosis (ICMSF 1996). The combined effect of reduced water activity and pH has been found to reduce and/or eliminate Brucellae during the production of hard cheeses, however, Brucellae may survive conditions during the production of other types of cheeses such as soft cheeses.

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5.4 *Campylobacter jejuni/coli*

Campylobacter cells are mostly slender, spirally curved rods, with a single polar flagellum at one or both ends of the cell, and typically motile with a characteristic rapid darting corkscrew-like mobility (Smibert, 1984; Vandamme, 2000). They are Gram-negative and non-spore forming bacteria. Their cells are 0.2-0.8 μm wide and 0.5 to 5 μm long. Campylobacters are classified under *Campylobacteraceae*, a bacterial family comprised of genera *Campylobacter*, *Arcobacter* and *Sulfurospirillum* (Vandamme, 2000).

Among the 16 species and six subspecies of *Campylobacter*, two are most commonly isolated from stool samples of human gastroenteritis (campynet[41]; (Vandamme, 2000). They are *Campylobacter jejuni* subspecies *jejuni* (*C. jejuni*) and *Campylobacter coli* (*C. coli*). *C. jejuni* accounts for approximately 95% of *Campylobacter* caused human gastroenteritis, and *C. coli* is responsible for approximately 3-4% of the human illness. Other species causing human gastroenteritis include *C. lari*, *C. upsaliensis*, *C. fetus* subsp. *fetus*, and subsp. *venerealis*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. concisus*, *C. jejuni* subsp. *doylei*. All these species share a common feature, which is their ability to grow at, or tolerate 42^{o}C. As such, these pathogenic *Campylobacter* species are collectively referred to as thermophilic campylobacters. For the purpose of this risk evaluation, *C. jejuni* and *C. coli* are collectively referred as campylobacters in the following text.

Campylobacters are normal intestinal flora of young cattle, sheep, goats, dogs, rabbits, monkeys, cats, chickens, turkeys, ducks, seagulls, pigeons, blackbirds, starlings and sparrows (Smibert, 1984), pigs (Nielsen et al., 1997), and in blood and faecal material from humans with *Campylobacter* enteritis. They are also found in the reproductive organs and oral cavity of man and animals[42]. Healthy puppies and kittens, rodents, beetles and houseflies may also carry campylobacters (Hartnett et al., 2002). *C. jejuni* is predominantly associated with poultry and *C. coli* is found predominantly in pigs.

5.4.1 Growth characteristics

Most campylobacters possess oxidase, are able to utilise all the organic acids in the tricarboxylic acid cycle, and are capable of deamination of glutamate and aspartate. Campylobacters however do not catabolise carbohydrates, have no lipase or lecithinase activity and do not require serum or blood for growth. They do not hydrolyse gelatin, casein, starch or tyrosine, but can reduce nitrate to nitrite.

Campylobacters require microaerophilic conditions for growth and different degrees of oxygen tolerance (3 to 5%) exist among different species of *Campylobacter* (Forsythe, 2000). For optimal growth, campylobacters require microaerophilic condition with 5% oxygen and 2-10% carbon dioxide (Park, 2002; CFSAN[43]). Most *Campylobacter* strains do not grow in the presence of air, other than a few that occasionally may grow slightly under aerobic conditions. Some species can grow under anaerobic conditions with fumarate, formate and fumarate, or fumarate and hydrogen in the medium ((Smibert, 1984; Vandamme, 2000).

[41] http://campynet.vetinst.dk/ accessed 05 May 2004.
[42] http://campynet.vetinst.dk/ accessed 05 May 2004.
[43] Centre for Food Safety and Applied Nutrition, US Food & Drug Administration. http://www.cfsan.fda.gov/~mow/chap4.html Accessed 02 April 2004.

Campylobacters grow optimally at 42-43^{o}C. *C. jejuni* can grow in the temperature range of 30-45^{o}C, in the pH range of 4.9-9.5 and at water activity above 0.990. At 32^{o}C, *C. jejuni* may double its biomass in approximately 6 hours (Forsythe, 2000). Campylobacters do not multiply at temperatures below 30^{o}C (Park, 2002), which means that the number of campylobacters in foods will not increase at normal room temperatures (20 – 25^{o}C). Although unable to grow below 30^{o}C, campylobacters remain metabolic active, are able to generate ATP, and are motile at temperatures as low as 4^{o}C (Park, 2002).

Although thermotolerant, campylobacters are sensitive to heat and readily inactivated by pasteurisation treatment or domestic cooking processes. Cooking at 55-60^{o}C for several minutes readily destroys campylobacters. The D value for *C. jejuni* at 50^{o}C is 0.88-1.63 minutes (Forsythe, 2000).

Low temperature treatment leads to inactivation of campylobacters. Zhao et al. (Zhao et al., 2003) reported that a short-term (72 hours) exposure to –20^{o}C and –30^{o}C can lead to 1.3 $\log_{10}$ and 1.8 $\log_{10}$ reduction of campylobacters on the surface of poultry meat respectively. A long-term (52 weeks) exposure to –20^{o}C and –80^{o}C can result in 4 $\log_{10}$ and 0.5 $\log_{10}$ reductions respectively. The study noted however that either short-term or long-term freezer storage could leave sufficient levels of campylobacter organisms on the surface of poultry meat to cause illness in humans if the initial contamination was high.

Other than temperature, a range of other environmental factors including desiccation, oxidation, and osmotic stress influences the survival of campylobacters.

- Campylobacters are highly sensitive to desiccation and do not survive well on dry surfaces (Fernandez, 1985).
- The microaerophilic nature of campylobacters means that these organisms are inherently sensitive to oxygen and its reduction substances (Park, 2002).
- Campylobacters are much less tolerant to osmotic stress than a number of other food-borne pathogenic bacteria. For example, campylobacters are not capable of multiplication in an environment where sodium chloride concentration is 2% or higher (Doyle et al., 1982).

Due to their sensitive nature to environmental conditions and inability of growth at temperatures below 30^{o}C and under air, the ability of campylobacters to multiply outside of an animal host is severely restricted. Campylobacters are not normally capable of multiplication in food during either processing or storage, although they have the ability to survive outside optimal growth conditions. (Park, 2002).

5.4.2 Pathology of illness

Both *C. jejuni* and *C. coli* cause fever and enteritis in human. *Campylobacter* enteritis is acute inflammatory diarrhoea with clinical signs similar to those of other acute bacterial infections of the intestinal tract, such as salmonellosis or shigellosis. Detecting campylobacters in the faeces is the only way to confirm the diagnosis.

Principal symptoms caused by campylobacters are diarrhoea, abdominal pain, fever, myalgia, headache, vomiting and blood in faeces with approximate mean frequencies of 84%, 79%, 50%, 42%, 41%, 15% and 15% respectively (Lastovica et al., 2000). Nausea is also a common symptom of *Campylobacter* infection.

The onset of symptoms is often abrupt with cramping abdominal pains quickly followed by diarrhoea. Mean incubation period of *Campylobacter* enteritis is approximately 3.2 days with a range of 18 hours to 8 days. A particular feature of *Campylobacter* infection is abdominal pain, which may become continuous and sufficiently intense to mimic acute appendicitis. This is the most frequent reason for admission of *Campylobacter* enteritis patients to hospital (Skirrow et al., 2000).

An Australian multi-centre case control study identified the following symptoms of *Campylobacter* infection (Table 1). The study suggests that approximately 13.3% of *Campylobacter* enteritis patients are hospitalised, and remained in hospital for 3 nights per person (median). This figure is similar to the estimation of FoodNet (13.2%)[44]. The study also indicates that 84% of people developed *Campylobacter* enteritis misses 5 days per person (median) from work/school/recreational/holiday activities(Hall, 2003)[45]).

Although incidents are rare, campylobacters have been implicated in causing a range of extra-intestinal infections including appendicitis, haemolytic ureamic syndrome, abortion, hepatitis, cholecystitis, pancreatitis, nephritis and others (Skirrow & Blaser, 2000). *C. jejuni* may cause septicaemia, meningitis and serious neurological disorders such as Guillain-Barré syndrome (GBS), an acute neuromuscular paralysis (Nachamkin, et al., 2000), and reactive arthritis such as Reiter syndrome.

Table 1: Clinical symptoms of *Campylobacter* infection[46]

Symptoms	**% (case number 881)**
Diarrhoea	100
Stomach cramps	88
Fever	72
Nausea	70
Muscle/body aches	66
Headache	63
Vomiting	35
Blood in stool	34

Other than *C. jejuni* and *C. coli*, *C. fetus* subsp. *fetus* has been found in cases of human diarrhoea, septicaemia, abortion and meningitis. *C. hyointestinalis* subsp. *hyointestinalis*, *C. lari*, *C. concisus*, *C. jejuni* subsp. *doylei* have been found in association with human enteritis. *C. fetus* subsp. *venerealis*, *C. hyointestinalis* subsp. *hyointestinalis*, *C. lari*, *C. consisus and C. jejuni* subsp. *doylei* have been found in association with human septicaemia (Lastovica et al., 2000). *C. upsalienesis* has been isolated from cases of human diarrhoea, septicaemia, spontaneous abortion and haemolytic-uremic syndrome. A number of *Campylobacter* species such as *C. concisus*, *C. curvus*, *C. rectus*, *C. showae* and *C. sputorum* occur in human oral cavity causing periodontal diseases[47].

5.4.3 Mode of transmission

In ascertaining transmission of *Campylobacter* cases in the USA, Friedmann et al., examined data from 111 outbreaks of *Campylobacter* enteritis due to food or water occurred in the

[44] http://www.cdc.gov/foodnet/annual/2002/2002executive_summary.pdf accessed 7 July 2004
[45] Personal communication (Russell Stafford, July 2004)
[46] Personal communication (Russell Stafford, July 2004)
[47] http://campynet.vetinst.dk/ accessed 05 May 2004.

period of 1978 to 1996. Other than unknown foods, milk and water are the most common vehicles associated with transmission of *Campylobacter* (Table 2). Raw (unpasteurised) milk is largely responsible for dairy-related transmission. Of four milk-borne outbreaks of *Campylobacter* enteritis in the period of 1990 to 1992, three were caused by raw cows' milk and raw goats' milk[48].

Surveys in other developed economies, including the United Kingdom, Sweden, Germany, New Zealand, Denmark, US and Norway, indicate milk is the most frequent cause of food-borne *Campylobacter* infection (Friedman et al., 2000). Outbreak data of food-borne campylobacteriosis recorded in Australia (Table 3) between 1992 and 2001 present a similar picture to the above where approximately 42% of recorded outbreaks were the result of consumption of milk, and among this, raw milk accounts for approximately 80% of milk-borne Campylobacter outbreaks.

Table 2: Transmission vehicles for *Campylobacter* enteritis (US, 1978-1996)[49]

Vehicle	Proportion of total outbreaks
Unknown food	38%
Milk	27%
Water (community and others)	11%
Multiple food	9%
Fruits	4%
Poultry meat (chicken and turkey)	3%
Other meat	2%
Beef	1%
Eggs	1%
Other foods	4%
Total	100%

Table 3: Reported outbreaks of *Campylobacter* enteritis in Australia

Year	Number falling ill	Vehicle	Location	Reference
2001	10	A number of foods*	Restaurant	(Raupach et al., 2003)
2001	3	Chicken kebabs	Takeaway food premise	NRVP[50]
2000	12	Milk (raw)	Farm – retail dairy	(Sumner, 2002)
2000	3	Chicken kebabs	Takeaway food premise	(Anonymous, 2000)
2000	~25	Milk (raw)	Farm – school camp	(Anonymous, 2001)
1999	16	Unknown	Caterer – function	NRVP
1998	9	Milk or water	Food caterer	NRVP#
1997	171	Chicken, beef salad	Food caterer – function	NRVP
1996	40	Unknown	Residential college	(Liddle, 1997)
1995	78	Cucumber salad	Catering facility – camp	(Kirk et al., 1997)
1993	21	Milk (raw)	Church caterer – camp	(Watson et al., 1993)
1992	4	Milk (raw)	Prison	(Bates et al., 1992)

* Most likely foods were a chicken dish, spring rolls and fried rice.
# *Clostridium perfringens* was part of the cause of food-borne outbreak.
^ Other than *Campylobacter*, *Salmonella* Virchow PT 34 and *S*. Typhimurium PT 64 were involved.

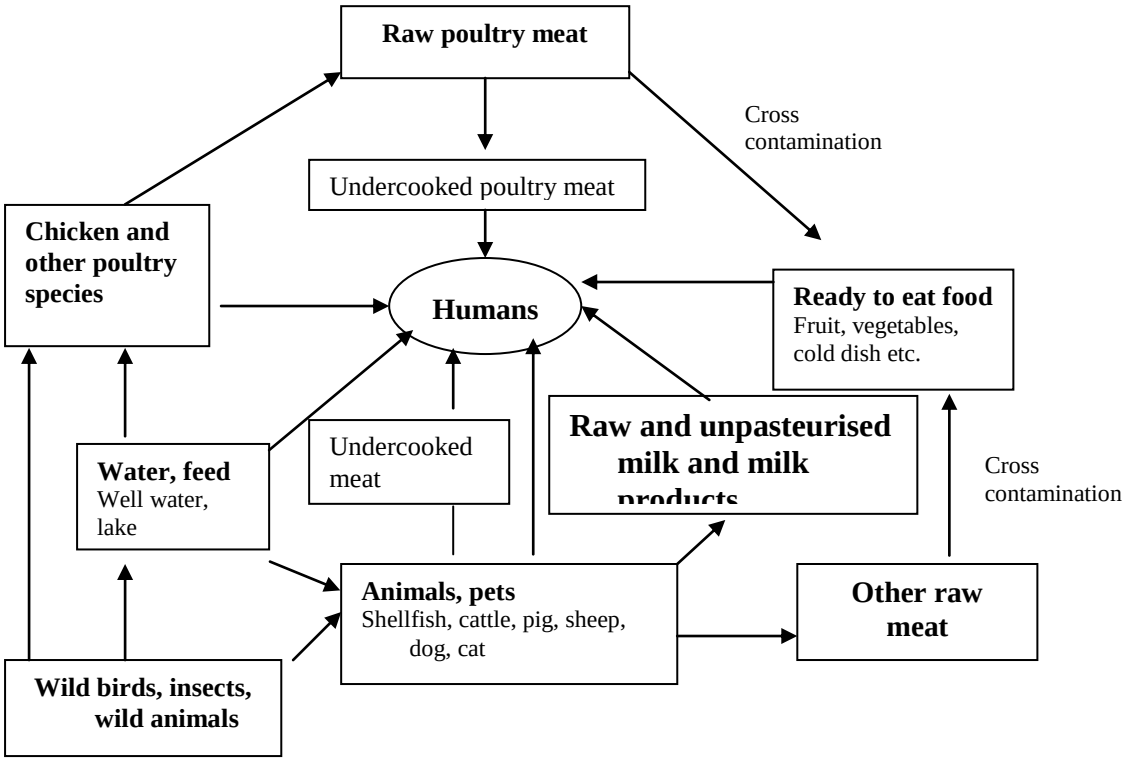
[48] http://www2.cdc.gov/ncidod/food-borne/OutbreaksReport.asp accessed 9 July 2004.
[49] Modified from Friedmann et al., 2000.
[50] NRVR for National risk validation report ((Food Science Australia & Minter Ellison Consulting, 2002)

Published information suggests that major routes of *Campylobacter* transmission to humans (Eberhart-Phillips et al., 1997; Friedman et al., 2000; Vellinga et al., 2002; World Health Organisation, 2000) are:

- consumption of food contaminated with *Campylobacter* spp., including consumption of raw and unpasteurised milk and milk products, consumption of undercooked meat such as poultry meat and consumption of raw seafood;
- consumption of water contaminated with *Campylobacter* spp.;
- bathing or swimming in a *Campylobacter* spp. contaminated lake or pool;
- direct contact with infected farm animals, such as cattle, sheep, chicken, etc.; and
- contact with infected domestic animals, such as a pet dog, cattle and bird.

These possible routes of transmission of *Campylobacter* spp. are summarised in Figure 1. In any case of the mentioned routes of transmission, *Campylobacter* infection is a result of oral ingestion of *Campylobacter* through food or water or animals, and faecal contamination is the common source of campylobacter transmission.

Figure 1: Possible route of transmission of Campylobacter spp. to humans[51]



5.4.4 Incidence of illness

Among different pathogenic bacteria associated with food-borne illness, campylobacters cause the highest number of human gastroenteritis cases in developed economies, such as Australia (Fig. 2), United Kingdom (Park, 2002) and the US (Mead et al., 1999).

[51] Adopted from Friedmann et al., (2000) and "Campylobacter" data sheet of Ministry of Health, New Zealand (May 2001).

In the USA, approximately 80% of all the cases of human campylobacteriosis are food-borne (Mead et al., 1999). Campylobacteriosis accounts for approximately 2 to 2.4 million cases of food-borne illness annually in the US (Friedman et al., 2000; Mead et al., 1999). *Campylobacter* caused food-borne illness accounts for approximately 47% of all the food-borne illnesses caused by bacteria associated with food, some 29% of hospitalisation and about 8% of death due to food-borne illness caused by bacteria.

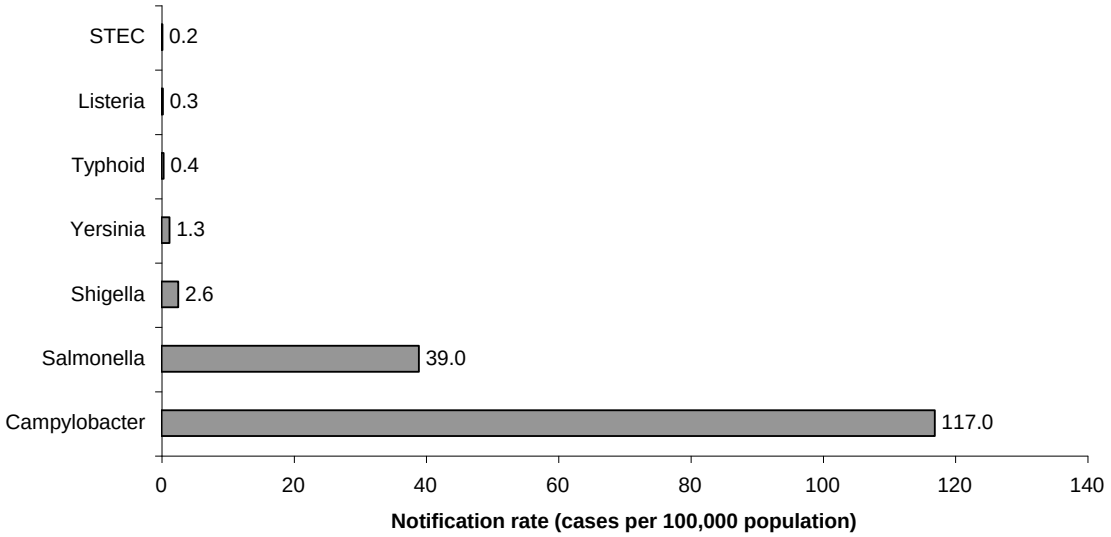


Figure 2: Notification rate for infections in Australia, 2004 (OzFoodNet, 2005)

From 2000 onwards, the notification rate of campylobacteriosis has been 100 – 120 cases per 100,000 population (Fig. 3).

Applying a multiplier of 15 (Food Science Australia & Minter Ellison Consulting, 2002) to account for underreporting of food-borne illness, the total number of campylobacteriosis cases would be at approximately 333,600 per annum in Australia. Assuming 80% of the total number of campylobacteriosis cases is transmitted by food (Food Science Australia & Minter Ellison Consulting, 2002; Mead et al., 1999), the number of food-borne campylobacteriosis cases in Australia is approximately 266,880 per year[52]. This figure is comparable to 247,351 cases of food-borne campylobacteriosis cases estimated by the Australian national risk validation project.

Although *Campylobacter* leads bacterial pathogens in causing the highest number of food-borne illness in developed economies, it is a low profile pathogen because most patients infected by campylobacters recover without treatment and large outbreaks of campylobacteriosis case are uncommon[53].

The trend of nationwide notified cases of *Campylobacter* enteritis (Fig. 4) suggests that the extent of campylobacteriosis as a communicable disease in Australia is yet to show clear signs of abating.

[52] This estimate is influenced strongly by the use of underreporting multiplier of 15. The underreporting multiplier may vary from 7.6 to 100 as suggested by the Preliminary Report of "Hazard identification, hazard characterization and exposure assessment of *Campylobacter* spp. in broiler chickens" prepared by the Joint FAO/WHO activities on risk assessment of microbiological hazards in foods (2001)

[53] http://www.fda.gov/fdac/features/1999/599_bug.html, accessed 07 July 2004

Figure 3: Notification rate for Campylobacteriosis in Australia (cases per 100,000)[54]

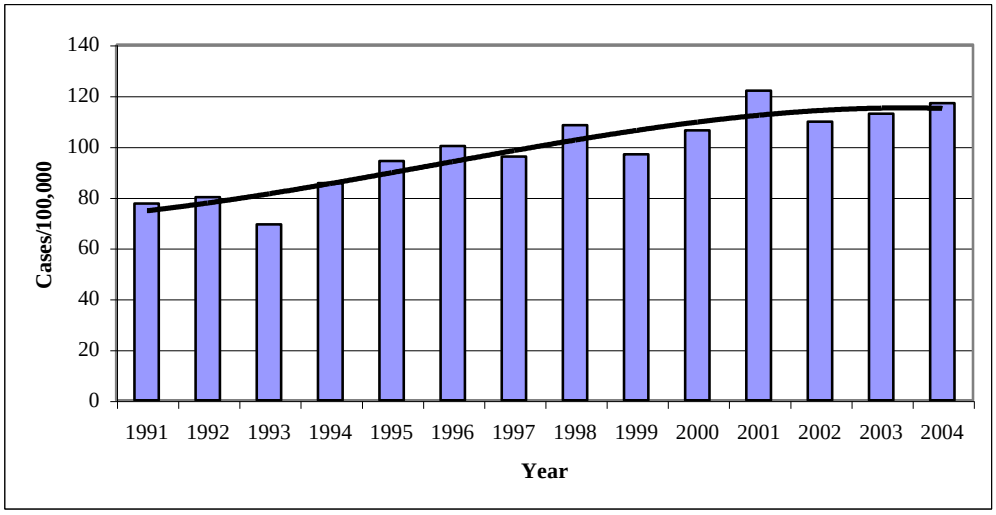
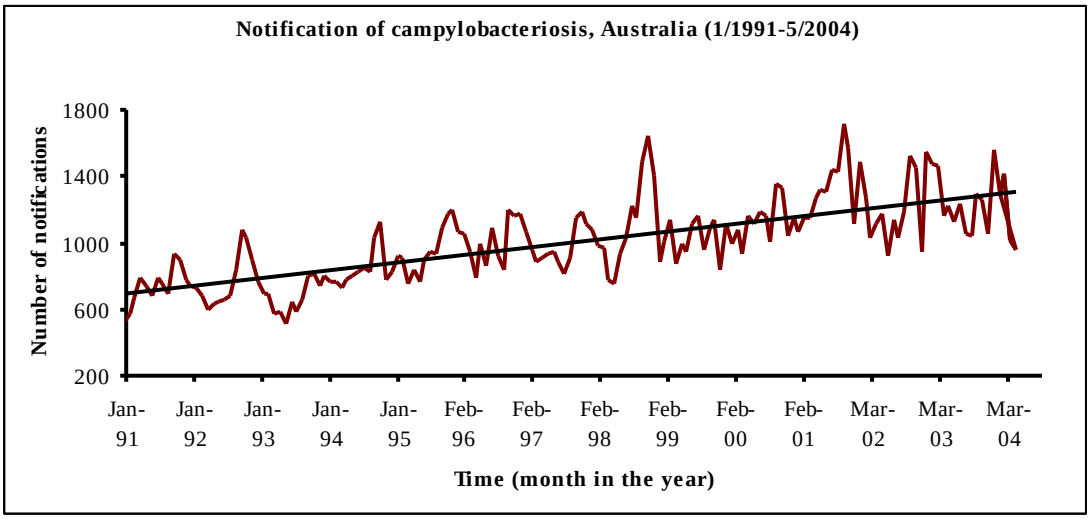


Figure 4: Annual cases of notified Campylobacteriosis in Australia[55]



There have been no reported cases of campylobacteriosis associated with the consumption of pasteurised milk or from dairy products made with pasteurised milk in Australia. A number of outbreaks of campylobacteriosis have been reported in the UK that were associated with consumption of raw milk or improperly pasteurised milk (Table x).

[54] National Notified Diseases Surveillance System. http://www1.health.gov.au/cda/Source/CDA-index.cfm Accessed 05 July 2004. Data presented does not include the State of New South Wales where campylobacteriosis is not reported separately. Population for the year 2003 is based on the estimation of Australian Bureau of Statistics.

[55] National Notified Diseases Surveillance System. http://www1.health.gov.au/cda/Source/CDA-index.cfm Accessed 05 July 2004. Data presented does not include the State of New South Wales where campylobacteriosis is not reported separately.

Table x: Outbreaks of campylobacteriosis resulting from consumption of unpasteurised and improperly pasteurised milk in the United Kingdom in 1987-1989 (Sockett, 1991) and 1992-1996(Djuretic et al., 1997).

	Number of outbreaks	Total number of cases
Unpasteurised milk	11	484
Improperly pasteurised milk	4	650

5.4.5 Occurrence in foods

Foods potentially contaminated with *Campylobacter* spp. include raw and unpasteurised milk and milk products, raw poultry, raw beef, raw pork and raw shellfish (Institute of Food Technologists, 2002).

5.4.6 Virulence and infectivity of campylobacters

The pathogenic mechanisms of *Campylobacter* causing human illness have not been fully elucidated. Two possible mechanisms are reflected in the literature. One is the genetic heterogeneity, *i.e.* different strains of *Campylobacter* may possess different ability to cause diseases (Park, 2002). The other is the involvement of microbial toxins in causing diseases. Published information indicates *Campylobacter* infection may involve production of microbial toxins. An enterotoxin[56] (Wassenaar, 1997), abbreviated, as CJT for *C. jejuni* toxin, is immunologically similar to the *Vibrio cholerae* toxin and the *E. coli* heat-liable toxin. At least six cytotoxins[57] have been observed in campylobacters. They are a 70-kDa cytotoxin, a Vero/HeLa[58] cell cytotoxin, a cytolethal distending toxin (CDT), a shiga-like toxin, a haemolytic cytotoxin and a hepatotoxin. The CDT toxin has been shown to cause dramatic distension of human tumour epithelial cells, which leads to cell disintegration (Pickett, 2000). Active CDT toxin has been found in roughly 40% of the over 700 *Campylobacter* strains tested (Johnson & Lior, 1988). However, the role of enterotoxin and the cytotoxins in *Campylobacter* pathogenesis has not been fully identified.

5.4.7 Dose Response

Campylobacter infection has been induced with a minimum dose of 800 cells in an experimental human feeding trial (Black et al., 1988). Taking into consideration the limited data in the human feeding trial and an infection rate of 50% resulting from the minimum dose, it has been proposed that the lowest infective dose would be somewhere close to 100 cells (Tribble[59]). This prediction is comparable with epidemiological data of campylobacteriosis where the number of milk-borne and waterborne outbreaks of *Campylobacter* enteritis is high.

[56] Enterotoxins are defined as secreted proteins with a capacity to bind to a cellular receptor, enter the cell and elevate intracellular cyclic AMP levels.

[57] Cytotoxins are defined as proteins that kill target cells. Cytotoxinis can act intracellularly or form pores in the cells.

[58] Vero cells refer to African green monkey kidney cells and HeLa cells are human tumour epithelial cells used in cell toxicological studies.

[59] Tribble D (1998) Suitability of experimental infections in volunteers to measure pathogenesis of food-borne pathogens. http://www.foodriskclearinghouse.umd.edu/Aug1988/Talks/tribbletalk.htm Accessed 11 February 2004

Based on the human trail data (Black et al., 1988), dose-response relationships discussed or established in various risk assessments of *Campylobacter* in poultry meat (Teunis et al., 1996, Hartnett et al., 2002, Rosenquist et al., 2003) came to a conclusion that (1) a single pathogen cell has the ability to initiate an infection and (2) the probability of causing infection increases as the level of the pathogen increases. Such dose-response relationship differs to some degree from the traditional dose-response relationship where an infection/illness is not established until a minimum dose is ingested.

Dose-response relationships have been developed based on results from human feeding studies, whereby human volunteers are fed known numbers of Campylobacter cells and then monitored for their response (Black et al., 1988). These models make the assumption that (1) a single pathogen cell has the ability to initiate an infection and (2) the probability of causing infection increases as the level of the pathogen increases.

Data from human trial experiments indicates that *Campylobacter* infection correlates proportionally to the dose ingested and gradually reaches saturation. For example, when the dose ingested increased from 3.9 log to 5.9 log (a 100 fold increase in cell numbers), *Campylobacter* infection[60] increased correspondingly by a rate of 13%[61]. Despite a direct dose-response relationship being observed for the probability of infection, the probability of illness following from infection was independent of the dose ingested. The FAO/WHO Joint Expert Group on Microbiological Risk Assessment proposed a conditional probability of illness based on the probability of infection. Beta distribution of this conditional probability (Hartnett et al., 2002) suggests that the probability of illness is 20% to 50% after the establishment of an infection by campylobacters.

5.4.8 Immune status

The incidence of *Campylobacter* infection in patients with AIDS has been calculated to be 40-fold higher than that in the general population (Sorvillo et al., 1991). In addition, 16% of *Campylobacter* infections resulted in bacteraemia in these immuno-compromised patients, a rate much higher than those occurring in the general population.

Literature data suggest that people with existing diseases have a higher susceptibility to campylobacteriosis than the general population. Pigrau et al., (1997) demonstrated in a study involving 58 patients with bacteraemia resulting from *Campylobacter* infections, 54 of the patients had existing diseases including human immunodeficiency virus infection, immunosuppressive therapy, liver cirrhosis and neoplasia.

Available data suggests that young children under the age of four and young adults in the age range of 20 to 30 years old are most susceptible to *Campylobacter* infection. Population groups that are very young (0-4 years), and that have an existing immuno-suppressed condition due to another serious disease, are likely to suffer more severe consequences as a result of *Campylobacter* infection.

[60] Infection is determined by positive detection of campylobacters in stool sample(Black et al., 1988).
I[61] illness is determined by signs of diarrhea or fever(Black et al., 1988)

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5.5 *Clostridium* spp.

Most of the clostridia are saprophytes, and only a few species are pathogenic for humans. Those that are pathogens also have primarily a saprophytic existence in nature and, in a sense, are opportunistic pathogens. For the purposes of this assessment, *Clostridium botulinum* and *Clostridium perfringens* are considered to be the principal species likely to be transmitted to humans by the food-borne route. Less common pathogenic species, such as *C. difficile*, will not be explicitly considered. *Clostridium tyrobutyricum*, a late-blowing agent in high pH and semi-hard cheeses (Klijn *et al*., 1995; Ingham *et al*., 1998; Rilla *et al*., 2003), is a food spoilage organism and is considered non-pathogenic to humans.

C. botulinum is an anaerobic, gram positive, spore-forming rod shaped bacterium that produces a potent neurotoxin. Seven types of *C. botulinum*, (types A-G) are recognised, grouped according to the antigenic specificities of their toxins (Szabo and Gibson, 1997). *C. botulinum* has also been classified phenotypically into Groups I-IV, with food-borne botulism mainly associated with Group I (proteolytic) or Group II (non-proteolytic) strains (Szabo and Gibson, 1997). The organism is ubiquitous and is found in almost all foods, whether of plant or animal origin. Spores of *C. botulinum*, although usually in low numbers, are widely distributed in soil, the sediments of lakes and coastal waters and in the intestinal tracts of fish and animals. It can cause illness in cattle (Cobb *et al*., 2002).

C. perfringens is an anaerobic (microaerophilic) Gram-positive bacillus with a square-ended rod morphology and oval central or sub-terminal spores. It is widely distributed in soil and vegetation and is part of the normal intestinal flora of humans and animals (Labbe, 1989).

C. perfringens is grouped into five types (A - E) according to the particular soluble antigens (exotoxins) produced (Labbe, 1989). Only types A, C and D are human pathogens, and only types A and C have been associated with food-borne illness (Bates and Bodnaruk, 2003). *C. perfringens* types A and C also produce an enterotoxin (*Clostridium perfringens* enterotoxin; CPE) associated with the acute abdominal pain, nausea and diarrhoea of *C. perfringens* food poisoning.

5.5.1 Growth characteristics

C. botulinum

C. botulinum requires anaerobic conditions for growth. Both the spores and the toxins of *C. botulinum* are tolerant of freezing, while toxin is destroyed rapidly at temperatures of 75–80°C (ICMSF, 1996). All strains of *C. botulinum* produce toxin to about pH 5.2 under optimal conditions (ICMSF, 1996).

Group I (proteolytic) *C. botulinum* strains have a temperature range for growth of about 10-50°C, with an optimal range of 35-40°C. Toxin production is very slow below about 12°C (ICMSF, 1996). Group I spores are the most heat-resistant of all *C. botulinum* spores and this property led to the development of the botulinum cook or '12D process' for low-acid canned foods.

Strains of Group I will not grow below pH 4.6 or if the water phase NaCl concentration exceeds 10 per cent (a_w = 0.935). Toxin production is very slow below an a_w of 0.95 (ICMSF, 1996).

Group II strains such as *C. botulinum* type E are capable of growth and toxin production at refrigeration temperatures (≥ 3.3°C) (ICMSF, 1996) but generally need weeks of growth to produce sufficient toxin to cause food-borne illness (Lyon and Reddmann, 2000). Optimum temperature for growth is 28-30°C.

Strains of Group II will not grow below pH 5.0 or if the NaCl concentration exceeds 5 per cent (a_w = 0.97) in the water phase (ICMSF, 1996).

<u>*C. perfringens*</u>
C. perfringens also requires anaerobic conditions for growth. Cells of *C. perfringens* will grow between 12°C and 50°C, with an optimum temperature of 43-45°C (Solberg and Elkind, 1970; Labbe, 1989). The organism is capable of rapid growth. Generation times as short as 7.1 min at 41°C were reported in a study of a number of strains having an average generation time of 13 min at 40°C (Willardsen *et al*., 1978). Vegetative cells die rapidly below 10°C. In experiments in laboratory media it has been shown that the thermal resistance of vegetative cells increases as the growth temperature increases (Roy *et al*., 1981). It has also been suggested that temperature stability is enhanced in foods, perhaps due to a protective effect of fats (Bradshaw *et al*., 1977; Labbe, 1989).

Optimum pH for growth is in the range 6.0 to 7.0, with growth inhibited below pH 5.5 and cell death occurring slowly below pH 5.0 (Bates and Bodnaruk, 2003). Growth is also inhibited below an a_w of 0.93.

In general, conditions for sporulation are more limited than for growth. The optimal temperature range is 35 - 40°C, and good sporulation can be obtained between pH 6.0 and 8.0 (Labbe and Duncan, 1974). The a_w must be above 0.98 for sporulation to occur (Labbe, 1989). A large amount of enterotoxin formation accompanies sporulation, so the optimal conditions for sporulation and enterotoxin formation are similar. In food-borne outbreaks, sporulation occurs primarily in the small intestine (Labbe, 1989).

There is a wide range of thermal resistance in spores of *C. perfringens* strains. In water, $D_{90°C}$ can be as long as 27.5 minutes (Adams, 1973), and thermal stability is greater in cooked meats than in water (Collee *et al*., 1961).

Germination in some strains of *C. perfringens* is improved by a moderate heat shock, in the range of 65-80°C, usually for up to ten minutes (Labbe, 1989). Strains implicated in food poisoning are more likely to require heat-activation of germination.

5.5.2 Pathology of illness

<u>*C. botulinum*</u>
Illness caused by *C. botulinum* can be of three types: food-borne, so-called “infant”, and wound botulism (Anon 2003). Food-borne botulism is caused by ingestion of preformed toxin. The mortality rate depends on the type of *C. botulinum* toxin ingested. Infant botulism affects infants under the age of 12 months and results from the ingestion of spores, which release cells that subsequently colonise the alimentary tract and produce toxin. Wound botulism occurs after infection of a wound with *C. botulinum* cells, or germination of spores within a wound, followed by toxin production.

C. botulinum neurotoxin causes muscle paralysis, beginning in the upper body and progressing downward, paralysing the chest muscles, eventually leading to asphyxiation and death. Mortality rates are still quite high, in the range 5-15% in most countries but up to 40% in some (Hauschild, 1989).

Onset of symptoms in food-borne botulism is usually 18–36 hours after ingestion of the food containing the toxin, although cases have varied from 4 hours to 8 days. Early signs of intoxication consist of marked lassitude, weakness and vertigo, usually followed by double vision and progressive difficulty in speaking and swallowing. Difficulty in breathing, weakness of other muscles, abdominal distension and constipation may also be common symptoms (Anon 2003). All people are believed to be susceptible to the food-borne intoxication.

<u>*C. perfringens*</u>
Only the symptoms of *C. perfringens* food poisoning and enteritis necroticans are described in this section. Symptoms of *C. perfringens* food poisoning include diarrhoea and abdominal cramps (sometimes severe), typically without fever. There is normally no vomiting, fever, shivering, headache or nausea. Onset of symptoms is usually within 8-24 hours after ingestion, and full recovery occurs within 24-48 hours. Unlike other toxin-mediated food-borne pathogens, toxin production occurs after the organism has been ingested, and is excreted during the process of sporulation.

Symptoms of enteritis necroticans include abdominal pain and swelling, vomiting, profuse and often bloody diarrhoea, and patchy necrosis of the upper small intestine that can lead to obstruction requiring surgical intervention. It can be fatal.

5.5.3 Mode of transmission

<u>*C. botulinum*</u>
Food-borne botulism, the ingestion of preformed toxin in foods, typically results from the presence of vegetative cells or spores of *C botulinum* in raw foods due to their presence in the growing environment or through contamination. Subsequent germination and/or growth and toxin production occur prior to ingestion of the food.

Infant botulism usually results from ingestion of spores, generally associated with the consumption of honey.

<u>*C. perfringens*</u>
C. perfringens is transmitted by the faecal-oral route and by contamination of food from the environment.

C. perfringens produces spores which vary in their heat resistance. Those spores which are highly heat resistant will be more likely to cause food poisoning due to survival and subsequent outgrowth during and after cooking. The food vehicles are usually cooked meat and poultry dishes stored for long periods of time at ambient temperature after cooking.

Spores may survive normal cooking procedures, with germination being triggered by the heat shock received during cooking. Slow cooling and non-refrigerated storage can permit growth of vegetative cells to high numbers, particularly in anaerobic environments in cooked meat and poultry dishes.

Outgrowth of spores commonly occurs after the heat shock encountered during cooking, and is favoured in anaerobic microenvironments within the food. The high number of vegetative cells produced under these conditions allows some to survive through the acidic environment of the stomach to reach the intestine, where sporulation is accompanied by production of the enterotoxin.

Type A strains also cause gas gangrene, a wound necrosis associated with poor hygiene which was widespread in troops in both world wars (Labbe, 1989).

5.5.4 Incidence of illness

C. botulinum

Botulism caused by consumption of commercial foods has been rare, with most cases involving improperly canned food (usually home-canned) and semi-preserved foods (Hauschild, 1992). Data for the years 1994-2003, inclusive, from the Food-borne Outbreak Response and Surveillance Unit of the United States Centers for Disease Control and Prevention indicate that this trend has continued (CDC 2003), with most outbreaks occurring in the private home setting and many involving home-canned and home-preserved foods.

However, outbreaks associated with commercial foods occur occasionally, *e.g.* a 1993 outbreak of type A botulism associated with a commercial cheese sauce (Townes *et al*., 1996) and a 2001 outbreak associated with inadequately refrigerated "chili" (Kalluri *et al*., 2003).

In Canada 61 outbreaks occurred in the period 1971–84, most (113/122) cases involving native peoples eating raw, parboiled or 'fermented' meats from marine mammals. Fermented salmon eggs or fish were responsible for 23 per cent of these outbreaks (Hauschild and Gauvreau, 1985). A similar pattern of illness occurs in Alaska.

In Italy, a 1996 outbreak of botulism affecting 8 people (1 death) was associated with consumption of a commercial cream cheese (mascarpone), either alone or as the (uncooked) ingredient of a dessert, tiramisu (Aureli *et al*., 2000).

In New Zealand, there have been two cases of illness (one death) due to botulism type A involving home-bottled fermented mussels and watercress, a traditional Maori food (Hauschild, 1992).

There have been no reported cases of food-borne botulism in Australia since national notification commenced in 1991 (Blumer *et al*., 2003). From 1942–83 there were five reported outbreaks of botulism in Australia (Hauschild, 1992), of which one (two cases) was linked to consumption of Australian canned tuna (Murrell, 1979).

C. perfringens

Outbreaks of *C. perfringens* food poisoning are usually associated with inadequately heated or reheated meats, pot pies, stews, or gravies. Spores become activated by the temperature shock of cooking, and if the food is not cooled to below 15°C rapidly enough, vegetative cells are able to rapidly multiply to high levels, as competing bacteria are greatly reduced in numbers by the cooking.

A summary of the epidemiology of food-borne disease outbreaks in Australia from 1995 to 2000 reported that *C. perfringens* was the responsible agent in 30 outbreaks (14% of 214 identified outbreaks) involving 787 cases (10% of the total reported food-borne illness cases) and 1 death (Dalton *et al*., 2004). The median number of cases per outbreak was 25, with a range from 2 to 171. Meats were the food vehicles in 60% (18 of 30) of those outbreaks. The outbreak settings were approximately equally split between restaurants, commercial caterers, institutional and 'other' settings. Dairy products were implicated in one outbreak (27 cases).

In 2001-2002 OzFoodNet, Australia's enhanced food-borne disease surveillance network, catalogued a further 10 outbreaks of *C. perfringens* food poisoning involving 102 cases. Dairy products were not implicated in any of these outbreaks.

The US Centres for Disease Control and Prevention (CDC) listings of food-borne disease outbreaks for 1990 to 2002 (CDC 2003), as reported to CDC through the Food-borne Disease Outbreak Surveillance System, demonstrate that *C. perfringens* was responsible for about 6% of outbreaks (10% of cases) of food-borne illness of confirmed aetiology during that period. The number of outbreaks due to *C. perfringens* ranged from 10 to 30 each year. Approximately 70% of the *C. perfringens* outbreaks were attributable to meat products or dishes. One outbreak (1995; 9 cases) was due to hard cheese and one was due to white sauce (1997; 7 cases).

Vegetable dishes are only rarely implicated in outbreaks of *C. perfringens* poisoning. In an analysis of several databases, only 1 outbreak due to *C. perfringens* related to a vegetable product was identified in the period 1969 to 1998 (Roach and Sienko, 1992; Carlin *et al*., 2000).

Outbreaks are often in institutional or mass-catering settings, where the large volumes of food prepared and/or inherent difficulties in maintaining appropriate standards of hygiene and sanitation may lead to improper cooking, cooling, holding and handling of potentially hazardous food. Because of the specific conditions leading to sporulation and growth of *C. perfringens* to high levels, it is believed that relatively few sporadic cases occur.

There are few data on the incidence of enteritis necroticans (also known as pigbel or darmbrand) due to *C. perfringens*. The disease is most commonly encountered in developing countries and is associated with poor nutrition and protein-poor and/or trypsin-inhibitor rich diets. These conditions allow for survival of the β-toxin of type C strains, a protein which is usually rapidly proteolysed in healthy and well-nourished individuals.

5.5.5 Occurrence in foods

C. botulinum

There is a large amount of data relating to the presence of *C. botulinum* in fish, and some data relating to the level and prevalence of spores in meat, meat products, fruit and vegetables (Dodds, 1992). The incidence and level of contamination of prepared fish in Europe and Asia appears to be much lower than that in North America, but fish from Scandinavia and the Caspian Sea appear to be exceptions (Dodds, 1992).

There is a small possibility that *C. botulinum* spores may be present in raw milk from infected animals (Cobb *et al.,* 2002; Bohnel *et al*., 2005). Silage is also a significant source of contamination of raw milk with *C. botulinum* spores (Giffel *et al*., 2002).

A survey of the presence of *C. botulinum* spores in 236 samples of infant foods (honey, dry cereal, non-fat dry milk, evaporated milk, canned formula, and canned baby food) in New York City found that none of the products was contaminated (Guilfoyle and Yager, 1983).

A survey prompted by the 1996 outbreak of botulism due to mascarpone in Italy found a high prevalence (32.5%; 331/1017) of *C. botulinum* spores in mascarpone, and 7 (0.8%) of the 878 samples produced at the plant involved in the outbreak also contained toxin type A. In addition, 2.7% of 260 other dairy products tested contained spores (Franciosa *et al*., 1999).

C. perfringens
C. perfringens spores and vegetative cells are likely to be present in uncooked foods of animal origin, vegetables exposed to soil, dust or faecal material, and in some dried spices (ICMSF, 1996).

During the mid-1990s, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture conducted a number of surveys of the microbiological status of raw meat products. The results for *C. perfringens* showed a high prevalence of contamination in poultry meat products, at relatively low levels, while for pork and beef the prevalence was lower but the level of contamination was generally higher (Anon 2004).

C. perfringens contamination has been found at relatively high prevalence, but usually at low levels, in some dried spices (ICMSF, 1998; Banerjee and Sarkar, 2003).

A review of the scientific literature on the incidence of pathogenic spore-forming bacteria (including *C. perfringens*) in vegetables, spices and foods containing vegetables found that, of 4040 samples, 3998 had <2 log cfu per gram *C. perfringens*, and the remaining 42 samples had less than 5 log cfu per gram (Carlin *et al.,* 2000).

5.5.6 Virulence and infectivity

C. botulinum
While most of the virulence factors in *C. botulinum* are chromosomally encoded (Shukla and Sharma, 2005), toxin production is complex and strain-dependent, with phages and plasmids implicated in control mechanisms (Hauschild, 1989; ICMSF, 1996).

C. perfringens
There are four major *C. perfringens* exotoxins, α, β, ε and ι (iota), and eight minor ones. All strains produce the α-toxin, a phospholipase C (lecithinase C) which causes enzymatic degradation of bilayer phospholipids (Bernheimer and Rudy, 1986) leading to disruption of cell membranes and cell lysis of erythrocytes, leukocytes, platelets, fibroblasts, and muscle cells (Titball, 1993). Several of the other toxins possess enzymatic activities, including a protease (λ-toxin), a deoxyribonuclease (υ-toxin) and a collagenase (κ-toxin). The β-toxin is implicated as the necrotic factor in enteritis necroticans (‘pigbel’).

C. perfringens types A and C also produce an enterotoxin (*Clostridium perfringens* enterotoxin; CPE) associated with the acute abdominal pain, nausea and diarrhoea of *C. perfringens* food poisoning.

5.5.7 Dose Response

C. botulinum
A very small amount (a few nanograms) of botulinum toxin can cause illness (Anon 2003). As little as 0.1–1.0 µg of type A toxin has been found to cause death in humans (ICMSF, 1996).

C. perfringens
Ingestion of a large number of vegetative cells is required to cause *C. perfringens* food poisoning. From outbreak investigations, it has been estimated that levels of around 10^6 to 10^8 cfu/g in implicated foods will cause illness (Bates and Bodnaruk, 2003). Volunteer feeding studies have suggested a total dose of $5x10^9$ cells is required to cause illness (Hauschild and Thatcher, 1967). Ingestion of 8-10 mg of purified enterotoxin induces symptoms of gastroenteritis (Skjelkvale and Uemura, 1977a; Skjelkvale and Uemura, 1977b). However, food poisoning usually occurs from production of the enterotoxin in the gut, rather than ingestion of preformed toxin, so those levels may not represent a toxic dose under normal conditions of food poisoning.

5.5.8 Host Factors

C. botulinum
All people are believed to be susceptible to botulinal food-borne intoxication. Infant botulism is typically seen in children under 6 months of age, although adults have also been known to suffer intestinal colonisation prior to intoxication (McCroskey *et al*., 1991; Szabo and Gibson, 1997).

C. perfringens
C. perfringens food poisoning may be more serious in the elderly and debilitated, but fatal cases are rare (Bates and Bodnaruk, 2003).

5.5.9 Food Matrix

C. botulinum
Botulism is primarily a concern when processes are used to extend the shelf life of a food, such as canning and vacuum- or modified atmosphere-packing. If *C. botulinum* spores survive treatment processes prior to packaging, they have the ability to proliferate and produce toxin, especially if the food is subjected to temperature abuse. The anaerobic environment produced by the canning process may further encourage the outgrowth of spores.

C. perfringens
Germination and outgrowth of *C. perfringens* is enabled by the generation of microaerophilic environments in foods cooked for long periods of time with poor heat penetration and inadequate aeration and/or prolonged holding of food at insufficient temperatures to prevent growth and/or toxin production (Bates and Bodnaruk, 2003).

It has been suggested that the temperature stability of *C. perfringens* vegetative cells is enhanced in foods, perhaps due to a protective effect of fats (Bradshaw *et al.,* 1977; Labbe, 1989).

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5.6 *Corynebacterium ulcerans*

Corynebacteria are non-motile, rod-shaped, Gram-positive bacteria related to the genus Actinomycetes. They do not form spores or branch, but may form irregular shaped, club-shaped or V-shaped arrangements in normal growth (TOTB 2002). They may be aerobic or facultatively anaerobic and are catalase-positive (Frassetto 2004).

The genus *Corynebacterium* comprises a diverse group of bacteria, including both animal and plant pathogens. Some *Cornyebacterium* are part of the normal flora of humans (TOTB 2002).

The genus is composed of the species *Corynebacterium diphtheriae*, the causal agent of the disease diphtheria and the best known and most widely studied of the corynebacteria (TOTB 2002), and the nondiphtheria corynebacteria, collectively referred to as diphtheroids. Nondiphtheria corynebacteria have recently been recognised as pathogenic, particularly in immunocompromised hosts (Frassetto 2004).

The nondiphtheria coryenbacterium include *C. ulcerans*, *C. pseudotuberculosis*, *C. pyogenes*, *C. haemolyticum*, *C. aquaticum*, *C. pseudodiphtheriticum* (also known as *C. hofmannii*), *C. urealyticum* (Group D2), Group E and *C. jeikeium* (Group JK) (Frassetto 2004).

C. ulcerans is found more commonly in cattle than in other animals and can carry the same bacteriophage that codes for toxin elaborated by toxigenic strains of *C. diphtheriae* (Anon. 1997). In dairy cattle, *C. ulcerans* is a cause of mastitis. Infected cows may produce milk containing *C. ulcerans* for many months or even years (Hedlund and Pohjanvirta 1989; Hommez et al., 1999).

5.6.1 Growth characteristics

Corynebacterium spp. are classified as thermoduric, psychrotrophic bacteria (Coghill 1982; Shin *et al.* 1993). Very little published information is available on the specific growth characteristics of *C. ulcerans*.

5.6.2 Pathology of illness

C. ulcerans, a cause of bovine mastitis (Hedlund and Pojmanvirta 1989; Watts 1988), was not a recognised species until 1995 (Riegel 1996). In humans, *C. ulcerans* causes a zoonotic infection similar to diphtheria. Usually the symptoms are milder than the illness caused by *C. diphtheria*, however some strains of *C. ulcerans* produce potent diphtheria toxin and may cause severe symptoms (Hatanaka *et al.* 2003; Kisely *et al.* 1994). In addition to the usual respiratory and pharyngitis symptoms, *C. ulcerans* may also causes skin infections (Frassetto 2004).

5.6.3 Mode of transmission

C. ulcerans infections have occurred in humans after drinking unpasteurised milk or after coming into contact with infected dairy animals or their waste (Barrett 1989; Bostock *et al.* 1984; Frassetto 2004). Cats with nasal discharge have also been found to have diphtheria-toxin-producing *C. ulcerans* and may transmit the bacteria to humans via scratches (Taylor *et al.* 2002; Hatanaka *et al.* 2003). Person-to-person transmission has not been reported (Anon. 1997), and in some cases of infection, the route may not be clear (Pers 1987).

5.6.4 Incidence of illness

Infections with the nondiphtheria corynebacteria have been reported throughout the world (Frassetto 2004). Two cases of poisoning due to *C. ulcerans* were reported in England and Wales in 1981. Both cases were attributed to consumption of raw milk (Anon 1982). A further two sporadic cases of *C. ulcerans* infections were reported in the UK in 1983 (Barrett 1986). Neither patient had signs of toxigenic diphtheria. One patient had been immunised as a child. Raw milk from a pet goat had been consumed prior to the infection in one case. In the other case, illness was associated with consumption of cows' milk that was thought to be improperly pasteurised due to a malfunction at the processing believed. Illness due to *C. ulcerans* has also been associated with consumption of unpasteurised milk in the US (Bostock *et al.* 1984).

Between 1993 and 1999, *C. ulcerans* caused five of the 10 cases of phyaryngeal diphtheria in the UK (CDR Weekly 2000). In January 2000, three apparently unrelated cases of infection with toxigenic *C. ulcerans* were identified in the Northern and Yorkshire NHS Region. All three cases lived in rural areas, denied exposure to raw milk and had not travelled overseas. The three cases spanned a wide age range. All cases had similar symptoms, presenting with sore throats. One case, an elderly woman, was admitted to hospital with pneumonia and a pharyngeal membrane. This case was fatal. The other two cases, a girl and a woman who worked at a riding school, did not require hospitalisation (Anon. 2000).

For a number of reported cases of illness associated with *C. ulcerans*, the vehicle or source could not be identified. For example, a case of respiratory diphtheria caused by a toxin-producing strain of *C. ulcerans* occurred in Terre Haute, Indiana in 1996. The patient did not report consumption of unpasteurised milk products or exposure to farm animals. The health authorities indicated that acquisition of the organism occurred locally in the state. In a case in Japan, a previously healthy 52-year-old woman was reported suffering illness due to *C. ulcerans* infection in 1991 (Hatanaka *et al.* 2003). Again, the patient did not report prior consumption of unpasteurised milk. The source of the infection was thought to be a scratch from a stray cat, which had rhinorrhea and sneezing. The cat died before the patient became ill. The cat was thought to have picked up the bacterium at one of the more than 10 dairy farms in the vicinity of the patient's home.

5.6.5 Occurrence in foods

Very few surveys have been conducted in recent times for the presence of *Corynebacterium spp.* in food. *Corynebacterium* spp. has been isolated from 0.5% of 200 raw milk samples taken from a bulk tanker in Korea (Shin *et al.* 1993). In a separate study, *Corynebacterium* spp. was the predominant species isolated from raw farm bulk tank milk collected from dairy farms in the Kyungii area of Korea during the period of July to December 1996, accounting for 28% of standard plate counts (Choi *et al.* 1998).

Corynebacterium spp. have been isolated also from raw camel milk produced in Riyadh, Saudi Arabia (Zahran and Al-Saleh 1997).

5.6.6 Virulence and infectivity

Virulence of *C. ulcerans* is primarily associated with the production of diphtheria-like toxins (Wong and Groman, 1984).

5.6.7 Dose response

There is a lack of information on the dose-response relationship for *C. ulcerans*.

5.6.8 Host factors

Immunocompromised hosts are more susceptible to infection with the nondiphtheria cornyebacteria, as are the very young and the elderly (Frassetto 2004).

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5.7 *Coxiella burnetii*

Coxiella burnetii is a gram-negative-like (non staining) species of rickettsia. The organisms are variously described as coccobacillus (Vanderlinde 2004b) or rod-like (Weiss and Moulder 1984) and are of the size 0.2-0.4 μm by 0.4-1.0 μm. Coxiella is distributed globally and causes the zoonotic illness Q fever (Vanderlinde 2004b). The usual animal reservoirs of *C. burnetii* are cattle, sheep and goats. Coxiella is also carried by ticks (Weiss and Moulder 1984), with transmission to animal hosts occurring through contact, blood sucking and contaminated tick faeces (Hilbink *et al.* 1993). Wild mammals and birds are also likely to be infected, however, isolations from these animals have been rare (Weiss and Moulder 1984). In Australia, several isolations have been made from the bandicoot, *Isodon torosus* (Derrick 1953). Other domesticated animals may also become infected, for example, sheep dogs (Hilbink *et al.* 1993).

Infection in animals is usually subclinical but infected animals can shed large quantities of bacteria into the environment. Infected females can shed very large quantities during parturition and the bacteria can survive harsh environmental conditions.

5.7.1 Growth characteristics

C. burnetii is an obligate intracellular micro-organism - it will not grow in foods or outside host cells. However, it is able to survive in a desiccated form in soil and the environment for several months (Hilbink et al. 1993). This may be due its ability to form spore-like structures (Marrie 2003).

Coxiella has a high resistance to drying, elevated temperatures and chemical agents including many common disinfectants (Vanderlinde 2004b; Weiss and Moulder 1984). Complete inactivation may not be attained at 63ºC for 30 minutes, or at 85-90ºC for a few seconds (Weiss and Moulder 1984). Studies conducted by Enright et al. (1957) with milk containing 100,000 guinea pig units (10 times that considered the maximum possible in cow's milk) became non-infectious when held at 62.7ºC for 30 minutes, but holding milk at 61.6ºC for the same period of time was insufficient to inactivate the organism. They strongly recommended pasteurisation at 72ºC for 15 seconds to be sure of complete elimination of viable *C. burnetii* from whole raw milk. It is also able to retain viability at 4ºC for one or more years in dried fomites such as tick faeces or wool.

5.7.2 Pathology of illness

Of those people infected with *C. burnetii*, only about half develop clinical signs of illness. Symptoms of acute infection may include the sudden onset of one or more of the following: high fever, severe headache, general malaise, myalgia, confusions, sore throat, chills, sweats, non-productive cough, nausea, vomiting, diarrhoea, abdominal pain, chest pain (Vanderlinde 2004b). If a fever is present, it may last 1 to 2 weeks. Longer term symptoms include persistent weight loss, pneumonia (30-50% of cases), abnormal liver function tests and hepatitis. The majority of patients will make a full recovery without any treatment. Tetracycline compounds are the antibiotics of choice for treatment if required (Weiss and Moulder 1984). The mortality rate in patients with acute Q fever is 1-2% (Vanderlinde 2004b).

Although uncommon, Q fever infection may persist beyond the acute phase of six months and develop into the more serious situation of a chronic illness. This may develop as soon as a year after initial infection, or may occur as long as 20 years later. The chronic form may manifest as endocarditis. Those at risk of developing chronic Q fever are those with a pre-existing valvular heart disease, vascular graft, other transplant patients, patients with cancer and those with chronic kidney disease. The mortality rate for patients with chronic Q fever is as high at 65% (Vanderlinde 2004b).

5.7.3 Mode of transmission

Infection in humans usually occurs via inhalation of the organisms from air containing dust contaminated by dried biological fluids from infected herd animals. Ingestion of contaminated raw milk or raw milk products is also suggested as a route of transmission although this is considered a minor route for human infection (Vanderlinde 2004b; Maurin and Raoult 1999).

5.7.4 Incidence of illness

Reliable estimates of the number of cases of Q fever worldwide are unavailable. This is due to the illness being rare and possibly under reported, with many human infections being subclinical (Vanderlinde 2004b).

Infected herd animals do not usually exhibit clinical disease. Abortion in goats and sheep may occur in some instances of infection though. Organisms are excreted in milk, urine and faeces. High numbers of the organism are present in amniotic fluids and placenta during birthing.

The incidence rate of Q fever in France is estimated at 50 cases per 100,000 inhabitants per year (Maurin and Raoult 1999). The number of clinical cases of disease increased from one reported case in France in 1982, to 107 reported in 1990 (Tissot-Dupont *et al.* 1992). The majority (61%) of these cases presented with hepatitis, which is linked with oral exposure rather than aerosol exposure (Vanderlinde 2004a).

In 1985, five cases of hepatitis were reported from workers at a meat packing plant in California. Further investigation of the workforce found that 31 of 42 persons tested were positive during serological testing for Q fever rickettsiae, with eight of these having recently experienced clinical symptoms of Q fever (MMWR 1986). Exposure was concluded to be due to the handling of sheep carcasses.

The notification rate for Q fever in Australia 1999 - 2002 was between 2.7 and 3.9 cases per 100,000 population (Australian Institute of Health and Welfare, 2004).

In Australia, the incidence rate was estimated to be between 3.11 and 4.99 cases per 100,000 inhabitants for the period 1991-4, whilst the hospital morbidity data for 2001-02 indicates a case rate of 1.3 cases per 100,000 (Australian Institute of Health and Welfare).

Despite the close proximity with Australia, New Zealand is generally believed to be free of Q fever, with the disease not being established in the ruminant population (Hilbink *et al.* 1993).

5.7.5 Occurrence in foods

C. burnetii has been associated with consumption of unpasteurised goats milk and cheese in Europe, Canada and the USA (Rampling 1998).

On average, 5% of sheep in France tested positive for *C. burnetii* in seroprevalence studies (Rousset *et al.* 2001), with *C. burnetii* recovered from 50% of milk samples collected from infected ewes (Berri *et al.* 2000). Infected animals may not show overt signs of clinical infection (Vanderlinde 2004a).

Tests on milk samples from Uttar Pradesh, India found 18 of 260 cows' milk samples and 2 of 84 buffaloes' milk samples were positive for antibodies to *C. burnetii*. The organism was isolated from 1 of 4 pooled buffaloes' milk samples and 1 of 8 pooled cows' milk samples from the LRC Dairy in Nagla (Sethi *et al.* 1978).

Milk from 20 herds of dairy cows from Zaria, Nigeria were screened for *C. burnetii*, with 16 (80%) of the herds containing cows shedding the organism in their milk. Of 169 individual cows tested, 41 (24%) were shedders of *C. burnetii* (Adesiyun *et al.* 1985).

Of 1052 dairy cows from 22 premises across 17 Californian counties, 82% tested positive to serum agglutinating antibodies to rickettsia. 51% of 1634 cows had specific agglutinating antibodies in their whey and 23% of 840 cows were actively shedding *C. burnetii* (Biberstein *et al.* 1974).

5.7.6 Virulence and infectivity

The incubation period for Q fever is dependent upon the number of organisms that initially infected the patient, with greater numbers of organisms resulting in a shorter incubation period. On average, most patients will exhibit symptoms within 2-3 weeks of exposure. Lifelong immunity against re-infection may be attained should a person fully recover from the infection (Vanderlinde 2004b).

5.7.7 Dose response

As humans are often very susceptible to the disease, very few organisms may be required to cause infections. Vanderlinde (2004b) reports the inhalation of as few as 10 organisms may result in disease in humans. MMWR Weekly (1986) indicates a single inhaled organism is sufficient to initiate infection. No information is available on the number of organisms required to cause infection via ingestion.

5.7.8 Host factors

Persons at greatest risk of exposure to *C. burnetii* fever include those occupationally exposed such as farmers, veterinarians, livestock transport workers, abattoir workers, those in contact with dairy products, laboratory personnel performing *Coxiella burnetii* culture and others working with *C. burnetii*-infected animals.

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5.8 *Cryptosporidium*

Cryptosporidium is an intestinal protozoan parasite that induces gastrointestinal symptoms when ingested by humans. Being an obligate parasite, the organism requires a host to reproduce, and is transmitted to humans via ingestion of the environmental stage of its life cycle, the oocyst. The oocysts are approximately 4 – 6 μm in diameter and are shed in the faeces of infected hosts in large numbers.

Cryptosporidium was discovered in 1907 but the first recognised case of human *Cryptosporidium* infection was in 1976 (Berkelman, 1994). During the early 1980's the population most at risk of infection were immunodeficient individuals, such as those suffering from HIV/AIDS. Cryptosporidosis, the disease caused by infection from *Cryptosporidium*, is a severe diarrhoeal disease. In immunocompetent individuals the disease is self-limiting, usually lasting less than ten days. It is often accompanied with abdominal pain, nausea, vomiting, general malaise and low -rade fever (Berkelman, 1994). For immunocompromised individuals, however, the disease can be prolonged and life threatening (Duffy and Moriarty, 2003).

Many species of *Cryptosporidium* have been identified. Some strains appear to be adapted to certain hosts but cross-strain infectivity occurs and may or may not be associated with illness. The most important species in relation to human illness is *C. parvum*, however this species also infects and causes disease in a range of mammals, particularly cattle and sheep (Dawson, 2003).

5.8.1 Growth and survival characteristics

Cryptosporidium will not grow outside an animal host. *Cryptosporidium* oocysts appear to be sensitive to heat, loosing infectivity rapidly at >60°C (Rose, 1997). Standard high-temperature-short-time (HTST; 72°C/15 sec) pasteurisation has been demonstrated to be sufficient to destroy the infectivity of *C. parvum* in milk and water (Harp et al., 1996).

Low temperatures have also been shown to reduce oocyst infectivity. Fayer and Nerad investigated the infectivity of *C. parvum* oocysts stored at low temperatures (suspended in deionised water) in mice. Oocysts stored at 5°C and –10°C remained infective for seven days, the duration of study. At temperatures below –15°C, infectivity reduced after 1 day and no infection was noted by 7 days.

Oocysts will survive and remain infective in moist conditions for long periods of time. *C. parvum* oocysts have been shown to be able to survive up to 176 days in drinking water or river water stored at 4°C, with inactivation between 89% and 99% of the population (Robertson et al., 1992).

Desiccation is detrimental to oocyst survival and low water activity has been reported to result in reduced viability (Rose and Slifko, 1999). A study by Robertson et al (1992) showed air-drying at room temperature resulted in 97% inactivation within 2 hours and 100% inactivation within 4 hours (Robertson et al., 1992).

A number of studies have demonstrated survival of *C. parvum* oocysts in different media (such as yoghurt) down to pH 4.0 (Deng and Cliver, 1999; Dawson et al., 2004).

5.8.2 Pathology of illness

Symptomatic cryptosporidiosis is usually characterised by profuse watery diarrhoea, often leading to rapid weight loss and dehydration. Other symptoms can include abdominal cramping, nausea, vomiting, low grated fever and headache (Smith, 1993). The disease is usually self-limiting, with symptoms normally lasting for two to four days (FDA 2003). Severity and duration of symptoms is considered greater for immunocompromised individuals. In these susceptible populations, infection may extend to other organs including the lungs and the bile duct and is considered life threatening (Dawson, 2003).

5.8.3 Mode of transmission

Cryptosporidium is transmitted via the faecal-oral route. Person-to-person contact to oocysts is of particular concern in settings such as childcare centres (Berkelman, 1994). The majority of documented cryptosporidiosis outbreaks have been associated with waterborne transmission.

5.8.4 Epidemiological data

Cryptopsoridiosis became a notifiable disease in Australia in 2001. A total of 3,255 (16.6 cases per 100,000 population) cases were notified to health authorities during 2002 (Yohannes et al., 2004). Children under the age of four have the highest cryptosprodiosis notification rate (129 cases per 100,000 population). This may reflect an increased susceptibility of children to *Cryptosporidium* and/or increased likelihood of exposure.

The most prominent waterborne outbreak occurred in Milwaukee in 1993 and resulted in an estimated 403,000 cases of illness (Mac Kenzie et al., 1994). *Cryptosporidium* oocysts are resistant to many disinfection techniques (Korich et al, 1990). It is for this reason that conventional water treatment plants are not always effective in removing the oocysts.

Although the majority of reported cryptosporidiosis outbreaks are waterborne, a number of food-borne outbreaks have occurred. For example an outbreak was observed in Maine, US that was associated with consumption of fresh-pressed apple cider (Millard et al., 1994). *Cryptosporidium* oocysts were detected in the apple cider, on the cider press and in the stool specimen of a calf on the farm that supplied the apples. The secondary transmission rate to other household members was 15%. Outbreaks have also been linked to consumption of unwashed green onions (Anon, 1998).

Two outbreaks of cryptosporidiosis occurred in Australia during 2001 which were associated with the consumption of unpasteurised cow's milk (Ashbolt et al., 2002). One outbreak consisted of 8 children developing cryptosporidiosis following consumption of milk labelled as "unpasteurised pet milk" (Harper et al., 2002). For the other outbreak, it was suspected consumption of unpasteurised milk during school camp was cause of infection.

A cryptosporidiosis outbreak (n = 48) occurred at a school in the UK during 1995 that was associated with consumption of pasteurised milk (Gelletlie et al., 1997). It was suggested that the milk may have been inadequately pasteurised to inactivate the *Cryptosporidium* oocysts.

5.8.5 Occurrence in foods

Food may be contaminated via a number of sources such as direct contact with faecal material during production (eg slaughtering or during milking), exposure to contaminated water or exposure via infected food handlers. Once contaminated, *C. parvum* oocysts can survive in wet/moist foods, however they are not able to grow.

Very few studies have been undertaken to determine the prevalence of *C. parvum* oocysts in food. Of the data that is available, it is hampered by the lack of consistent methodologies to isolate oocysts from samples, methods of detection and viability assays.

5.8.6 Virulence and infectivity

Cryptosporidium is considered highly infective. Once ingested, oocysts excysts in the small intestine and release sporozoites that attach to the gut epithelium. The sporozoites undergo several asexual and sexual reproduction cycles within the epithelium, resulting in the formation of both thick- and thin-walled oocysts. Thin-walled oocysts reinfect the same host and start a new life cycle, which can lead to severe tissue damage and changes to the absorptive properties of the small intestine. Thick-walled oocysts are excreted in the faeces.

5.8.7 Dose-response

DuPont et al (1995) developed an exponential dose-response relationship for *Cryptosporidium* infection based on data from a feeding study using healthy adult volunteers. The median infectious dose (ID50) was determined mathematically to be 132 oocysts. At the lowest dose of 30 oocysts, a probability of infection of 20% was observed.

When data was fitted with an exponential model, the probability of infection is described by:

$$P_i = 1 - e^{-rD}$$

where,

P_i = Probability of infection
r = 0.004005
D = dose ingested

5.8.8 Host factors

Severity and duration of cryptosporidiosis is generally more severe in immunocompromised individuals, including children under five. For example, it is estimated that approximately 1% of the immunocompetent population may be hospitalised with very little risk of mortality, *Cryptosporidium* infections are associated with high rates of mortality in the immunocompromised population (Rose 1997).

5.8.9 Food Matrix

Survival data for *Cryptosporidium* in different food and beverages is limited. Water activity and temperature appear to be major factors that determine oocyst survival (Rose and Slifko 1999). Studies have shown that *Cryptosporidium* oocysts are not able to survive the ice-cream making processes, largely due to its sensitivity to low temperature (Deng and Cliver

1999). Ooycsts inoculated into milk have been found to survive the yoghurt-making process (Deng and Cliver 1999).

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5.9 *Enterobacter sakazakii*

Enterobacter sakazakii is a gram-negative bacterium belonging to the Enterobacteriaceae family. This family contains a number of species of bacteria that are commonly found in the human and animal gut, and in the environment (Lehner and Stephan 2004). *E. sakazakii* are included within the coliform group of bacteria

E. sakazakii is considered an opportunistic pathogen and has been associated with sporadic cases or small outbreaks of meningitis, sepsis, cerebritis and necrotizing enterocolitis, especially in neonates and infants (FAO/WHO, 2004).

5.9.1 Growth characteristics

E. sakazakii generally grows between 6 – 45°C, although there have been reports of some strains being able to grow at as low as 3.4°C and as high as 47°C (Lehner and Stephan, 2004; Nazarowec-White and Farber, 1997). *E. sakazakii* is considered more heat tolerant than many other Enterobacteriacea, however, it is rapidly inactivated at temperatures obtained during HTST pasteurisation. Reported thermal inactivation rates for *E. sakazakii* vary between studies. Nazarowec-White and Farber (1997) calculated a $D_{72°C}$ of 1.3 seconds when heated in infant formula, whereas Iversen et al. (2004) calculated a $D_{71°C}$ of 0.7 seconds in infant formula.

Breeuwer et al. (2003) demonstrated that *E. sakazakii* cells are particularly tolerant to desiccation, which may provide a competitive advantage in dry environments such as those found in dried milk powder. It has also been demonstrated that *E. sakazakii* has the ability of forming biofilms on a range of surfaces which may act as a reservoir of infection (Iversen et al., 2004).

5.9.2 Pathology of illness

E. sakazakii has been implicated in cases of meningitis and enteritis. Urmenyi and Franklin (1961) reported the first two known cases of neonatal meningitis caused by *E. sakazakii* infection in 1961. Although the frequency of cases of *E. sakazakii* is low, it is the severity of the illness that is of concern, with neonates and infants being particularly affected by this organism. Neonatal meningitis can result in ventriculitis, brain abscess or cyst formation and development of hydrocephalus. The reported fatality rate for neonatal infections has been reported to be as high as 50%, with over half the reported patients dying within one week of diagnosis. All neonatal patients that recover from the central nervous system infection suffer mental and physical delays (Lehner and Stephan 2004).

Another clinical manifestation of infection with *E. sakazakii* is the development of neonatal necrotising enterocolitis (NEC) following consumption of re-constituted contaminated powdered infant formula. This disease is characterised by intestinal necrosis and pneumatosis intestinalis. It is the most common gastrointestinal emergency in newborns (Lehner and Stephan 2004).

E. sakazakii has been isolated from clinical sites such as cerebrospinal fluid, blood, sputum, lower and upper respiratory tracts, digestive tract, superficial wounds and urine of infected individuals (Lehner and Stephan 2004).

5.9.3 Incidence of illness

E. sakazakii is not a notifiable disease in Australia, and some cases of infection may go undetected due to the difficulties of identifying the organism in the laboratory. The frequency of disease in infants appears to be low, with 2 cases over a 14-year period being recorded by the Victorian Hospital Pathogen Surveillance Scheme.

In July 2004, a premature baby in New Zealand developed meningitis due to *E. sakazakii* infection and subsequently died. The source of the organism was attributed to mishandling of contaminated powdered infant formula.

An outbreak of necrotising enterocolitis occurred in an intensive care unit in a hospital in Belgium in 1998, with 12 neonates contracting the disease between June and July that year (Van Acker *et al.*, 2001). A significant correlation was found between the development of NEC, the consumption of reconstituted powdered infant formula from a specific manufacturer, and the isolation of *E. sakazakii* in neonates.

In a review of *E. sakazakii*-induced illness in infants in the United States between 1961 and 2003 there were 48 reported cases (Lehner and Stephan, 2004). During 2001, a number of *E. sakazakii* infections were associated with consumption of reconstituted powdered infant formula in Tennessee, USA (Himelright et al., 2002). Of 49 infants screened for *E. sakazakii*, 10 were positive. Of these ten infants, one had a confirmed infection of cerebrospinal fluid (and died 9 days post infection), two had suspect tracheal infections and there were seven cases of infection identified by *E. sakazakii*-positive stool and/or urine samples.

5.9.4 Occurrence in foods

Although *E. sakazakii* has been isolated from a wide range of food commodities, most research has been undertaken on the presence of the organism in dried infant formula (Lehner and Stephan, 2004). Muytjens et al. (1988) analysed 141 powdered infant formulas and isolated *E. sakazakii* from 20 (14%) of them (limit of detection 1 cfu/100g). Iversen and Forsythe (2004) isolated *E. sakazakii* in two of 82 powdered infant formulae sampled. Nazarowec-White and Farber (1997) reported the prevalence of *E. sakazakii* in powdered infant formulae made from five different manufacturers to be 0 – 12%.

E. sakazakii has also been isolated from cheese, meat, vegetables, grains, herbs and spices and ultrahigh-temperature milk (Lehner and Stephan, 2004).

5.9.5 Virulence and infectivity

Although not fully understood, virulence of *E. sakazakii* has been associated with the ability to produce enterotoxin (Pagotto et al., 2003). Another key mechanism required for extraintestinal infection of *E. sakazakii* is thought to be the ability to penetrate the epithelial layer of the intestinal mucosa.

5.9.6 Dose response

There is no epidemiological or experimental data to develop an accurate dose-response relationship for *E. sakazakii* infections in humans. It is assumed that the ingestion of one *E. sakazakii* cell has the ability, albeit small, to cause illness in infants at risk.

Using this assumption, R-values (the probability of one ingested organism causing illness) have been estimated between 8.9×10^{-6} to 2.5×10^{-6} (EFSA, 2004).

Reported levels of *E. sakazakii* present in samples of powdered infant formula associated with outbreaks are often low. Counts between 1 - 20 coliforms/g have been observed (EFSA).

5.9.7 Host factors

Neonates and infants have been particularly affected by this organism. The outcome related to adult disease seems to be significantly milder than that for children. There have only been a few reports of infections in adults, with most adult patients with *E. sakazakii* infection also having serious underlying diseases such as malignancies (Lehner and Stephan 2004).

5.9.8 Food matrix

Stationary-phase *E. sakazakii* is remarkably resistant to osmotic stress and desiccation and can therefore survive in dry environments such as those observed in dried milk powder (Breeuwer et al., 2003)

E. Sakazakii can grow in the reconstituted products if stored at temperatures above 5ºC for a sufficient time and multiply very rapidly at room temperatures.

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5.10 Pathogenic *Escherichia coli*

Escherichia coli (*E. coli*) are members of the family Enterobacteriaceae and are a common part of the normal intestinal flora of humans and other warm-blooded animals. The organisms are described as gram-negative, facultatively anaerobic rod shaped bacteria (Desmarchelier and Fegan, 2003). Although most strains of *E. coli* are considered harmless, the species does contain certain strains that can cause severe illness in humans (Bell and Kyriakides, 1998). Strains of *E. coli* are differentiated serologically, based on O (somatic) and H (flagella) antigens (Lake et al., 2003).

This assessment is primarily concerned with human pathogenic *E. coli*. Pathogenic *E. coli* are characterised into specific groups based on virulence properties, mechanisms of pathogenicity and clinical syndromes (Doyle et al., 1997). These groups include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC) and enterohaemorrhagic *E. coli* (EHEC). Many synonyms are used to describe EHEC, including Shiga toxin-producing *E. coli* (STEC), Shiga-like toxin-producing *E. coli* (SLTEC), and verocytotoxin-producing *E. coli* (VTEC).

E. coli O157:H7 is the best known and most widely studied serotype of *E. coli.* One of its natural habitats is the intestines of cattle, which creates the potential for contamination of milk and dairy products. In spite of this risk, milk and dairy products have only occasionally been implicated in outbreaks of *E. coli* O157:H7 food poisoning, and even more rarely does an outbreak involve a pasteurised product (Kirk and Rowe, 1999).

5.10.1 Growth and Survival

Growth and survival of pathogenic *E. coli* is dependent on the simultaneous effect of a number of environmental factors such as temperature, pH and water activity (a_w). In general, pathogenic *E. coli* strains behave similarly to non-pathogenic strains, however certain EHEC strains have been found to have a higher tolerance to acidic conditions than other groups of *E. coli* (Desmarchelier and Fegan, 2003).

The optimum temperature for growth of *E. coli* is 37°C, and it can grow within the range of 7-8°C to 46°C (ICMSF, 1996). Heat sensitivity of pathogenic *E. coli* is similar to that of other Gram-negative bacteria and is dependent on the pH, a_w and composition of the food (Bell and Kyriakides, 1998). Due largely to its importance as a cause of food-borne illness in the United States, most studies on the growth and/or survival of pathogenic *E. coli* have been undertaken with *E. coli* O157:H7 (an EHEC organism). Studies on the thermal sensitivity of *E. coli* O157:H7 have revealed that it is no more heat sensitive than *Salmonella* (Doyle and Schoeni, 1984). Therefore, heating a product to kill typical strains of *Salmonella* will also kill *E. coli* O157:H7.

Numbers of pathogenic *E. coli* O157:H7 have been shown to remain stable in ground beef stored at –20°C for over 9 months (Doyle and Schoeni 1984). In contrast, a 10-fold reduction of non-pathogenic *E. coli* has been observed in ground beef stored at –25.5°C for 38 weeks (ICMSF, 1996).

Studies have demonstrated that some EHEC strains are acid-tolerant and can survive for at least five hours at pH 3.0 - 2.5 at 37°C (Benjamin and Datta, 1995; Large *et al*., 2005). Stationary phase and starved pathogenic *E. coli* have been found to have an increased acid tolerance compared with exponential growth phase organisms (Arnold and Kaspar, 1995).

Pathogenic *E. coli* may therefore be able to survive and/or grow in food products previously considered too acidic to support the survival of other food-borne pathogens. The effect of pH on *E. coli* survival is, however, dependent on the type of acid present. For example, *E. coli* O157:H7 can survive in a medium adjusted to pH 4.5 with hydrochloric acid but not when adjusted to the same pH with lactic acid (ICMSF, 1996).

The minimum water activity (a_w) required for growth of pathogenic *E. coli* is 0.95, or approximately 8% sodium chloride (ICMSF, 1996). In sub-optimal temperature or pH conditions, the a_w required for growth increases (Desmarchelier and Fegan, 2003).

5.10.2 Pathology of illness

EPEC causes illness primarily in infants and young children in developing countries. Symptoms include watery diarrhoea, with fever, vomiting and abdominal pain. The diarrhoea is usually self-limiting and of short duration, but can become chronic (more than 14 days). EPEC is also recognised as a food and water-borne pathogen of adults, where it causes severe watery diarrhoea (with mucus, but no blood) along with nausea, vomiting, abdominal cramps, fever, headache and chills. Duration of illness is typically less than three days (Doyle and Padhye, 1989; Dalton et al., 2004).

ETEC is another major cause of diarrhoea in infants and children in developing countries, as well as being recognised as the main cause of 'travellers diarrhoea' (Doyle and Padhye, 1989). Symptoms include watery diarrhoea, low-grade fever, abdominal cramps, malaise and nausea. In severe cases, the illness resembles cholera, with severe 'rice-water' diarrhoea and associated dehydration. Duration of illness is from three to 21 days (Doyle and Padhye, 1989).

EIEC cause a dysenteric illness similar to shigellosis. Along with profuse diarrhoea, symptoms include chills, fever, headache, muscle pain and abdominal cramps. Onset of symptoms is usually rapid (<24 hours), and may last several weeks (Doyle and Padhye, 1989).

EHEC infection normally results in diarrhoea like symptoms. Haemorrhagic colitis, an acute illness caused by EHEC organisms, is characterised by severe abdominal pain and diarrhoea. This diarrhoea is initially watery but becomes grossly bloody. Symptoms such as vomiting and low-grade fever may be experienced. The illness is usually self-limiting and lasts for an average of 8 days. The duration of the excretion of EHEC is about one week or less in adults, but it can be longer in children (ICMSF, 1996).

Complications resulting from EHEC infections vary. About 5 per cent of haemorrhagic colitis victims may develop haemolytic ureamic syndrome (HUS) (European Commission, 2000). This involves the rupture of red blood cells (haemolysis), subsequent anaemia, low platelet count and kidney failure. The case-fatality rate of HUS has been reported to be 3–7 per cent (Codex Alimentarius Commission, 2002). Shiga toxins produced by EHEC attack the lining of the blood vessels throughout the body, predominantly affecting the kidney. However other organs such as the brain, pancreas, gut, liver and heart are also affected and may result in further complications such as thrombotic thrombocytopenic purpura.

Table 4.1 Clinical, pathological and epidemiological characteristics of disease caused by the five principal pathotypes of *E. coli* (Robins-Brown 1987)

Pathotype	Clinical symptoms	Intestinal pathology	Susceptible population
ETEC	Watery, cholera-like diarrhoea	No notable change	Children in developing countries; travellers to those countries
EIEC	Bacillary dysentery	Inflammation and disruption of the mucosa, mostly of the large intestine	All ages; more common in developing countries
EPEC	Non-specific gastroenteritis	Attaching-effacing lesions throughout the intestine	Children under 2 years of age in developing countries
EHEC	Bloody diarrhoea	"Haemorrhagic colitis"; attaching-effacing lesions confined to the large intestine; necrosis in severe cases	Children and the elderly in developed countries.
EAEC	Persistent diarrhoea	Inflammation, cytotoxic changes in enterocytes (data from experimental studies)	Children in developing countries; travellers to those countries

5.10.3 Mode of transmission

Pathogenic *E. coli* are transmitted by the faecal-oral route. Sources of transmission include person-to-person, food-borne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals.

5.10.4 Incidence and outbreak data

Infection with pathogenic *E. coli* is a cause of significant morbidity and mortality worldwide. Outbreaks caused by EPEC, ETEC and EIEC occur infrequently in developed countries (ICMSF, 1996). In contrast, outbreaks caused by EHEC are more common, with a number of large food-borne outbreaks being reported in many countries, including Australia (Goldwater and Bettelheim, 1998). In developing countries, the incidence of EHEC infection is reported to be much lower than that of ETEC and EPEC infection (Nataro and Kaper 1998).

EIEC stains have been isolated with low frequency from diarrhoeal cases in both industrialised and less developed countries (Nataro and Levine, 1994). Outbreaks have occurred in hospitals, on a cruise ship, and from contaminated water (Desmarchelier and Fegan, 2003).

ETEC stains are a major cause of diarrhoea in infants and young children in developing countries, particularly in the tropics, and are a leading cause of travellers' diarrhoea (Gross and Rowe, 1985; Doyle and Padhye, 1989; Nataro and Levine, 1994). Although uncommon, a number of food-borne outbreaks due to ETEC have occurred internationally (Olsvik et al., 1991). Mead et al. (1999) estimated that ETEC infection is responsible for approximately 0.4% of food-borne illnesses in the US. In 1983 a multi-state ETEC outbreak occurred in the US that was associated with consumption of imported Brie and Camembert cheese (Anon, 1984; MacDonald et al., 1985). More recently, contaminated parsley was implicated in two ETEC outbreaks in Minnesota, USA during 1998 (Naimi et al., 2003). The source of the contamination was believed to be inadequately chlorinated wash water used on-farm.

A large ETEC outbreak, affecting over 800 people, occurred in Japan during 1996. The outbreak occurred at four elementary schools and was associated with consumption of tuna paste (Mitsuda et al., 1998). Analysis of patient stool samples and samples of the implicated tuna paste confirmed *E. coli* O25:NM as the cause of illness.

EPEC stains have caused infantile diarrhoea in hospitals and nurseries in the United Kingdom and the United States (Robins-Brown, 1987; Nataro and Levine, 1994). In developing countries, EPEC stains are still responsible for a high incidence of sporadic infant diarrhoea. Limited information is available on food-borne outbreaks associated with EPEC. An outbreak of EPEC (serotype O111) occurred amongst people on a coach trip to France, although no specific food was identified, the infection was believed to have been the result of consuming food at a restaurant in northern France (Wight et al., 1997). Outbreaks associated with consumption of contaminated cold pork and meat pies have been reported in Britain (Doyle and Padhye, 1989).

Since its identification as a human pathogen in 1982, and implication in a number of outbreaks in the United States, *E. coli* O157:H7 has become identified as the most predominant cause of EHEC related disease (WHO/FAO, 2002). It is estimated that 85% of EHEC infections in the United States are food-borne (Mead et al., 1999).

In the United States, consumption of undercooked hamburger meat has been an important cause of EHEC outbreaks (Nataro and Kaper 1998). A large multi-state *E. coli* O157:H7 outbreak involving consumption of contaminated hamburgers occurred in December 1992 – January 1993 with 732 cases identified, of which 195 were hospitalised and 4 died (Nataro and Kaper 1998).

Food-borne outbreaks of *E. coli* O157:H7 have also been associated with consumption of contaminated fresh produce. In the United States, outbreaks occurred in 1995 and 1996 (70 and 49 cases respectively), which were traced to consumption of lettuce (Tauxe, 1997). Studies have shown that *E. coli* O157:H7 can be transmitted to lettuce plant tissue from soil contaminated with manure and contaminated irrigation water (Solomon et al., 2002). Another large *E. coli* O157:H7 outbreak occurred in the US in 1996 which was linked to apple juice. Although the low pH of fruit juices will generally not allow the survival and growth of many Enterobacteriaceae, some strains of *E. coli* O157:H7 may survive due to their high acid-tolerance.

In 2002, an outbreak of E. coli 0157:H7 in Canada was attributed to the consumption of unpasteurised Gouda cheese (Honish et al, 2005).

Over 200 non-O157 STEC serotypes have been isolated from humans, with the WHO identifying O26, O103, O111 and O145 as the most important food-borne non-O157 serogroups worldwide (WHO, 1998).

STEC has been a notifiable disease in most Australia States and Territories since August 1998 (Roche et al., 2001). During the period of 2001 – 2005, the notification rate for STEC (excluding HUS cases) in Australia has been 0.2 – 0.3 cases per 100,000 population (Ashbolt *et al*., 2002; OzFoodNet, 2003; OzFoodNet, 2004, OzFoodNet, 2005). *E. coli* O157 has been the most commonly reported serotype.

Significant variations in notifications exists between states and territories, and part of this variation is likely to be a result of different practices employed by pathology laboratories when screening faecal samples for toxin producing *E. coli* (OzFoodNet, 2003).

A large EHEC outbreak occurred in South Australia during 1995, which resulted in approximately 200 cases of illness. Twenty-two people aged between 4 months and 12 years developed haemolytic uraemic syndrome (HUS) and were hospitalised and a 4-year-old child died. Investigations of the outbreak identified EHEC strain O111:NM (or strain O111:H-, NM for non-motile) as the principal cause of the outbreak. A locally produced uncooked, fermented mettwurst was identified as the vehicle for the pathogen. The product was found to contain a variety of EHEC strains in addition to O111 (Paton and Paton, 1998).

5.10.5 Occurrence in food

Humans appear to be the primary reservoir of EIEC, ETEC and EPEC organisms (Desmarchelier and Fegan, 2003). Therefore, contamination of food with these organisms is often due to human faecal contamination, either directly from an infected food handler or indirectly via contaminated water. Very little information is available on the occurrence of these organisms in food. The detection of these organisms in food is difficult, requiring sophisticated methodology and therefore food is not routinely screened for these organisms.

In general, EPEC and ETEC organisms are more commonly isolated in foods from developing countries and their presence is associated with poor hygiene (Desmarchelier and Fegan, 2003). EPEC has been isolated from milk products in Iraq as well as from a variety of raw and cooked food in Malaysia (Abbar and Kaddar, 1991; Norazah et al., 1998). In Brazil, EPEC has been isolated from 21.1% of soft cheeses sampled (n=45) and has frequently been isolated from pasteurised milk (da Silva et al., 2001; Araújo et al., 2002).

EIEC have only sporadically been isolated from foods (Olsvik et al., 1991).

In addition to being a major cause of infantile diarrhoea in developing countries, ETEC organisms are a leading cause of traveller's diarrhoea, which has been linked to the consumption of contaminated food and water (Nataro and Kaper, 1998). ETEC have been isolated from Brazilian fish and shrimp which were harvested from waters contaminated with raw sewage (Teophilo et al., 2002). ETEC have also been detected in sauces at Mexican-style restaurants, and in chilli sauce sold by street vendors in Mexico (Adachi et al., 2002; Estrada-Garcia et al., 2002). In general, these sauces had been prepared and handled under poor hygienic conditions.

The major reservoir of EHEC organisms appears to be the intestinal tract of ruminants, in particular cattle and sheep (Desmarchelier and Fegan, 2003). *E. coli* O157:H7 and other EHEC species have been isolated from both healthy and diarrhoeic animals, and individual animals can carry more than one serotype (Anon, 1998). Foods derived from these animals may become contaminated via exposure to faecal material during processing.

Prevalence of STEC in raw milk has been determined in a limited number of studies (Table 4.2). Caution must be exercised when comparing results between independent studies due to differences in sample size, stage of production where the samples were taken and different methodologies used to isolate the organisms. *E. coli* O157:H7 is the most widely studied EHEC serovar due to it being associated with a large number of outbreaks worldwide.

In general, prevalence of STEC in raw milk is low. Adequate pasteurisation will ensure that STEC is inactivated.

Very little information is available of the prevalence of EHEC organisms in food in Australia. Of the limited studies undertaken, the prevalence of *E. coli* O157:H7 in beef and sheep meat appears to be low, however, the prevalence of non-O157:H7 EHEC serotypes is unknown (Vanderlinde et al., 1998; Vanderlinde et al., 1999; Phillips et al., 2001a; Phillips et al., 2001b).

Table 4.2 EHEC isolation rates from a variety of dairy products

Sample	Organisms Isolated	Country	No. Sampled	% Positive	Reference
Raw goat's milk	STEC	Switzerland	344	16	(Muehlherr *et al*., 2003)
Raw ewe's milk	STEC	Switzerland	63	13	(Muehlherr *et al.,* 2003)
Raw cow's milk (bulk tank)	O157:H7	USA	268	0.75	(Murinda *et al*., 2002)
Raw cow's milk	O157:H7	UK	329	0	(Mechie *et al*., 1997)

5.10.6 Virulence and infectivity

Clinical, pathological and epidemiological characteristics of disease caused by pathogenic *E. coli* vary between pathotypes and is discussed below.

EPEC have technically been defined as "diarrhoeagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related either to heat-labile enterotoxins or heat-stable enterotoxins or to Shigella-like invasiveness" (Edelman & Levine 1983). EPEC cause characteristic attaching and effacing lesions in the intestine, similar to those produced by EHEC, but do not produce Shiga toxins. Attachment to the intestinal wall is mediated by a plasmid-encoded outer membrane protein called the EPEC Adherence Factor in type I EPEC. However, pathogenicity is not strictly correlated to the presence of the EPEC Adherence Factor, indicating that other virulence factors are involved (ICMSF, 1996).

ETEC that survive passage through the stomach adhere to mucosal cells of the proximal small intestine and produce a heat-labile toxin (LT) and/or a heat-stable toxin (ST). The heat-labile toxins are similar in structure and mode of action to cholera toxin, interfering with water and electrolyte movement across the intestinal epithelium (Desmarchelier and Fegan, 2003). If the volume of accumulated fluid exceeds the normal absorptive capacity of the large intestine, the excess is evacuated as watery diarrhoea.

EAEC strains are defined as *E. coli* strains that do not secrete LT or ST. These strains adhere to cultured human epithelial cells in a characteristic aggregative or "stacked-brick" pattern (Yatsuyanagi et al., 2002). The mechanisms causing enteric disease are not fully understood, however EAEC have been associated with persistent diarrhoea, primarily in infants and children (Desmarchelier and Fegan, 2003).

Following ingestion, EIEC invade epithelial cells of the distal ileum and colon. The bacteria multiply within the cytoplasm of the cells, causing cell destruction and ulceration. Pathogenicity is associated with a plasmid-encoded type III secretory apparatus and other plasmid-encoded virulence factors (Desmarchelier and Fegan, 2003).

The EHEC group of *E. coli* comprises a subset of Shiga toxin-producing *E. coli* (STEC). The Shiga toxins (Stx1 and Stx2) are closely related, or identical, to the toxins produced by *Shigella dysenteriae*. Additional virulence factors allow the organism to attach tightly to intestinal epithelial cells, causing what is commonly referred to as attaching-and-effacing lesions.

5.10.7 Dose response

EPEC: It is thought that only a few EPEC cells are necessary to cause illness in children (FDA 2003). Volunteer studies in adults demonstrated that illness could be caused by ingesting 10^6–10^{10} cells with sodium bicarbonate to neutralise stomach acidity (Doyle and Padhye, 1989).

ETEC: Volunteer studies have shown that 10^8–10^{10} cells of ETEC are necessary for illness in adults (DuPont *et al*., 1971), although the infective dose is probably less for infants and children (FDA 2003).

EIEC: Volunteer studies have shown that 10^8 EIEC cells are necessary to cause illness in adults, with the infectious dose reduced to 10^6 when ingested with sodium bicarbonate (DuPont *et al.*, 1971). However, the United States Food and Drug Administration (FDA) suggest that as few as 10 cells may be needed to cause illness in adults, based on the organisms similarity with *Shigella* (FDA 2003).

The dose-response relationship for EHEC is complicated by the large number of serotypes and the association of EHEC with a variety of foods. Haas *et al*. (2000) developed a dose-response relationship for *E. coli* O157:H7 based on data from a prior animal study undertaken by Pai *et al*. (1996), which involved oral administration of bacterial suspension to infant rabbits. The model was validated by comparison with two well-documented human outbreaks, one food-borne and the other waterborne. The model estimated that the dose required to result in 50% of the exposed population to become ill was 5×10^5 organisms. The corresponding probability of illness for the ingestion of 100 organisms was 2.6×10^{-4}.

Dose-response relationships for *E. coli* O111 and O55 have been developed from human feeding trial data (Haas *et al*., 1999). The relationship estimated a dose required for 50% of the exposed population to become ill was 2.55×10^6 and the probability of illness for ingestion of 100 organisms was 3.5×10^{-4}.

Investigations of other known outbreaks of food-borne illness due to *E. coli* O157:H7 and systematic studies aimed at quantifying the dose–response relationship suggest as few as 1–700 EHEC organisms can cause human illness (FDA, 2003).

5.10.8 Host susceptibility

A variety of host factors may be important in the pathogenesis of specific *E. coli* serotypes. In general, the young and the elderly appear to be more susceptible to pathogenic *E. coli* infection. Epidemiological studies have identified that children are at higher risk of developing post-diarrhoeal HUS than other age groups (Cummings et al., 2002).

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5.11 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, non-spore forming rod-shaped bacteria that may be isolated from a variety of sources including soil, silage, sewage, food-processing environments, raw meats and the faeces of healthy humans and animals (USFDA CFSAN, 2004a). *L. monocytogenes* belongs to the genus *Listeria* along with *L. innocua, L. welshimeri, L. selligeri, L. ivanovii and L. grayi*. Thirteen serotypes are associated with *L. monocytogenes* (1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab,4b, 4c, 4d, 4e, 7).

5.11.1 Growth characteristics

Growth of *L. monocytogenes* in foods is influenced by a variety of factors, including the nature and concentration of essential nutrients, pH, temperature, water activity, the presence of food additives that could enhance or inhibit growth and presence of other microbial flora (Lovett et al., 1990). The limits and optima for key factors are summarised in Table 4.7.

Table 4.7 Growth conditions for *L. monocytogenes* (ANZFA unpublished)

	Minimum	Optimum	Maximum
Temperature (°C)	-1.5	37	45
pH	4.39	7.0	9.4
Water activity (a_w)	0.90	-	-

Under conditions outside the growth range, the bacteria may survive and growth may recommence once suitable conditions are encountered.

Temperatures of >50ºC are lethal to *L. monocytogenes*. When in a suitable medium, *L. monocytogenes* can grow between ~0-45°C. Although *L. monocytogenes* does not grow below –1.5ºC, it can readily survive at much lower temps. Nonetheless, freezing and frozen storage will cause a limited reduction in the viable population of *L. monocytogenes*. Optimal conditions for growth are between 30 and 37ºC (Ryser, 1999).

L. monocytogenes will grow in a broad pH range with the upper limit being approximately 9.3 and the lower limit being 4.6-5.0 (ICMSF, 1996). Although growth at pH <4.3 has not yet been documented, *L. monocytogenes* appears to be relatively acid tolerant. It has been suggested that food fermentations, which involve a gradual lowering of pH, could lead to acid adaptation of *L. monocytogenes*.

Like most bacterial species, *L. monocytogenes* grows optimally at a water activity (a_w) of approximately 0.97. However, when compared with most food-borne pathogens, the bacterium has the unique ability to multiply at a_w values as low as 0.90. While it does not appear to be able to grow below 0.90, the bacterium can survive for extended periods at lower values (Ryser, 1999).

L. monocytogenes is reasonably tolerant to salt and can grow in NaCl concentrations up to 10% (European Commission, 2003). Extended survival occurs at a wide range of salt concentrations and *L. monocytogenes* has survived for up to eight weeks in a concentration of 20% NaCl (Sutherland et al., 2003). Survival in the presence of salt varies with storage temperature and studies have indicated that survival of *L. monocytogenes* in concentrated salt solutions can be increased dramatically by lowering the incubation temperature (Ryser and Marth, 1999).

L. monocytogenes grows well under both aerobic and anaerobic conditions (Ryser and Marth, 1999; Sutherland et al., 2003).

The listericidal effect of preservatives is strongly influenced by the interactive effects of temperature, pH, type of acidulant, salt content, water activity, and type and concentration of food additives present in the food. For example the ability of potassium sorbate to prevent growth of *L. monocytogenes* is related to temperature and pH. The lower the storage temperature and pH of the medium, the greater the effectiveness of sorbates against *L. monocytogenes*. Sodium benzoate is more inhibitory to *L. monocytogenes* than is either potassium sorbate or sodium propionate. Inhibition and inactivation of *L. monocytogenes* in the presence of sodium benzoate is affected by temperature (more rapid at higher than lower incubation temperatures), concentration of benzoic acid (more rapid at higher than lower concentrations) and pH (more rapid at lower rather than higher pH values) as well as the type of acid used to adjust the growth medium (Ryser and Marth, 1999).

5.11.2 Pathology of illness

There are two main forms of illness associated with *L. monocytogenes* infection; listerial gastroenteritis, where usually only mild symptoms are reported, and invasive listeriosis, where the bacteria penetrate the gastrointestinal tract and invade normally sterile sites within the body (USFDA Centre for Food Safety and Applied Nutrition, 2004a).

Symptoms of the mild form of *L. monocytogenes* infection are primarily those generally associated with gastrointestinal illness: chills, diarrhoea, headache, abdominal pain and cramps, nausea, vomiting, fatigue, and myalgia (FDA et al., 2003). The onset of illness is usually greater than 12 hours (Anon, 2004).

Invasive listeriosis is clinically defined when the organism is isolated from blood, cerebrospinal fluid or an otherwise normally sterile site (*e.g.* placenta, foetus). The manifestations include septicaemia, meningitis (or meningoencephalitis), encephalitis, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion in the second or third trimester, or stillbirth (USFDA CFSAN, 2004a). The onset of these manifestations is usually preceded by influenza-like symptoms including persistent fever. Gastrointestinal symptoms such as nausea, vomiting and diarrhoea may also precede the serious forms of listeriosis. Listeriosis typically has a 2 to 3 week incubation time, but onset time may extend to 3 months (FDA et al., 2003).

It is estimated that approximately 2-6 percent of the healthy human population harbour *L. monocytogenes* in their intestinal tract, which suggests that people are frequently exposed to *L. monocytogenes* (Rocourt and Bille, 1997; Farber and Peterkin, 1991a). This may also suggest that most people have a tolerance to infection by *L. monocytogenes*, and given the relatively low number of reported cases, exposure rarely leads to serious illness in health individuals;(Anon, 2004; Hitchins, 1996; Marth, 1988).

5.11.3 Mode of transmission

Food-borne exposure is the primary route of transmission for listeriosis, however listeriosis can be transmitted vertically (*i.e.* mother to child), zoonotically and through hospital acquired infections.

5.11.4 Incidence of illness

Most cases of listeriosis are sporadic. The number of reported cases of invasive listeriosis in Australia between 2001 and 2004 varied between 61 – 72 cases (Ashbolt et al., 2002; Anon., 2003a, Anon., 2004a; Anon., 2005a), which equates to approximately 3 – 4 cases per million persons per year. In Australia, the exact mortality rate is not known, although the data available would suggest a rate of approximately 23%.

The reported incidence of invasive listeriosis in New Zealand over the period of 2002 - 2004 was between five – six cases per million (range of 19 – 26 cases per annum) of the general population per year (Anon., 2003b; Anon., 2004b; Anon., 2005b). The case fatality rate in New Zealand is approximately 17% (Anon, 2004).

Table 4.8 Outbreaks of listeriosis (US: 1970-2002; Outside US: 1970-2000) with known food vehicle(s) (FDA et al., 2003).

Year	Food Vehicle	Country	Cases	Deaths (% total)	Sero-type	Reference
Not Specified	Frozen vegetables	US	7	Unknown	4b	Simpson.D.M., 1996
1983-1987	Vacherin Mont d'Or cheese	Switzer-land	122	31 (25.4)	4b	Bille, 1990a; Bula et al., 1995
1987-1989	Pâté and meat spreads	England	355	94 (26.5)	4b	McLauchlin et al., 1991
1986-1987	Ice cream, salami, brie cheese	US	36	16 (44.4)	4b,1/2b, 1/2a	Schwartz et al., 1989
1986-1987	Raw eggs	US	2	Unknown	4b	Schwartz et al., 1989
1998-1999	Hot dogs, deli meats	US	101	21 (20.8)	4b	Mead, 2004
2000-2001	Homemade Mexican-style cheese (raw milk)	US	12	5 (41.7)	unknown	CDC, 2001
1978-1979	Vegetables (raw)	Australia	12	0 (0)	Unknown	Le Souëf and Walters, 1981
1989-1990	Semi-soft Cheese (blue)	Denmark	23	0 (0)	4b	Jensen et al., 1994
1994-1995	Smoked Seafood (finfish and molluscs)	Sweden	9	2 (22.2)	4b	Ericsson et al., 1997
1998-1999	Butter	Finland	25	6 (0)	3a	Lyytikainen et al., 2000
1999-2000	Pigs tongue in aspic	France	26	7 (0)	Unknown	Dorozynski, 2000
1979	Raw vegetables or cheese	US	20	3 (15.0)	4b	Ho et al., 1986
1980	Raw seafood (finfish and mollusks)	New Zealand	22	6 (27.3)	1b	Lennon et al., 1984
1981	Miscellaneous Dairy Products	England	11	5 (45.5)	1/2a	Ryser, 1999
1981	Vegetables (raw)	Canada	41	17 (41.5)	4b	Schlech, III et al., 1983
1983	Pasteurized fluid milk	US	32	14 (43.8)	4b	Fleming et al., 1985
1985	Mexican-style cheese (raw milk)	US	142	48 (33.8)	4b	Linnan et al., 1988
1986	Unpasteurised milk, organic vegetables	Austria	28	5 (17.9)	Unknown	Allerberger and Guggenbichler, 1989
1987	Butter	US	11	Unknown	Unknown	Ryser, 1999
1990	Pâté and meat spreads	Australia	11	6 (54.5)	1/2a	Ryser, 1999
1991	Smoked mussels	Australia	4	0 (0)	1/2a	Mitchell, 2001; Misrachi et al., 1991
1992	Smoked mussels	New Zealand	4	0 (0)	1/2	Brett et al., 1998
1992	Pork tongue in jelly	France	280	63 (22.5)	4b	Jacquet et al., 1995
1993	Rillettes	France	38	11 (28.9)	4b	Goulet et al., 1998
1995	Soft Ripened Cheese, >50% moisture (brie, feta, camembert, mozzarella)	France	33	4 (20.0)	4b	Jacquet et al., 1995; Goulet et al., 1995
1996	Cooked chicken	Australia	5	1 (20.0)		Sutherland, 2003Hall et al., 1996
1997	Pont l'Eveque cheese	France	14	0 (0)	4b	Ryser, 1999
1999	Pâté	US	11	unknown	1/2a	Carter, 2000
2000	Deli turkey meat	US	29	7 (24.1)	unknown	CDC, 2000
2002	Deli turkey meat, sliceable	US	63	7 (11.1)	unknown	CDC, 2002

The estimated incidence of invasive listeriosis in European countries has been reported to between 0.3-7.5 cases per million of the general population per year (European Commission, 2003). In France, the estimated incidence is sixteen cases per million (general population) per year (ICMSF, 1996; Bille, 1990b). The annual incidence of listeriosis in the United States has been estimated to range from 3.4 per million (Centers for Disease Control and Prevention, 2000) to 4.4 per million (Tappero et al., 1995). Of all food-borne pathogens, *L. monocytogenes* results in the highest hospitalisation rate in the United States, with fatality rates of 20-30% being common (WHO/FAO, 2004).

Outbreaks of invasive listeriosis have been linked to Hispanic-style soft cheeses; soft, semi-soft and mould-ripened cheeses; hot dogs; pork tongue jelly; processed meats; pate; salami; pasteurised chocolate flavoured milk; pasteurised and unpasteurised milk; butter; cooked shrimp; smoked salmon; maize and rice salad; maize and tuna salad; potato salad; raw vegetables; and coleslaw (FDA/FSIS, 2003). In addition, sporadic cases have been linked to the consumption of raw milk; unpasteurised ice cream; ricotta cheese; goat, sheep and feta cheeses; soft, semi-soft and mould-ripened cheeses; Hispanic-style cheese; salami; hot dogs; salted mushrooms; smoked cod roe; smoked mussels; undercooked fish; pickled olives; raw vegetables; and coleslaw (WHO/FAO, 2004).

An outbreak of listeriosis associated with consumption of pre-cooked, diced chicken occurred in South Australia during 1996 (Hall et al., 1996). There were five confirmed cases of listeriosis, including one death. The majority of cases were patients of health care facilities. Between September 1997 and January 1999, nine cases of listeriosis (resulting in six deaths) were reported in the Hunter region of NSW (Anon., 2000). All individuals were either immunocomprimised or elderly. Fruit salad was reported as the likely source of infection.

5.11.5 Occurrence in foods

L. monocytogenes has been found in foods such as milk, dairy products (particularly soft-ripened cheeses), meat, poultry, seafood and vegetables.

The worldwide prevalence of *L. monocytogenes* spp. in raw milk is estimated to be around 3-4% (Doores and Amelang, 1988; Hayes et al., 1986; Lovett et al., 1987). In Australian surveys on soft and surface ripened cheeses and ice-cream, *L. monocytogenes* has been isolated from 2% of locally produced cheese samples and 6% of ice-cream samples (Sutherland et al., 2003). 7% of imported cheeses, camembert and blue vein were positive for *L. monocytogenes* (Sutherland et al., 2003). 25% of European soft and surface-ripened cheeses have been found to be positive for *L. monocytogenes* (Terplan, 1988).

The incidence of *L. monocytogenes* in slaughter animals is generally low (0-9%) (Farber and Peterkin, 1999). Overseas studies have shown the prevalence of *L. monocytogenes* contamination in raw meat to be in the range 5-20% (Farber and Peterkin, 1999). In Australia, levels of 24% in beef, 16% in lamb and 10% in pork have been found (Ibrahim and MacRae, 1991). Other meat products from which *L. monocytogenes* has been isolated include minced meat products, sausages, salami, ham, mettwurst, pate, frankfurters and vacuumed packed meat (Farber and Peterkin, 1991b).

Prevalence in poultry meat products ranges from 12-60% (Ojeniyi et al., 2004), and has been isolated from fresh, frozen, cook-chilled and precooked ready to eat chicken products (Cox et al., 1999).

L. monocytogenes has been detected in fresh, frozen and processed seafood. Prevalence in fresh processed seafood ranges between 4-12% in published surveys (Sutherland et al., 2003).

Types of vegetable produce where the organism has been detected include radishes, cucumbers, cabbage, potatoes, lettuce, frozen broccoli and cauliflower and endive (Brackett, 1999; Heisick et al., 1989). Levels of 44% have been detected on fresh cut salad vegetables in the Netherlands, and 9% in prepared salads in Ireland (Harvey and Gilmour, 1993). Recent European surveys show the presence of *L. monocytogenes* to be less than 10% (Brackett, 1999).

5.11.6 Virulence and infectivity of L. monocytogenes

When ingested, *L. monocytogenes* penetrates the intestinal tissue and is taken up by macrophages and non-phagocytic cells in the host. *L. monocytogenes* is disseminated throughout the host via blood or lymphatic circulation to various tissues. Its presence intra-cellularly in phagocytic cells permits access to the brain and probably transplacental migration to the foetus in pregnant women. The pathogenesis of *L. monocytogenes* relies on its ability to survive and multiply in phagocytic host cells.

Not all strains appear to be equally virulent. The 4b and occasionally 1/2a and 1/2b serovars account for most cases of human listeriosis (ICMSF, 1996).

The virulence of *L. monocytogenes* is increased when the bacterium is grown at low rather than high temperatures. The possibility exists that cold storage may enhance virulence of some *L. monocytogenes* strains isolated from refrigerated foods (Ryser and Marth, 1999).

5.11.7 Dose Response

Cases of non-invasive listeriosis (also referred to as febrile listerial gastroenteritis) have been observed during outbreaks, involving symptoms such as diarrhoea, fever, headache and myalgia, generally following a short incubation period (WHO/FAO, 2004), Insufficient quantitative data is available to develop a dose-response model for this milder form of listeriosis, however, outbreak situations have generally involved the ingestion of high doses of *L. monocytogenes*.

The dose-response relationship for invasive listeriosis is highly dependent on a number of factors, such as the virulence characteristics of the organism, the number of cells ingested, the general health and immune status of the host, and the attributes of the food matrix that may alter the microbial or host status. FDA et al. (2003) and WHO/FAO (2004) developed separate dose-response models for both healthy and susceptible populations by combining data from surrogate animal models with epidemiological data. For the healthy population (classified as "intermediate-age") the median mortality rate from ingestion of 10^9 organisms was estimated to be 1.0×10^{-6} (FDA et al., 2003). For neonatal and elderly groups the mean mortality rate at the same dose was estimated to be 1.4×10^{-3} and 3.3×10^{-6} respectively.

The infectious dose is unknown but it is believed to vary with strain and susceptibility of the individual. There is a lack of information concerning the minimal infectious dose, although it is generally thought to be relatively high (>100 viable cells) (ICMSF, 1996). From cases contracted via raw or inadequately pasteurised milk, it is assumed that for susceptible individuals, ingestion of fewer than 1,000 organisms may cause disease (FDA et al., 2003).

It is thought the consumption of food with exceptionally high levels of *L. monocytogenes* ($>10^7$/g) is required to cause the mild gastrointestinal form of illness in healthy persons (Sutherland et al., 2003).

5.11.8 Host factors

Specific sub-populations at risk for invasive listeriosis include pregnant women and their foetuses, neonates, the elderly and persons with a compromised immune system, whose resistance to infection is lowered (*e.g.* transplant patients, patients on corticosteroid treatments, HIV/AIDS patients and alcoholics). Less frequently reported, diabetic, cirrhotic, asthmatic and ulcerative colitis patients are also at more risk (USFDA CFSAN, 2004a).

Another physiological parameter thought to be relevant to susceptibility is a reduced level of gastric acidity (WHO/FAO, 2004).

5.11.9 Food Matrix

To date, the properties of the food vehicle have been viewed as having little effect on the infective dose of *L. monocytogenes*. However, it is possible that food vehicles with high buffering capacity may protect the bacteria from inactivation by the pH of gastric acids in the stomach. In general, there are insufficient data available as to whether the food matrix affects the dose-response curve for *L. monocytogenes* (WHO/FAO, 2004).

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5.12 *Mycobacterium bovis*

The genus Mycobacterium comprises approximately 95 species, of which over 30 have been associated with disease in humans (Katoch, 2004). Mycobacterium species are also pathogens of food producing animals such as cattle, sheep, other ruminants and fish, and some of those species have zoonotic potential in humans (Sutherland, 2003).

Mycobacteria are aerobic, non-sporeforming, Gram-positive (though difficult to stain) acid-fast rod-shaped bacilli without flagellae. They are slow growing and difficult to culture, having fastidious and nutritionally-exacting growth requirements (Anon, 1998).

The mycobacteria are widely distributed in the environment, being found in soil and water. They readily form biofilms in drinking water distribution systems (Falkinham, III, 2002; Sutherland, 2003). Mycobacteria have particularly hydrophobic cell walls, giving them a propensity to form aerosols, to clump together in liquid media and to form biofilms (Sattar et al., 1995; Anon 1998; Woelk et al., 2003).

M. bovis is related to *M. tuberculosis*, the agent of human pulmonary tuberculosis. *M. bovis* causes systemic infections in cattle and other animals, where it initially infects the gastrointestinal tract before spreading to other parts of the body, including the lungs. *M. bovis* can be shed directly from infected mammary glands into milk, and subsequently transmitted to humans via consumption of contaminated milk (Lake et al., 2002). Multidrug resistance is common. The current requirements for holding (batch) and high temperature short time (HTST) pasteurisation were developed, in part, to manage the risk to human health from the transmission of *M. bovis* through the milk supply.

M. bovis was introduced into Australia at the time of European settlement. A program to eradicate bovine tuberculosis began in 1970 and Australia was declared free of the disease in December 1997 (Animal Health Australia, 2005).

5.12.1 Growth characteristics

Mycobacteria are slow growing obligate aerobes which are difficult to culture as they do not grow on ordinary microbiological media (Anon 1998). Colonies are rarely visible to the naked eye in under 4 weeks of incubation on Dorset egg medium (Anon 1998).

This slow and fastidious growth habit, allied with the short shelf life of foods they are associated with, means that mycobacteria are unlikely to grow to any significant extent in food during production, processing, distribution and storage (Lake et al., 2002).

M. bovis is inactivated by sunlight (Lake et al., 2002). It has also been found to be relatively resistant to a wide range of disinfectants used in medical / hospital settings (Rutala et al., 1991; Gregory et al., 1999; Lake et al., 2002).

5.12.2 Pathology of illness

Symptoms associated with *M. bovis* gastrointestinal infection include fever, chills, weight loss, abdominal pain, diarrhoea or constipation. Symptoms of further infections depend on the organs infected. Symptoms may last for months or years, and death may result (Lake et al., 2002).

Due to the slow growing nature of the organism, the onset time to elaboration of symptoms may be years after initial infection, and even in immunocompromised individuals the onset time may be several months (Lake et al., 2002).

5.12.3 Mode of transmission

M. bovis is considered to be transmitted to humans primarily through aerosols from infected animals and consumption of unpasteurised milk and dairy products (O'Reilly and Daborn, 1995; Cousins and Dawson, 1999; Lake et al., 2002; Anon 2005). Water is not considered to be a source of human infection with *M. bovis* (Lake et al., 2002), although transmission of other waterborne mycobacteria can occur through drinking or via inhalation as a result of aerosolisation.

There is disagreement as to whether consumption of meat from infected (tuberculous) cattle can lead to human infection.

5.12.4 Incidence of illness

M. bovis was responsible for 2.4% of human TB patients in Santa Fe province, Argentina, in the period 1984-1989 (Zumarraga et al., 1999). Eleven of 19 *M. bovis* strains isolated from humans were from rural or slaughterhouse workers.

M. bovis subsp. *caprae* was responsible for one third of 166 human isolates of *M. bovis* from TB cases in Germany between 1999-2001, and *M. bovis* was present in approximately 1% of human TB cases (Kubica et al., 2003).

The prevalence of human tuberculosis (TB) due to *M. bovis* was determined in urban areas in Madagascar in 1994-1995. A prevalence of *M. bovis* of 1.25% was observed among sputum smear-positive patients and 1.3% among extra-pulmonary TB patients (Rasolofo-Razanamparany et al., 1999).

M. bovis was isolated in 0.5% (38/7075) of cases of bacteriologically confirmed tuberculosis notified to the National Reference Centre (CNR) in France in 1995. Incidence rates increased with age, and were approximately equally split between pulmonary and extra-pulmonary sites. Occupational exposure was identified in 13 cases and ingestion of non pasteurised milk in three (Robert et al., 1999).

M. bovis was responsible for approximately 1% of cases of TB in the Australian population during 1970-1994 (at least 236 cases). The majority of cases (74%) involved pulmonary disease. Most cases were apparently due to reactivation of infection acquired through occupational exposure and had histories of employment in meat and/or livestock industries (Cousins and Dawson 1999).

About 1% of clinically diagnosed cases of TB in the UK are attributed to *M. bovis* (Gibson et al., 2004).

33.9% of 180 culture-positive paediatric cases (<15 years old) of TB in San Diego during 1980 to 1997 were attributed to *M. bovis* (Dankner and Davis, 2000).

Between 1994 and 2000, 6.7% (129/1931) of all cases of culture-positive TB in San Diego County were identified as *M. bovis*, and 90% of these occurred in the Hispanic population (LoBue et al., 2003).

Lumb et al., (2002) and Lumb et al. (2003) reported that *M. bovis* accounted for only 2 of 765 new diagnoses of disease caused by tuberculosis-causing mycobacteria in 2000. In 2001, only 1 of 771 cases were due to *M. bovis* (Lumb et al., 2002; Lumb et al., 2003).

M. bovis subsp. caprae accounted for less than 1% (4/640) of human isolates of Mycobacterium species from patients in western Austria in the period 1994-2001 (Prodinger et al., 2002).

A study of 35 culture-confirmed cases of tuberculosis caused by *M. bovis* in New York, USA during 2001 – 2004, raw milk cheese from Mexico was implicated as the likely source of infection (Anon., 2005).

5.12.5 Occurrence in foods

Very few surveys have been conducted in recent times for the presence of *M. bovis* in pasteurised milk, presumably due to the expectation that current pasteurisation practices are sufficient to eliminate the pathogen from milk. A survey of milk samples in Brazil identified culturable *M. bovis* in 1 of 78 raw milk samples and no isolates from pasteurised and UHT milk (Leite et al., 2003). It is generally accepted that current pasteurisation practices are sufficient to inactivate *M. bovis* at the levels commonly found in raw milk.

5.12.6 Virulence and infectivity

The virulence factors of the mycobacteria remain largely unknown (Collins et al., 1995; Collins, 1996). Mycobacteria are intracellular pathogens, able to grow and multiply inside macrophage cells, thus effectively avoiding attack by the host's immune system. The unique structure of the mycobacterial cell wall, particularly a cell wall glycolipid containing mycolic acid, is thought to contribute to protecting the invading organism from the host's defence mechanisms. Pathology may also be due to the direct action of toxic chemical components of the mycobacterial cell wall.

The other main virulence factor identified is a catalase-peroxidase (KatG gene: (Collins 1996), which appears to help the cells to resist destruction by macrophages.

Most mycobacteria do not secrete exotoxins.

5.12.7 Dose Response

Results from animal experiments indicate that *M. bovis* infection via the oral route requires thousands or millions of organisms compared to less than ten via the inhalation route (O'Reilly and Daborn 1995; Lake et al., 2002).

5.12.8 Host Factors

The group at greatest risk of infection with *M. bovis* is those with a compromised immune system, whose resistance to infection is lowered

Biet et al. (2005) report that those at greatest risk of infection with *M. bovis* (and mycobacterial infections generally) include the very young and old, those with compromised immune systems (*e.g.* transplant patients, patients on corticosteroid treatments, HIV/AIDS patients and alcoholics), and those exposed due to occupation or lifestyle. Reactivation can occur under stress or in old age, when latent mycobacterial infections may become subject to less stringent control by host systems.

5.12.9 Food Matrix

M. bovis is a very slow growing, microaerophillic organism and given the usual short shelf-life of products that may be associated with transmission, eg raw milk and raw meat, it would appear that growth in foods would be insignificant.

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5.13 *Mycobacterium avium* subsp. *paratuberculosis*

The genus Mycobacterium comprises approximately 95 species, of which over 30 have been associated with disease in humans (Katoch, 2004). Mycobacterium species are also pathogens of food producing animals such as cattle, sheep, other ruminants and fish, and some of those species have zoonotic potential in humans (Sutherland, 2003).

Mycobacteria are aerobic, non-sporeforming, Gram-positive (though difficult to stain) acid-fast rod-shaped bacilli without flagellae. They are slow growing and difficult to culture, having fastidious and nutritionally-exacting growth requirements (Anon, 1998).

The mycobacteria are widely distributed in the environment, being found in soil and water. They readily form biofilms in drinking water distribution systems (Falkinham, III, 2002; Sutherland, 2003). Mycobacteria have particularly hydrophobic cell walls, giving them a propensity to form aerosols, to clump together in liquid media and to form biofilms (Sattar et al., 1995; Anon 1998; Woelk et al., 2003).

The position of *M. avium* subsp. *paratuberculosis* (hereafter *M. avium* subsp. *paratuberculosis* or MAP) as a human pathogen is still unclear. Debate centres on the possible role of MAP in Crohn's disease, a chronic intestinal enteritis in humans. Similarities have been observed between Crohn's disease and Johne's disease in cattle and sheep, a disease which is known to be caused by MAP (Anon 1998). The debate is characterised by firmly entrenched opinions on either side, and the subject has been comprehensively reviewed several times (Chiodini, 1989; Thompson, 1994; Anon 1998; Harris and Lammerding, 2001; Lipiec, 2003; Chacon et al., 2004).

5.13.1 Growth characteristics

MAP is an obligate parasite and is absolutely dependent on mycobactin, an iron-chelating siderophore, for in vitro growth (Anon 1998; Motiwala et al., 2004). The temperature range for growth of MAP is 25ºC to 45ºC with an optimum of around 39ºC (Anon 1998). Batch (63ºC for 30 min) and HTST (72ºC for 15 sec) pasteurisation are sufficient to inactivate high levels of pathogenic mycobacteria in milk, although they will survive thermisation (treatment at 62ºC for 15 sec for cheese production) (Stabel and Lambertz, 2004).

MAP does not grow in the presence of 5% sodium chloride but is able to grow in microbiological media at pH 5.5. The organism is resistant to drying and may survive in faeces on pasture land for one year or so (Anon 1998).

5.13.2 Pathology of illness

Although there is ongoing disagreement regarding the role of MAP in human Crohn's disease, the following brief description of the disease is included for information. Crohn's disease is a chronic, granulomatous inflammatory disease of humans, which primarily affects the terminal ileum and colon (reviewed by: Anon 2000; Rubery 2002). The disease is characterised by periods of activity interspersed with periods of remission. The clinical signs of Crohn's disease include weight loss, abdominal pain, diarrhoea, reduced appetite and fatigue. Crohn's disease has also been associated with arthritis, skin lesions, anaemia and, in the younger age group, reduced growth rate(Anon 2004).

It has also been observed that mycobacterial illnesses can reactivate many years after recovery from overt illness (Rutala et al., 1991; Gregory et al., 1999; Kubica et al., 2003; Gibson et al., 2004).

5.13.3 Mode of transmission

MAP is excreted primarily in the faeces of infected animals and is excreted during both the sub-clinical and clinical stages of disease. In dairy animals, MAP can be transmitted both vertically through the placenta to the foetus in advanced infection and also through the calf ingesting colostrum, milk or faeces from an infect animal. MAP is also transmitted horizontally through the faecal-oral route (Streeter et al., 1995; Sweeney, 1996, Scientific Committee on Animal Health and Animal Welfare, 2000; Anon., 2004). Young animals are most susceptible to MAP infection (Morgan, 1987).

Although there has been concern that MAP could survive the time and temperature combinations routinely used for batch and HTST milk pasteurisation, recent studies have confirmed the efficacy of these processes (Pearce et al., 2001; Stabel and Lambertz 2004; Pearce et al., 2004). However, the potential for its presence and survival in unpasteurised dairy products still exists. Other potential sources of human infection include water supplies, raw vegetables and undercooked meat, although there are no definitive studies on these routes of exposure (Anon 1998). Pickup et al., 2005 demonstrated the survival of MAP in river water and inferred a link to clusters of Crohn's disease. DNA fingerprinting studies have indicated that water was the source of *Mycobacterium avium* infection in AIDS patients (von Reyn et al., 1994).

Goats' milk, which is often drunk unpasteurised, may also contain MAP and may therefore pose a potential source of human exposure (Anon 1998; Muehlherr et al., 2003).

Human to human transmission occurs rarely, mainly among immuno-compromised patients suffering pulmonary symptoms (Kubica et al., 2003; Gibson et al., 2004).

5.13.4 Incidence of illness

There is ongoing uncertainty regarding any role of MAP in human Crohn's disease. The current estimated prevalence of Crohn's disease in Australia is 50 per 100,000 (estimated 1 per 1,000 in western countries world-wide: (Selby, 2003; Anon 2004). The incidence of Crohn's disease is highest in the 15-35 year age group, followed by the 55-65 year age group. Crohn's disease incidence appears to be increasing worldwide. However, this may be due to more sensitive diagnostic measures and an increased awareness of the disease. There is currently no cure for Crohn's disease (Rubery 2002).

5.13.5 Occurrence in foods

Infected cattle may shed MAP in their faeces at levels up to 10^8 cfu/g. MAP has been cultured from the milk of 35% of infected cattle and 11.6% of asymptomatic carriers, the latter having been found to contain 2-8 cfu/50ml of milk (Sweeney et al., 1992; Anon 1998).

Concern regarding the ability of MAP to survive pasteurisation has been prompted by a number of surveys for the organism in pasteurised milk. Interpretation of the results of these surveys is complicated because of large discrepancies between results of polymerase chain reaction (PCR) methods (detecting the presence of DNA) and culture methods (detecting viable organisms).

For example, 15% (110/710) of retail milk samples collected in southwest Ontario, Canada, tested positive for the presence of MAP DNA by PCR, although broth and agar culture of 44 of those positives failed to demonstrate any survivors (Gao et al., 2002).

A survey of MAP in milk in England and Wales conducted by (Millar et al., 1996) raised significant concern regarding the possible survival of MAP during pasteurisation. Seven percent (22/312) of samples tested positive by PCR, and the authors concluded that since the positive PCR signal segregated to either (or both) the pellet and/or cream fractions, the results were indicative of the presence of intact mycobacterial cells. Fifty percent of PCR positive samples and 16% of PCR negative samples yielded MAP-positive cultures. However, other workers questioned the conclusion drawn that MAP could survive pasteurisation (*e.g.* see Stabel, 2000).

A survey of 104 samples of raw sheep and goat's milk from bulk tanks on farms throughout England, Wales and Northern Ireland identified 1 goat milk sample positive by PCR and no positive MAP culture results (Grant et al., 2001).

(Grant et al., 2002) tested a total of 814 cows' milk samples, 244 bulk raw and 567 commercially pasteurised (228 whole, 179 semiskim, and 160 skim) over a 17-month period to July 2000. MAP DNA was detected by PCR in 19 (7.8%) and 67 (11.8%) of the raw and pasteurised milk samples, respectively. Confirmed MAP isolates were cultured from 4 (1.6%) and 10 (1.8%) of the raw and pasteurised milk samples, respectively. The authors noted that pasteurisation conditions complied with the legal requirement for the HTST process, and considered that post-process or laboratory contamination was unlikely to have occurred, leading them to conclude that viable MAP is occasionally present at low levels in commercially pasteurised cows' milk in the United Kingdom.

A similar 13-month study (to November 2001) of bulk raw (n = 389) and commercially pasteurised (n = 357) liquid-milk supplies was conducted in Ireland (O'Reilly et al., 2004). MAP DNA was detected by PCR in 50 (12.9%) of raw-milk samples and 35 (9.8%) of pasteurised-milk samples. Confirmed MAP was cultured from one raw-milk sample and no pasteurised-milk samples. It was concluded that MAP DNA is occasionally present at low levels in both raw and commercially pasteurised cows' milk but, since no viable MAP was isolated from pasteurised milk samples, current pasteurisation procedures were considered to be effective.

5.13.6 Virulence and infectivity

Virulence factors of MAP remain largely unknown (Collins et al., 1995; Collins, 1996). MAP is an intracellular pathogen, able to grow and multiply inside macrophage cells, thus effectively avoiding attack by the host's immune system. A major distinguishing feature of MAP is its requirement of exogenous mycobactin for growth. Mycobactin is an iron-chelating agent produced by all other mycobacteria, which MAP does not produce, or produces an insufficient amount.

The other main virulence factor identified is a catalase-peroxidase which appears to protect the cells from destruction by macrophages (Collins 1996).

5.13.7 Dose Response

As described earlier, there is ongoing debate around the role of MAP in human Crohn's disease. There is no data available on a likely dose-response relationship.

5.13.8 Host Factors

It has been well documented that there is a genetic component associated with developing Crohn's disease (Rubery, 2002). It has been linked to mutations in the NOD2 gene (chromosome 16) which regulates the activity of macrophages against bacterial pathogens (McGovern et al., 2001).

5.13.9 Food Matrix

Limited studies have investigated the survival of MAP in food, with most research being undertaken on dairy products. For cheddar cheese, Donaghy et al. (2003) observed an increased concentration of MAP in 1-day old cheese compared to the original concentration inoculated into the milk, then a gradual decrease during the ripening period. When numbers of MAP in day-old cheese was high (>3.6 $\log_{10}$), the organism was able to be cultured after a 27 week ripening period. D-values for a different MAP strains ranged from 90 – 107 days.

Spahr and Schafroth (2001) studied the survival of MAP in Swiss Emmentaler (hard) and Swiss Tisliter (semi hard) cheeses. For both cheeses, MAP numbers descreased steadily, although slowly, during ripening. Calculated D-values for the hard and semi-hard cheese were 27.8 and 45.5 days respectively. Based on ripening periods of between 90 – 120 days, the estimated reduction during the cheese making process would be between 3 – 4 $\log_{10}$. Factors that were identified as having the greatest impact on MAP survival were the temperatures applied during the cheese making process and the low pH at the early stages of ripening.

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5.14 *Salmonella* spp.

Salmonellosis is a leading cause of enteric illness, 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 food-borne salmonellosis. However, as the disease is primarily zoonotic, foods of animal origin have been consistently implicated as the main sources of human salmonellosis (FAO/WHO, 2002).

The genus *Salmonella* is currently divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori* (Brenner *et al*., 2000); Table 2.1). The subspecies of most concern in relation to food safety is *S. enterica* subsp. *enterica,* as over 99% of human pathogens 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 example *S. enterica* subsp. *enterica* serotype Typhimurium (formerly *Salmonella typhimurium*). For practical reasons, the shortened versions of these names are commonly used, such as *Salmonella* Typhimurium.

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 environment [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.

Some *Salmonella* serotypes are host-adapted to individual animal species. For example *S.* Typhi and *S*. Paratyphi are specifically associated with infections leading to severe illness in humans (Bell and Kyriakides, 2002).

5.14.1 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 temperature, pH, and water activity (a_w). Being facultatively anaerobic, salmonellae also have the ability to grow in the absence of oxygen. Growth and survival is also influenced by the presence of inhibitors such as nitrite and short-chain fatty acids (Jay *et al*., 2003).

Temperature
The growth of most salmonellae is substantially reduced at <15°C and prevented at <7°C (ICMSF, 1996). Growth generally does not occur at >46.2°C. The optimum temperature for growth is 35 – 43°C.

Freezing can be detrimental to *Salmonella* 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 freezing damage. However, at lower temperatures (-17 to -20°C) there is a significantly less rapid decline in the number of viable organisms. *Salmonella* have the ability to survive long periods of time at storage temperatures of < -20°C (Jay *et al*., 2003).

Heat resistance of *Salmonella* in foods is dependant on the composition, nature of solutes and pH, and water activity of the food (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).

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. However, growth can usually occur between pH 3.8 – 9.5 (Jay *et al*., 2003). The optimum pH range for growth is 7.0 – 7.5 (Table 2.2). Volatile fatty acids are more bactericidal than acids such as lactic and citric acid.

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 having a low a_w (such as black pepper, chocolate, gelatine). Exposure to low a_w environments can greatly increase the heat resistance of *Salmonella*.

Table 2.2: Limits for growth of *Salmonella* when other 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

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

In a study of 48,857 patients with gastroenteritis (of which 26,974 were salmonellosis) Helms *et al.*, (2003) found an association with increased short-term (mortality within 30 days of infection) and long-term risk of death (mortality within a year of infection) compared with controls.
In cases of acute gastroenteritis, the incubation period is usually 12-72 hours (commonly 12-36 hours) and is largely dependant on the sensitivity of the host and size of the dose ingested (FAO/WHO, 2002; Hohmann, 2001). 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, and approximately 1% of patients become chronic carriers (Jay *et al*., 2003).

Due to the general self-limiting nature of the disease, antibiotics are not usually recommended for healthy individuals suffering from mild to moderate *Salmonella* gastroenteritis (Hohmann, 2001). Antibiotics should be used, however, for those who are severely ill and for patients with risk factors for extraintestinal spread of infection, after appropriate blood and faecal cultures are obtained.

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-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 constitutes 8–9% of human *Salmonella* isolates in the USA. Sporadic human cases are reported in Australia, although these are commonly acquired overseas (Blumer *et al*., 2003). During 2001 an outbreak of *S.* Typhimurium DT104 occurred in Victoria and was linked to contaminated imported halva (a sesame seed product).

5.14.3 Mode of transmission

Salmonella are transmitted by the faecal-oral route. Sources of transmission include person-to-person, food-borne, waterborne (drinking water and direct contact with faecally contaminated water) and direct contact with infected animals.

5.14.4 Incidence and outbreak data

Salmonellosis is one of the most commonly reported enteric illnesses worldwide (FAO/WHO, 2002). Approximately 7,000-8,000 cases of salmonellosis per annum are formally notified to health authorities in Australia (Hall, 2003). Taking into account under-reporting it has been estimated (based on published rates of under-reporting) that 80,000 cases of food-borne salmonellosis occur annually (Hall, 2003).

The salmonellosis notification rate in Australia for 2002 was 40.3 cases per 100,000 population (Figure 1). This varies from 24.8 cases per 100,000 population in Victoria to 166.7 cases per 100,000 population in the Northern Territory (Anon, 2003). Children less than five years of age have by far the highest notification rate, with a rate of 210.6 cases per 100,000 population reported for 2002 (Yohannes *et al*., 2004). 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 seek medical care and be tested.

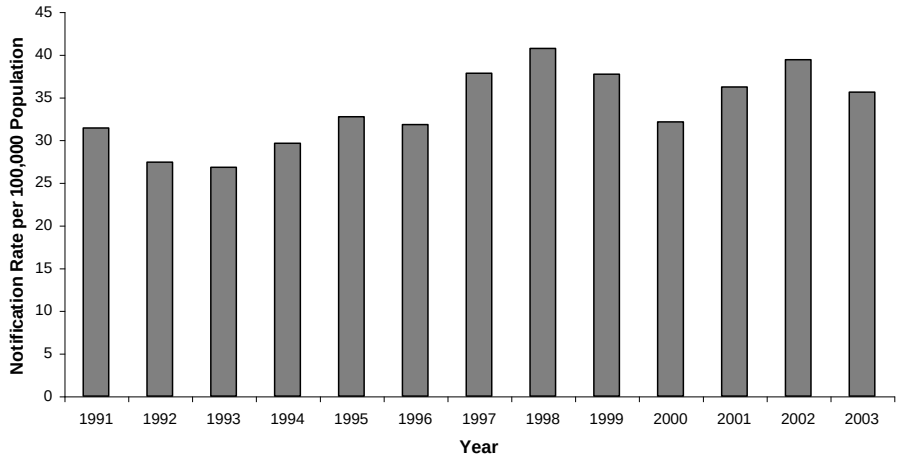

Figure 2.1 Salmonellosis notification rates in Australia by year (1991-2003; National Notifiable Diseases Surveillance System).

Of the total number of *Salmonella* serovars reported to Australian health authorities during 2002, *S*. Typhimurium 135 was the most commonly reported (Table 2.3). Distribution of *Salmonella* serovars varies geographically, with the most commonly reported serovars in Queensland, Tasmania and the Northern Territory being *S*. Virchow (10%), *S*. Mississippi (48%) and *S*. Ball (15%) respectively. Of the other States and Territories, *S*. Typhimurium was the most commonly reported serovar, representing 34% of cases in the Australian Capital Territory, 28% in New South Wales, 60% in South Australia, 66% in Victoria and 15% in Western Australia. Salmonellosis notifications in Australia fluctuate seasonally, from a low in August-September to a peak in January-March, with 36% of salmonellosis cases notified during this period (Yohannes *et al.*, 2004).

Table 2.3 Principal isolates in Australia, 2002 (Yohannes *et al.*, 2004)

	State or Territory									
	ACT	**NSW**	**NT**	**Qld**	**SA**	**Tas**	**Vic**	**WA**	**Aust**	**Total**
Organism										**%**
S. Typhimurium 135	11	238	8	117	14	18	178	91	675	8.8
S. Typhimurium 9	16	268	0	77	24	12	151	44	592	7.7
S. Typhimurium 170	5	161	0	135	1	1	152	3	458	5.9
S. Saintpaul	0	37	20	225	11	2	44	44	383	5
S. Virchow 8	0	21	0	268	0	0	11	2	302	3.9
S. Birkenhead	0	95	3	134	4	0	8	1	245	3.2
S. Typhimurium 126	1	62	2	28	39	4	61	8	205	2.7
S. Chester	1	29	16	82	11	2	5	32	178	2.3

Organism	State or Territory									
	ACT	NSW	NT	Qld	SA	Tas	Vic	WA	Aust	Total %
S. Hvittingfoss	1	17	6	110	3	1	13	2	153	2
S. Muenchen	0	20	12	55	9	3	9	24	132	1.7
Other	60	1136	248	1354	405	117	588	470	4378	56.8
Total	**95**	**2084**	**315**	**2585**	**521**	**160**	**1220**	**721**	**7701**	**100**

It has been estimated that in the United States (Mead, 1999) and England and Wales (Adak *et al*., 2002), 95% and 91.6% respectively of salmonellosis cases are food-borne. Other sources of infection may be via contaminated water, person-to-person transmission and direct contact with infected animals.

Based on results from national and international epidemiological data (primarily outbreak investigations) a wide range of foods have been implicated in human salmonellosis (Table 2.4). It is clear from Tables 2.4 and 2.5 that foods of animal origin (*e.g.* meat, eggs, dairy) are important sources of human salmonellosis.

Table 2.4 Major food-borne outbreaks of human salmonellosis (from D'Aoust, 1994)

Year	Country(ies)	Vehicle	Serovar	Number	
				Cases[a]	Deaths
1973	Canada, US	Chocolate	*S*. Eastbourne	217	0
1973	Trinidad	Milk powder	*S*. Derby	3,000[b]	NS
1974	United States	Potato salad	*S*. Newport	3,400[b]	0
1976	Spain	Egg salad	*S*. Typhimurium	702	6
1976	Australia	Raw milk	*S*. Typhimurium PT9	>500	NS
1977	Sweden	Mustard dressing	*S*. Enteritidis PT4	2,865	0
1981	Netherlands	Salad base	*S*. Indiana	600[b]	0
1981	Scotland	Raw milk	*S*. Typhimurium PT204	654	2
1984	Canada	Cheddar cheese	*S*. Typhimurium PT10	2,700	0
1984	France, England	Liver pâté	*S*. Goldcoast	756	0
1984	International	Aspic glaze	*S*. Enteritidis PT4	766	2
1985	United States	Pasteurised milk	*S*. Typhimurium	16,284	7
1987	China	Egg drink	*S*. Typhimurium	1,113	NS
1987	Norway	Chocolate	*S*. Typhimurium	361	0
1988	Japan	Cuttlefish	*S*. Champaign	330	0
1988	Japan	Cooked eggs	*Salmonella* spp.	10,476	NS
1991	US, Canada	Cantaloupe	*S*. Poona	>400	NS
1991	Germany	Fruit soup	*S*. Enteritidis	600	NS
1993	France	Mayonnaise	*S*. Enteritidis	751	0
1993	Germany	Paprika chips	*S*. Saintpaul, *S*. Javiana, *S*. Rubislaw	>670	0
1994	United States	Ice cream	*S*. Enteritidis	>645	0
1994	Finland, Sweden	Alfalfa sprouts	*S*. Bovismorbificans	492	0

[a] Confirmed cases unless stated otherwise.
[b] Estimated number of cases.
[c] Jay *et al*., 2003. NS = not specified.

Following notifications of salmonellosis to Australian health authorities, over 50 epidemiological investigations are initiated each year in an attempt to identify a common source of infection (Anon 2003). It is often difficult, however, to confirm a single food commodity as a source due to the difficulty of investigating commonly consumed foods, conducting traceback, and lack of systematically collected microbiological data from foods.

In a review of reported food-borne disease outbreaks in Australia during 1995 – 2000, meats, in particular poultry meat, were associated with 33% of identified salmonellosis outbreaks (Dalton *et al*., 2004; Table 2.5). A large outbreak (consisting of 502 cases) of *S.* Typhimurium 135a occurred in 1999 and was associated with consumption of unpasteurised commercial orange juice (Roche *et al*., 2001). In 2001 a community-wide outbreak of *S.* Typhimurium 126 occurred in South Australia (Ashbolt *et al*., 2002). A subsequent case-control study associated illness with the consumption of chicken meat. This link was corroborated with microbiological testing of raw poultry, and the likely source of contaminated products was traced to a single poultry processing facility.

Table 2.5 Salmonellosis outbreaks in Australia, 1995-2000 (from Dalton *et al*., 2004)

Vehicle	Outbreaks		Cases	
	n	%	n	%
Meats	25	33	658	17
Chicken	10		335	
Beef	4		67	
Pork	2		37	
Processed meats – consumed cold	4		61	
Other meats*	5		158	
Eggs	8	11	701	17
Sandwiches	7	9	1,205	29
Desserts	6	8	254	6
Fruit	2	3	60	1
Seafood	2	3	14	<1
Dairy	1	1	26	<1
Fish	1	1	26	<1
Fruit juice	1	1	502	12
Salads	1	1	21	<1
Vegetables	1	1	54	1
Miscellaneous	18	24	573	14
Unknown	2	3	43	1
Total	**75**	**100**	**4,123**	**100**

* Includes meats in above categories that may be mixed together and meats not in above categories, or where type of meat was not known.

5.14.5 Occurrence of Salmonella *in food*

The primary reservoir of *Salmonella* is the intestinal tract of warm and cold-blooded vertebrates. Infected animals shed large numbers in their faeces, and this leads to contamination of the surrounding environment including soil, pasture, streams and lakes. *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* (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).

Table 2.6 summarises reported isolation rates of *Salmonella* from a variety of dairy products. It is difficult to directly compare results between different commodities due to variations in sample size, stage of production sampled and methodology used. Pasteurisation effectively inactivates *Salmonella* spp., however contamination of milk has occurred due to improper pasteurisation and/or post-processing contamination (Jay *et al*., 2003).

Table 2.6 Reported prevalence of salmonellae in dairy products.

Food	Country	Samples	% positive	Reference
Raw milk	Switzerland	456	0	(Bachmann and Spahr, 1995)
Raw goat's milk	Switzerland	344	0	(Muehlherr *et al*., 2003)
Raw ewe's milk	Switzerland	63	0	(Muehlherr *et al*., 2003)
Raw milk (bulk tank)	USA	131	6.1	(Jayarao and Henning, 2001)
Raw goat's milk	UK	100	0	(Little and De Louvois, 1999)
Raw ewe's milk	UK	26	0	(Little and De Louvois 1999)
Raw milk (bulk tank)	US	268	2.2	(Murinda *et al*., 2002)
Raw bovine milk	France	69	2.9	(Desmasures *et al*., 1997)

5.14.6 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.

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). 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 of the intestinal mucosal surface and general enteric symptoms and inflammation. For non-typhoidal *Salmonella*, infection is generally limited to a localised intestinal event.

5.14.7 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.C.W, 1951a; McCullough and Eisele.C.W, 1951b; McCullough and Eisele.C.W, 1951c; McCullough and Eisele.C.W, 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 FAO/WHO 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).

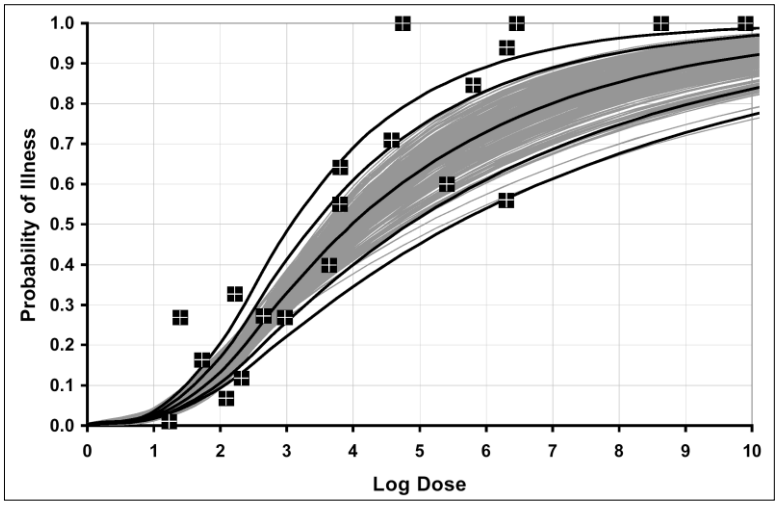

Figure 2.2 Uncertainty bounds for dose-response curves compared with expected value for the outbreak data (FAO/WHO, 2002).

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.

Table 2.7 Beta-Poisson dose-response parameters that generate the approximate bounds shown in Figure 2.2 (FAO/WHO, 2002).

	Alpha	Beta
Expected Value	0.1324	51.45
Lower Bound	0.0763	38.49
2.5^{th} Percentile	0.0940	43.75
97.5^{th} Percentile	0.1817	56.39
Upper Bound	0.2274	57.96

5.14.8 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 immunucompromised (organ transplant patients, cancer patients, AIDS patients) (Gerba *et al.*, 1996).

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5.15 *Shigella* spp.

Shigella is a genus of the Enterobacteriaceae family. The *Shigella* genus has four species, *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. *Shigella* spp. are Gram-negative, non-spore forming, rod shaped, non-motile, facultatively anaerobic organisms. All members of the species are able to cause bacillary dysentery. *Shigella* spp. share many biochemical and serological features with the genus of *Escherichia coli*.

5.15.1 Growth characteristics

Available information indicates *Shigella* species can survive and grow in a wide range of food including boiled rice, lentil soup, milk, cooked beef, cooked fish, raw cucumber, mashed potato, cheese, shredded lettuce, tofu, butter and margarine, lemon juice and wine (Lightfoot, 2003). Table 1 summarises the prevailing growth conditions of *Shigella* species. Under favourable conditions, the growth of *Shigella* in food is rapid. For example, *S. sonnei* can double its cells number in less than 1.5 hour when it is incubated with diced tofu at 32^{o}C (Lee et al., 1991).

Table 5 Growth conditions for *Shigella* species

	Minimum	**Optimum**	**Maximum**
Temperature (^{o}C)	6-7	Room temperature	45-47
pH	4.5	6-8	9.3
Water activity (a_w)	0.97	Not available	Not available

From (Lightfoot, 2003) and (International Commission on Microbiological Specification for Foods, 1996).

At temperatures around 65^{o}C, *Shigella* species are inactivated rapidly. At pH less than 4.0, shigellae die rapidly (International Commission on Microbiological Specification for Foods, 1996).

5.15.2 Pathology of illness

Shigellosis refers illness in humans caused by *Shigella*. It occurs principally as a disease to humans and rarely occurs in animals. Shigellosis is associated with symptoms of abdominal pain, cramps, diarrhoea, fever, vomiting, mucosal ulceration, rectal bleeding, and drastic dehydration. The onset time is between 12 to 50 hours. The fatality rate is high and for some of the virulent strains, the rate is close to 10-15%. Sequelae as a result of shigellosis include Reiter's disease, reactive arthritis and haemolytic ureamic syndrome (Lightfoot, 2003).

5.15.3 Mode of transmission

Shigellosis is prevalent in areas of inadequate sanitation and poor living conditions with overcrowding. Transmission of *Shigella* to humans is via the faecal-oral route. Faecal contaminated water and unhygienically prepared/handled food are the most common causes of transmission of *Shigella* organisms.

Contamination of food is generally the result of poor personal hygiene of food handlers. In Australia, contamination of food by an infected food handler resulted in a number of cases of *Shigella* infection in outbreaks of *S. flexneri* in Alice Springs in 1977 and in Wangaratta in 1978 (Lightfoot, 2003). Transmission of *Shigella* contamination can occur via contaminated flies. Inadequate treated contaminated water used of drinking or food preparation, inadequately disinfected swimming pool or recreational water contaminated by animal or human faeces are potential sources of transmission of shigellae.

Peron to person transmission is common, either via direct or indirect contact. There have been reports of *Shigella* infection in laboratory settings (Collins et al., 1999).

5.15.4 Incidence of illness

In 2003, 443 cases shigellosis of reported in Australia, representing 2.2 cases per 100,000 of the population. The majority of shigellosis infections probably were acquired by person-to-person transmission or overseas. Northern Territory recorded highest rate of notification, 67 cases per 100,000. Rates of shigellosis were considerably higher in the indigenous communities. The notified rate of shigellosis was 300 cases per 100,000 population in indigenous children aged 0–4 years of age in Western Australia (The OzFoodNet Working Group, 2004).

According to information of the Food-borne Outbreak Response and Surveillance Unit of the Centres for Disease Control and Prevention[62], during the period of 1990 and 1995, 39 outbreaks of food-borne illness caused by *Shigella* species were reported in the US. Average case number per outbreak was 53, ranging from the lowest number of 3 cases to the highest number of 400 cases in a single outbreak. Although the vehicle of transmission could not be identified for almost half of the outbreaks, food implicated as vehicles of transmission included vegetable or vegetable based food, turkey based noodles, raw oysters, wild rice salad, spaghetti salad, chicken meat salad, smoked salmon, cheese/lettuce/tomato mix and others. In reviewing *Shigella* outbreak investigations in the past 30 years, Lightfoot (Lightfoot, 2003) listed the following as main contributing factors to *Shigella* food-borne outbreaks, which are:

- poor hygiene in food preparation;
- food handlers who have developed shigellosis;
- use of uncooked food ingredient which may have been contaminated by *Shigella*;
- ready to eat food; and
- mass food service.

A typical example is the explosive outbreak of shigellosis caused by *S. sonnei* that affected an estimated 3175 people attending a five-day outdoor musical festival in August 1988 in the State of Michigan of the US. The vehicle of transmission was an uncooked tofu salad that was made with several hundred ponds of uncooked tofu and vegetables involving some 50 people in its preparation (Lee et al., 1991).

5.15.5 Occurrence in foods

Salads (lettuce, potato, tuna, shrimp, macaroni and chicken), raw vegetables, raw oysters, apple cider, milk and dairy products, and poultry have been identified as food sources that have been contaminated by *Shigella* organisms.

Fresh pasteurised milk cheese was implicated in an outbreak of shigellosis caused by *S. sonnei* in the winter of 1995-1996 in southwest Spain. More than 200 people from eight townships were affected during the outbreak. Investigation of the outbreak suggested that an infected food handler at the cheese factory might have been the source of contamination and the cheese processing method might have allowed cross contamination to occur post pasteurisation (Garcia-Fulgueiras et al., 2001).

[62] http://www2.cdc.gov/ncidod/food-borne/OutbreaksReport.asp Accessed 5 May 2005

5.15.6 Virulence and infectivity of Shigella *species*

Virulent *Shigella* organisms, once ingested and passed through the digestive system, attach to and penetrate the colonic epithelial cells of the intestinal mucosa. The organism then multiplies intracellularly, resulting in tissue destruction. Invasive strains of *S. dysenteriae* 1 produce high levels of cytotoxin referred as Shiga toxin. Shiga toxin has both enterotoxic and neurotoxic activity in addition to cytotoxiticity. Other serovars of *S. dysenteriae* and other *Shigella* spp. produce low levels of cytotoxic activities (Lightfoot, 2003).

5.15.7 Dose Response

Shigella spp. are considered relatively infectious, with an estimated infective dose as low as 10 cells (DuPont et al., 1989).

5.15.8 Immune status

Humans of all age and all health status are susceptible to *Shigella* infection. However, incidence of shigellosis is the highest among children of 1 to 4 years old (Clemens et al., 1999). The infants, the elderly and those suffering from immune deficiency, particularly people with AIDS and non-AIDS homosexual men are susceptible to the severest symptoms of shigellosis (CFSAN[63]).

5.15.9 Food Matrix

Shigella can survive in foods stored at –20^{o}C, at refrigerated temperature and at room temperature for long period of time. Their survival is greater at temperature of 25^{o}C or less. It has been shown that *Shigella* can survive in cheese for several weeks at room temperature (Nakamura, 1962) and in ground meat for at least 4 days at 6-8^{o}C (Smith, 1987). *S. flexneri* has been shown to be able to survive in prepared coleslaw, carrot and vegetable salad for at least 11 days and in crab salad for up to 20 days at 4^{o}C (Rafii et al., 1997).

Shigella species survive better in alkaline environment and there is a data to suggest that *S. sonnei* and *S. dysenteriae* can survive in acidic foods, such as grape juice, lemon juice and wine for 4 to 24 hours and in orange juice for six days and in carbonated beverages for a day (International Commission on Microbiological Specification for Foods, 1996).

S. flexneri and *S. sonnei* have been shown to be able to grow in less then 5.2% of sodium chloride and less than 700 mg/L of sodium nitrite (International Commission on Microbiological Specification for Foods, 1996).

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5.16 *Staphylococcus aureus*

The genus *Staphylococcus* is subdivided into 28 species and 8 subspecies. *S. aureus* is a non-motile, gram-positive, non-spore forming spherical bacterium. On microscopic examination, *S. aureus* appears in pairs, short chains, or bunched, grape-like clusters (Stewart, 2003).

S. aureus is ubiquitous and inhabits the mucous membranes and skin of most warm-blooded animals, including all food animals and humans. Up to 50% of humans may carry this organism in their nasal passages and throats and on their hair and skin (USFDA Centre for Food Safety and Applied Nutrition, 2004b).

S. aureus counts are often estimated by detecting coagulase-positive staphylococci, with further confirmatory tests required to specifically identify *S. aureus*. Nevertheless, the identification of coagulase-positive staphylococci or *S. aureus* is essentially an indicator test for the likelihood of enterotoxin production, as not all of these organisms have the ability to produce toxin (Stewart, 2003). In addition, some strains of enterotoxin-producing staphylococci do not possess the coagulase enzyme.

5.16.1 Growth characteristics

The temperature range for growth of *S. aureus* is 7-48°C with optimum growth occurring at 35-40^{o}C. The temperature range for toxin production is 10-48°C with the optimum temperature being from 40-45^{o}C. *S. aureus* grows over a wide a_w range (0.83-0.99) with an optimum a_w of >0.99. The pH range for growth is 4.0-10 and the pH range for toxin production is 4.5-9.6 (ICMSF, 1996). *S. aureus* is tolerable to salt up to 25% NaCl (a_w 0.85).

S. aureus grows under both aerobic and anaerobic conditions, however growth is better in the presence of oxygen. Toxins are also produced under both aerobic and anaerobic conditions with greatest toxin production in the presence of oxygen (Bergdoll, 1989). *S. aureus* is generally considered a poor competitor with other bacteria.

S. aureus is readily killed at cooking and pasteurisation temperatures, however heat resistance is increased in dry, high-fat and high-salt foods. In contrast, *S. aureus* enterotoxins are extremely resistant to heat. Heat resistance for enterotoxin B has been reported at D_{149}=100 min (a_w of 0.99) (ESR, 2001). Heat resistances for *S. aureus* vegetative cells have been reported at D_{60} = 0.43-8.0 min whereas a time/temperature equivalent for enterotoxin is 121^{o}C for 3-8 min (Baird-Parker, 1990; ICMSF, 1996). The enterotoxin is not affected by frozen storage.

Preservatives such as sorbate and benzoate are inhibitory to *S. aureus*, with their effectiveness increasing with a reduction in pH. Methyl and propyl parabens also have an effect on *S. aureus*, and high concentrations of carbon dioxide cause a substantial reduction in growth rates of *S. aureus* (Molin, 1985).

Most chemical sanitisers used routinely in food industry such as chlorine, other halogens and quaternary ammonium compounds destroy *S. aureus* on surfaces. However some strains, for example those that become established on poultry processing equipment, have increased resistance (Bolton *et al*., 1988).

5.16.2 Pathology of illness

Staphylococcal food-borne illness is caused by the ingestion of food that contains preformed toxins produced by *S. aureus*. Usually this occurs when *S. aureus* is introduced into a food that will support growth of the organism, and that food is stored under conditions allowing the organism to grow and produce sufficient quantities of enterotoxin (Ash, 1997).

Symptoms generally appear around 3 hours after ingestion but can occur in as little as 1 hour (range 1-6 hours) and are self-limiting (Stewart, 2003; Ash, 1997). Symptoms include nausea, vomiting, abdominal cramps of varying severity, and diarrhoea. Some individuals may not demonstrate all the symptoms associated with the illness. In severe cases, blood and mucus may be observed in stools and vomitus. Marked prostration, headaches and sweating accompany severe attacks and there may be fever or shock with subnormal temperatures and lowered blood pressure. Recovery is usually between 1-3 days requiring no medical treatment. Fatalities are rare, but are occasionally reported in young children and the elderly (Ash, 1997). All people are susceptible to staphylococcal food poisoning, however the intensity/severity may vary, depending of individual sensitivities.

S. aureus is also an opportunistic pathogen that causes infections via open wounds. *S. aureus* causes several types of infection including skin eruptions and inflammations (boils, acne, sties, etc.) and wounds. *S. aureus* can also cause respiratory infections or may become established in the gut causing enteritis.

S. aureus is an important bacterial cause of mastitis (an inflammatory disease of the mammary gland) in cows (Akineden et al., 2001). Mastitis in dairy cattle is characterised by changes in the udder tissue, clots and changes in milk quality, and is sometimes accompanied by heat and pain in the udder.

5.16.3 Mode of transmission

Staphylococcal food poisoning is caused by the consumption of food containing enterotoxins produced by certain stains of *S. aureus*. Despite the wide-spread association of *S. aureus* with animals, humans are the main reservoir for *S. aureus* involved in human disease (Jablonski and Bohach, 1997). Hand contact with ready-to-eat foods is an important means by which *S. aureus* may enter food supply by food handlers.

Foods that present the greatest risk of causing illness are those in which the normal flora has been destroyed (eg cooked meats) or inhibited (eg cured meats containing high salt content) (Stewart, 2003).

5.16.4 Incidence of illness

Food poisoning caused by *S. aureus* is one of the most common type of food-borne diseases world-wide. The incidence of staphylococcal food poisoning is often under-reported due largely to the self-limiting nature of illness, with most people recovering within 1-2 days without requiring medical attention. Foods commonly associated with staphylococcal food poisoning are meat and poultry, dairy products (particularly cheese and cream due to inappropriate handling as well as contaminated raw milk), salads, cream filled bakery products, and processed meat (especially ham, hot dogs, salami).

Improper storage/temperature abuse of food is greatest factor attributing to outbreaks (Homberg and Blake, 1984).

In July 2000, an extremely large outbreak of staphylococcal food poisoning occurred in Japan, with an estimate 13,420 people being affected (Asao et al., 2003). The source of the outbreak was traced to powdered low-fat milk produced at a single factory in Osaka and was used as an ingredient in a number of dairy products. Staphylococcal enterotoxin was detected in the implicated milk powder, however, viable *S. aureus* was not isolated. This suggests that staphylococci were able to produce enterotoxin in the milk prior to pasteurisation, and remained immunologically and biologically active despite being pasteurised three times at 130°C for 2 – 4 seconds.

Despite *S. aureus* not being a notifiable illness in Australia, in 2002, three outbreaks of food poisoning attributed to *S. aureus* were reported. In one outbreak, a meal of lamb, rice and potatoes was implicated, in which *Bacillus cereus* was also identified. Other outbreaks implicated rice served in a childcare centre and pizza as the causative agent (Anon 2003a; Ashbolt et al., 2002) . An outbreak was also reported in 2001 from consumption of BBQ chicken strongly suggesting an enterotoxin-producing bacterium as the causative agent, possibly *S. aureus* (Armstong *et al*., 2002). In 2003, *S. aureus* was also implicated in food-borne illness after the consumption of a rice, beef and black bean sauce meal (Anon, 2003b).

Mead *et al*. (1999) state that sporadic illness from *S. aureus* is not reportable in the US through either passive or active systems. The authors estimated 185,060 illnesses, 1753 hospitalisations and 2 deaths per year are attributed to *S. aureus* illness via contaminated food (Mead *et al*., 1999). Between 1975 and 1982, 36% of all reported *S. aureus* illness in the US was attributed to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and 1.4% attributed to milk products and seafoods. In 17.1% of cases, the food involved was unknown (Genigeorgis, 1989).

In Canada, the average number of cases of illness from *Staphylococcus* for the years 1975-1984 was 232 cases per year (Todd, 1992). Foods implicated included pork (ham), turkey, chicken, cheese, pasta, salads and sandwiches.

In France, *S. aureus* was attributed to 16 of 530 food-borne disease outbreaks recorded between 1999 and 2000 (Le Loir *et al*., 2003). Of these outbreaks, milk products and especially cheeses were responsible for 32% of cases, meats 22%, sausages and pies, 15%, fish and seafood 11%, eggs and egg products 11% and poultry 9.5% (Haeghebaert *et al*., 2002).

In the United Kingdom for the years 1969-81, 1-6% of all cases of bacterial food poisoning were attributed to *S. aureus*. From 1982-1990, 0.5-1% of all cases of bacterial food poisoning was attributed to staphylococcal food poisoning. For the years 1969-90 a study of 359 incidents of staphylococcal food poisoning was investigated (Table 4.4). Poultry and poultry products accounted for 22% of incidents, most attributed to cold cooked chicken and in nine incidents turkey was the food vehicle (Bertolatti *et al*., 1996; Wieneke *et al*., 1993).

Table 4.4: Foods implicated in staphylococcal food poisoning in the UK from 1969-1990 (Wieneke *et al*., 1993)

Type of Food	Number of incidents	
Ham	65	53%
Meat pies	25	
Corned beef	20	
Tongue	16	
Jars of meat, chicken or fish paste	12	
Other meats and meat containing products	43	
Meat dishes	9	
Poultry (chicken, turkey, duck)	64	22%
Poultry dishes	15	
Fish and shellfish	24	7%
Milk and desserts containing milk or cream	23	8%
Cheese	5	
Boiled eggs and egg dishes	13	3.5%
Other foods	20	5.5%
Not known	5	1%
Total	**359**	

5.16.5 Occurrence in foods

Animals carry *S. aureus* on various parts of their bodies. Cows udders and teats, and the tonsils and skin of pigs, chickens and turkeys are also known sources. Occurrence of staphylococci is common in raw milk. *S. aureus* in milk is related to the health status of the herd in respect to mastitis, and organisms numbers can range from <10 to several thousands per ml of milk with occasional counts of 10^5 cfu/ml (Asperger and Zangerl, 2002).

The prevalence of coagulase-positive staphylococci (which can include *S. aureus*, S. *intermedius* and some S. *hyicus*) in Australian beef and sheep carcasses and boneless beef and sheep surveyed in 1998 were 24.3% (beef carcasses), 24.1% (sheep carcasses), 17.5% (boneless beef) and 38.6% (boneless sheep) respectively (Phillips *et al*, a, b 2001).

5.16.6 Virulence and infectivity

S. aureus forms a wide range of substances associated with infectivity and illness, including the heat stable enterotoxins that cause food poisoning (Ash, 1997). Eleven antigenic types of staphylococcal enterotoxins are currently recognised, with types A and D being most commonly involved in food poisoning outbreaks.

To date, staphylococcal enterotoxins A, B, C1, C2, C3, D, E, G, H, I and J toxins have been identified (Balaban and Rasooly, 2000). These enterotoxins are single-chain proteins comprising a polypeptide chain containing relatively large amounts of lysine, tyrosine and aspartic and glutamic acids and characterised by containing only two residues of half cystine and one or two residues of tryptophan. Most of them possess a cystine loop required for proper conformation and which is probably involved in the emetic activity. They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They also resist chymotrypsine, rennin and papain (Bergdoll, 1989).

The production of enterotoxins is dependent on de novo synthesis within the cell. The quantity of toxin produced is variable and can be categorised by type of toxin produced. Although weakly antigenic, enterotoxin antibodies have been produced in a variety of animal hosts.

The mode of action of the toxin causing illness is not fully understood, although it is thought that the vomiting response to ingestion of preformed toxin is the result of the stimulation of local neuroreceptors in the intestinal tract which transmit the stimuli to the vomiting centre of the brain via the vagus and other parts of the sympathetic nervous system (ICMSF, 1996).

A number of studies have identified toxin genes present in *S. aureus* isolates from the milk of cows with mastitis (Akineden et al., 2001; Cenci-Goga et al., 2003; Lim et al., 2004; Zschöck et al., 2004; Loncarevic et al., 2005). The rate of enterotoxigenic *S. aureus* isolates from dairy cattle is highly variable and demonstrates the diversity of *S. aureus* strains (Cenci-Goga et al., 2003).

5.16.7 Dose response

The amount of enterotoxin that must be ingested to cause illness is not known exactly, but it is generally believed to be in the range 0.1-1.0 µg/kg (ICMSF, 1996). Toxin levels within this range are typically reached when *S. aureus* populations exceed 100,000/g (Ash, 1997).

5.16.8 Immune status

All people are believed to be susceptible to staphylococcal intoxication, but the severity of symptoms may vary depending on the amount of food ingested and the susceptibility of the individual to the toxin.

5.16.9 Food Matrix

The range of conditions that allow growth of staphylococci and the production of toxin vary with food type. The amount of starch and protein present in the food may enhance toxin production (Frazier and Westhoff, 1988).

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5.17 *Streptococcus* spp.

Streptococci are gram-positive, spherical or ovoid, non-motile bacteria. They grow aerobically or micro-aerophilically. Anaerobic species have no significance in food microbiology. The term 'streptococcus' was first used by Billroth (1874) to describe the chain-forming, coccoid bacteria that had been observed in wounds and discharges of animals (ICMSF 1996).

The classification of the genus *Streptococcus* has long been in a state of flux (Jones 1978), however current information groups them into pyogenic streptococci and enterococci. Pyogenic streptococci include *S. pyogenes* and *S. agalactiae.* Enterococci include *E. faecalis* and *E. faecium.*

The genus is sorted into Groups A, B, C, D, F and G on the basis of antigenic, haemolytic and physiological characteristics. Streptococci from Groups A and D can be transmitted to humans via food (Bad Bug Book 1992). *S. zooepidemicus* (Group C) has also been implicated in several episodes of human illness, including death, in the UK following consumption of raw milk (Barrett 1986).

Streptococcus agalactiae is a major cause of bovine mastitis (ICMSF). It is a highly contagious obligate parasite of the mammary gland (Martinez et al., 2000).

5.17.1 Growth characteristics

Group A streptococci grow poorly in raw milk, but there is some evidence that pyogenic streptococci may multiply in raw meat held at ambient temperature (Fraser *et al.* 1977).

Limits for growth of *S. pyogenes* (ICMSF 1996)

	Minimum	Optimum	Maximum
Temperature (ºC)	10 - 15	37	>40, <45
pH	4.8 - 5.3	7	<9.3
NaCl (%)	-	-	>4, <6.5

Experiments conducted by Obiger (1976) found that *S. pyogenes* would not survive exposure to 66ºC for 20-40s resulting in a calculated D-value at 66ºC of 0.1-0.2min.

Heat resistance figures reported by Stumbo (1973) included a D-value at 65.6ºC of 0.2-2.0 and a z value of 4.4-6.7ºC. Based on these figures, ICMSF (1996) conclude that pasteurisation at 62ºC for 30 minutes and 70ºC for 30s would ensure only a 1.6-2.3 decimal reduction of *S. pyogenes*. However, using the D-value at 66ºC of 0.2 as per Obiger (1976), pasteurisation would result in a 20 decimal reduction of *S. pyogenes* in milk.

5.17.2 Pathology of illness

The symptoms of group A streptococcal infection include sore and red throat, pain on swallowing, tonsillitis, high fever, headache, nausea, vomiting, malaise, rhinorrhea. A rash may occur within the first few days. Group A streptococci may also cause acute rheumatoid fever following infection of the upper respiratory tract, and acute glomerulonephritis after skin infection (ICMSF 1996).

Although rare, complications may occur when the bacteria enter the blood, muscles or lungs. These infections are termed "invasive group A streptococcal (GAS) disease". Two of the least common but most severe forms of GAS disease are necrotising fasciitis and Streptococcal Toxic Shock Syndrome (STSS). Necrotising fasciitis destroys the muscles, fat and skin tissue. Streptococcal Toxic Shock Syndrome causes blood pressure to drop rapidly and organs, such as the kidneys, liver and lungs, to fail. About 20% of patients with necrotising fasciitis and more than 50% with STSS die.

Group D streptococci infections may result in a clinical syndrome similar to staphylococcal intoxication. The symptoms commence within 2-36 hours of infection and include diarrhoea, abdominal cramps, nausea, vomiting, fever, chills and dizziness (Bad Bug Book 1992).

5.17.3 Mode of transmission

Humans are usually the source of contamination of pyogenic streptococcal infections. Transmission occurs from infected hosts to foods. The bacteria are generally spread via direct contact with mucus from the nose or throat of infected persons, or through contact with infected wounds or sores on the skin. Group A streptococci may be carried in the throat on the skin of people with no symptoms of illness.

5.17.4 Incidence of illness

Outbreaks of septic sort throat and scarlet fever were numerous prior to the introduction of milk pasteurisation. Most current outbreaks have involved foods such as salads, with the source of infection being an infected food handler.

An outbreak of food borne illness due to *S. zooepidemicus* (Group C) involving at least 11 cases occurred in the UK in 1984. Seven persons died during the outbreak. Unpasteurised milk from a dairy herd that had experienced intermittent mastitis was implicated as the source of infection (Edwards *et al.* 1988).

Outbreaks of Group D streptococcal infections are not common and have usually been the result of unsanitary preparation, storing or handling of food (Bad Bug Book 1992).

Sixteen cases of invasive group C streptococcal infection were identified in northern Mexico between July 25 and September 9 1983. The organism was isolated from the blood of 15 patients and from the pericardial fluid of one patient. A homemade white cheese produced from raw cows' milk at a small family dairy in northern Mexico was indicated as the food source of the infection, with samples testing positive for streptococci. The cows at the dairy were found to have mammary infections due to *S. zooepidemicus* (MMWR October 07, 1983).

In 1984, there was one outbreak of *S. zooepidemicus* associated with the consumption of raw milk in England. Twelve people were admitted to hospital with meningitis or endocarditis. Eight of the 12 died, although the infection was not necessarily the primary cause of death. Ten of the patients were aged over 70 years, and one was a one-day-old infant. Cows at a local dairy that had supplied the mild were subsequently found to be excreting *S. zooepidemicus* in their milk (Barrett 1986).

5.17.5 Occurrence in foods

Food associated with streptococcus Group A food-borne illness include milk, ice cream, eggs, steamed lobster, ground ham, potato salad, egg salad, custard, rice pudding and shrimp salad. Foodstuffs were allowed to stand at room temperature for several hours between preparation and consumption in almost all cases. Poor hygiene, ill food handlers or the use of unpasteurised milk were the main routes for streptococcus Group A into food (Bad Bug Book 1992).

Food sources for streptococcus Group D food-borne illness include sausage, evaporated milk, cheese, meat croquettes, meat pie, pudding, raw milk and pasteurised milk. Underprocessing and/or poor food preparation is the usual mechanisms for entrance into the food chain (Bad Bug Book 1992).

200 samples of raw milk from Zulia State, Venezuela were examined, with 19 samples testing positive for the presence of *Streptococcus* spp. Seventeen samples were positive for Enterococcus (Faria-Reyes *et al.* 2002).

Results from the microbiological testing of 77,172 milk samples submitted to the Wisconsin Veterinary Diagnostic laboratory from January 1994 until June 2001 were analysed. Milk samples obtained included cases of clinical and subclinical mastitis as well as samples obtained from mastitis surveillance programmes. The proportion of samples from which Streptococcus was isolated decreased from 8.1% in 1994 to 3.0% in 2001 (Makovec and Ruegg 2003)

Raw bulk tank milk samples from 48 dairy farms in New York State were tested over a five-month testing period. Streptococci accounted for 69% of the total bacterial counts. The most commonly identified streptococcal species were *S. uberis* (found in 81% of the bulk milk samples), *Aerococcus viridans* (found in 50% of the bulk milk samples) and *S. agalactiae* (found in 31% of the bulk milk samples) (Zadoks *et al.* 2004).

5.17.6 Virulence and infectivity

Pyogenic streptococci possess specific virulence proteins which enable the organism to adhere to epithelial cells and protect the streptococci from phagocytosis (ICMSF, 1996).

5.17.7 Dose response

The infectious dose for streptococcus Group A likely to be quite low, with less than 1,000 organisms required for infection (Bad Bug Book 1992). In contrast, it is estimated that food-borne Group D streptococcus has a high infectious dose of greater than 10^7 organisms.

5.17.8 Host factors

All individuals in a population are equally susceptible to streptococcal illness. (Bad Bug Book 1992). People with chronic illnesses such as cancer, diabetes and kidney dialysis and those using medications such as steroid have a higher risk of getting invasive GAS disease (CDC webpage).

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5.18 *Yersinia enterocolitica*

Yersinia is a facultative anaerobic organism, a member of the *Enterobacteriaceae* family (Farmer, 1995). Among 11 named species in the genus *Yersinia*, 3 are considered important human pathogens. *Y. pestis* is the cause of the plague. *Y. pseudotuberculosis* and *Y. enterocolitica* are enteropathogenic strains. *Y. pestis* and *Y. pseudotuberculosis* do not frequently infect humans. *Y. pestis* mainly infects rats and other rodents which are the prime reservoir for the bacteria. Fleas are the prime vectors carrying the bacteria from one species to another. They bite rodents infected with *Y. pestis*, then they bite people and so transmit the disease to them. Transmission of the plague to people can also occur from eating infected animals such as squirrels. Once someone has the plague, they can transmit it to another person via aerosol droplets. *Y. pseudotuberculosis* is primarily a zoonotic disease of wild and domesticated birds and mammals, with humans as incidental hosts. *Y. enterocolitica* is more commonly found in human clinical specimens.

Y. enterocolitica are Gram-negative, small rods with dimension in the range of 0.5-0.81µm x 1-3 µm. Young cells of *Y. enterocolitica* are oval or coccoid shape. The organism produces peritrichous flagella and is actively motile when it is grown at 25^{o}C but not at 35^{o}C(Forsythe, 2000). *Y. enterocolitica* is often isolated from faeces but also from wounds, sputum and mesenteric lymph nodes of patients and sick animals.

Y. enterocolitica are found in cows, pigs, cats, dogs, and birds, and in water, soil and a variety of food. However, they are not part of the normal human flora (CFSAN[64]).

5.18.1 Growth characteristics

Optimal growth temperature for *Y. enterocolitica* is at approximately 30^{o}C, but the organism can grow at refrigerated temperatures and ability of growth at –5^{o}C has been reported (Barton et al., 2003). *Y. enterocolitica* is able to grow in the presence or absence of oxygen, but growth in the absence of oxygen is retarded at refrigerated temperatures.

Table 1 Growth conditions for *Y. enterocolitica*

	Minimum	**Optimum**	**Maximum**
Temperature (^{o}C)	-5	22-28	44
pH	4.6	7- 8	10.0
Water activity (a_w)	0.945	Data not available	Data not available

It has been reported that *Y. enterocolitica* can survive in spring water stored at 4^{o}C for up to 64 weeks. Survival of *Y. enterocolitica* is enhanced at low temperatures when the environment pH is below the minimum allowing for its growth.

The D values for *Y. enterocolitica* are approximately 2 min at 55^{o}C, 0.5 min at 60^{o}C and 2 seconds at 65^{o}C (Forsythe, 2000). The D value for *Y. enterocolitica* in milk at 62.8^{o}C is 0.24 – 0.96 min (Lovett et al., 1982). As such, cells of *Y. enterocolitica* in milk are readily inactivated by pasteurisation.

[64] Yersinia enterocolitica, http://www.cfsan.fda.gov/~mow/chap5.html Accessed 26 April 2005.

5.18.2 Pathology of illness

Yersiniosis refers to the illness caused by *Y. enterocolitica*. Yersiniosis is characterised by gastroenteritis with diarrhoea and/or vomiting, fever and abdominal pain. Many patients seek medical attention for persistent fever, night sweats, or secondary features of the disease. Self-limiting enterocolitis is the most usual syndrome and often seen in young children (Barton et al., 2003). Mesenteric lymphadenitis caused by *Y. enterocolitica* shows symptoms similar to appendicitis, can be seen in older children or adolescents. Long-term sequelae as a result of infection by *Y. enterocolitica* include reactive arthritis, erythema nodosum, uveitis and others.

Incubation period for enterocolitis is 24-36 hours or longer and the illness lasts usually one to three days. Duration of excretion of the organisms in the stool of infected patients ranges from 14-97 days (Cover et al., 1989).

5.18.3 Mode of transmission

Cells of pathogenic *Y. enterocolitica* ingested and travelled through the gastrointestinal tract can bind to the epithelial cells of the ileum and penetrate the intestinal mucosa and colonise the Peyer's Patches. Cells multiplied may spread to the mesenteric lymph nodes via the lymphatics and in rare situations may spread to the bloodstream, liver and spleen (Barton et al., 2003).

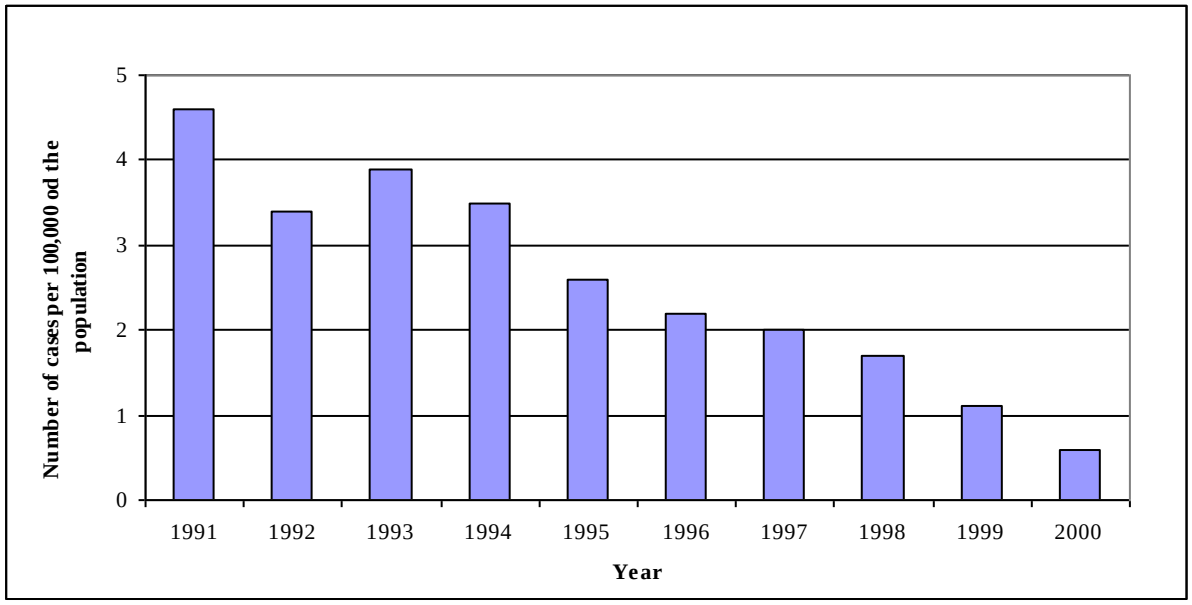
Pigs are the primary source of human infections of yersiniosis. *Y. enterocolitica* is carried in health pigs worldwide. Tonsils and oral cavities of pigs are generally heavily contaminated. Consumption and handling of raw pork meat are a primary source of human infection by *Y. enterocolitica* (Barton et al., 2003).

5.18.4 Incidence of illness

Since its peak in early 1990s, there has been a continuing decline in the number of yersiniosis in Australia, as reported by the National Notifiable Disease Surveillance Systems (Figure 1). As such, yersiniosis is no longer a notifiable disease since 2001 (Lin et al., 2002). The OzFoodNet recorded 117 cases of yersiniosis in 2002, representing 1.7 per 100,000 of the population.

Most cases of food-borne yersiniosis are sporadic but some outbreaks have been reported. In September and October of 1976, an outbreak of illness due to consumption of *Y. enterocolitica* contaminated chocolate milk in the US affected 218 people including 36 hospitalisation and 16 appendectomies. Investigations found that pasteurised milk was contaminated during the mixing by hand of chocolate syrup (Black et al., 1978). In October 1995, another outbreak in the US reported 10 cases of yersiniosis associated with consumption of pasteurised milk with 3 hospitalisations and 1 appendectomy. The research found that the pasteurised milk was possibly contaminated post-pasteurisation by unchlorinated rinsing water and dairy pigs were identified as the most likely source of *Y. enterocolitica* (Ackers et al., 2000). An investigation of an Australia outbreak of yersiniosis associated with consumption of pasteurised milk in 1987-1988 reported 11 cases of *Y. enterocolitica* enteritis among which three were presented as appendicitis (Butt et al., 1991). Other than milk, tofu (Tacket et al., 1985), pig meat products and bean sprouts have been implicated as vehicles of outbreaks of yersiniosis.

Figure 1: Notified cases of yersiniosis in Australia



The above data does not include those of NSW and ACT where yersiniosis was reported as either a "food-borne disease" or "gastroenteritis in an institution". (To be qualified as some of the years do include ACT reports but none of the figure includes NSW reports.)

Yersiniosis caused by *Y. enterocolitica* appears to be a particular health problem in northern Europe, Scandinavia, parts of North America, Japan and New Zealand (Barton et al., 2003). The number of reported yersiniosis is high in New Zealand where the incidence of yersiniosis is 15.1 per 100,000 in 1998 and 13.9 cases in 1999 (ESR 2001[65]). In Finland, the reported varied from 11.7 to 17.5 per 100,000.

5.18.5 Occurrence in foods

Y. enterocolitica is ubiquitous; frequently found in soil, water, animals, and can grow in a variety of foods even at refrigeration temperatures. They have been found in many food sources like raw milk and cream, meat and meat products, oysters, vegetables, fish, and poultry (Barton et al., 2003). They have also been isolated from well water, streams, lakes, and soil.

5.18.6 Virulence and infectivity of Y. enterocolitica

There are 5 biotypes (described as biotype 1A, 1B, 2, 3, 4 and 5) and at least 60 O-antigen[66] serological groups. Human infections are mainly caused by a small number of pathogenic bioserotypes that carry a plasmid encoding a number of virulence factors (Barton et al., 2003). Bioserotype 4,O:3 is the most common pathogenic *Y. enterocolitica* found in humans worldwide. In addition, bioserotype 2,O:9, 2,O:5,27 and 3,O:5,27 are important human pathogens reported in Northern Europe, and Bioserotype 1B,O:8; 1B,O:13a,13b, 1B,O:20, 1B,O:21 are important pathogens in North America. The North American biotypes are more virulent than those of the Northern Europe (Barton et al., 2003). The genes encoding for invasion of mammalian cells are located on the chromosome, and other virulence factors are associated with a 70-kb virulence plasmid in pathogenic bioserotypes (Forsythe, 2000).

[65] ESR (2001) Fact sheet of *Yersinia enterocolitica*.
[66] Refers to lipopolysaccharide-protein somatic antigens of the microorganism.

The North American biotype 1B strains carry a high pathogenicity island (HPI) on their chromosome, which enhances their virulence (Barton et al., 2003). In Australia, biotype O:3, O:6,30 have been reported in outbreak investigations (Butt et al., 1991).

5.18.7 Dose Response

Although the minimum infectious dose of *Y. enterocolitica* is not known (Forsythe, 2000), there is estimation that the infective dose is around 10^6 (Health Canada, 2001[67]) to 10^7 cells (Granum et al., 1995).

5.18.8 Immune status

Population most susceptible to yersiniosis and the subsequent complications are the very young, the debilitated, the very old and persons undergoing immunosuppressive therapy (CFSAN[68]). In 2000, notification rate of yersiniosis in Australia was 3.6 per 100,000 for the 0-4 years old sub-population (male) and 1.5 per 100,000 for 0-4 years old (female) sub-population, and the remaining populations was at 0 to 1 per 100,000 (Lin et al., 2002).

5.18.9 Food Matrix

Survival and growth of *Y. enterocolitica* in food is influenced by pH, water activity, salt content, temperature of storage, oxygen availability and carbon dioxide levels, competing microflora, and food additives in the food matrix. *Y. enterocolitica* has been found to multiply in cottage cheese that contained no sorbic acid. On the other hand, *Y. enterocolitica* could not be isolated from ripening hard goat's milk cheeses (Tornadijo et al., 1993) or Swiss-hard or semi-had cheeses made with raw milk (Bachmann et al., 1995). In the absence of competing microflora, *Y. enterocolitica* can multiply to high numbers in foods, such as pasteurised milk (Black et al., 1978). Presence of starter culture on the other hand, had an inhibitory effect on the growth of *Y. enterocolitica* in Turkish Feta cheese (Bozkurt et al., 2001). It has been demonstrated that the growth of *Y. enterocolitica* in milk could be inhibited by the presence of a bacteriocin producing *Y. kristensenii* (Toora et al., 1994) or propionicin producing *Propionibacterium thoenii* (Lyon et al., 1993).

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[68] http://www.cfsan.fda.gov/~mow/chap5.html, accessed 26 April 2005.

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APPENDIX 6

Previous Risk Assessments on Microbiological Pathogens in Dairy Products

Organism	International Risk Assessment
Bacillus cereus	**Effect of dissolved carbon dioxide on thermal inactivation of micro-organisms in milk**. Journal of Food Protection 65 (12) : 1924-1929, 2002 **Abstract:** Post pasteurisation addition of CO_2 inhibits growth of certain micro-organisms in dairy products, but few studies have investigated the effect of CO_2 on thermal inactivation of micro-organisms during pasteurization. Concn. of CO_2, ranging from 44 to 58 mM, added to raw whole milk significantly ($P < 0.05$) reduced the number of surviving standard plate count (SPC) organisms in milk heated over the range 67-93 degreesC. A decrease in thermal survival rates (D-values) for *Pseudomonas fluorescens* RI-232 and *Bacillus cereus* ATCC 14579 spores in milk was positively correlated with CO2 concn. (1-36mM). D50degreesC-values for *P. fluorescens* significantly decreased ($P < 0.05$) in a linear fashion from 14.4 to 7.2 min. D89 degreesC-values for B. cereus spores were significantly ($P < 0.05$) decreased from 5.56 min in control milk to 5.29 min in milk containing 33 mM CO2. The Weibull function was used as a model to describe the thermal inactivation of P. fluorescens, *B. cereus* spores and SPC organisms in raw milk. Nonlinear parameters for the Weibull function were estimated, and survival data fitted to this model had higher R2 values than when fitted to the linear model, further providing support that the thermal inactivation of bacteria does not always follow first-order reaction rate kinetics. Results suggest that CO2 could be used as a processing aid to enhance microbial inactivation during pasteurisation.
	A risk assessment study of Bacillus cereus present in pasteurized milk. Notermans S, Dufrenne J, Teunis P, Beumer R, te Giffel M, Peeters Weem P., Food Microbiology 14 (2) : 143-151, 1997 It is generally regarded that the presence of 105 toxigenic Bacillus cereus in pasteurized milk is hazardous to human health. A risk assessment study of the presence of B. cereus in pasteurized milk in the Netherlands was determined. Risk of exposure to B. cereus was determined using data collected on the storage conditions (temp. and time) of pasteurised milk in households and by storage tests at 6, 8, 10 and 12 degreesC. Milk in households was held at <5-13 degreesC and stored for 2-12 days. Storage tests revealed that spoilage occurred at 8, 10 and 12 degreesC after 12, 8 and 6 days storage, respectively. Results suggested that >105 B. cereus/ml would be present in approx. 7% of the total portions of milk consumed. It is concluded that more information is required to evaluate human exposure to B. cereus in milk.
	Biological variability and exposure assessment. Delignette-Muller ML, Rosso L. Int J Food Microbiol. 2000 Jul 15;58(3):203-12. Predictive models are now commonly used for exposure assessment, with growth parameters defined for each microbial species. In this study, we tried to take into account microbial growth variability among strains of a single species. Bacillus cereus in pasteurized milk was chosen to illustrate the influence of the biological variability on the outcome of exposure assessment. Each parameter of the exposure assessment (growth parameters, shelf-life conditions) was characterized by a probability distribution describing variability and/or uncertainty. The impact of the intra-species variability on the result of the exposure assessment was then quantified and discussed. Two simple domestic shelf life conditions were tested. The results confirm that the biological variability has a great impact on the accuracy of the result and should not be systematically neglected.

Organism	International Risk Assessment
	Bacillus sporothermodurans - a Bacillus forming highly heat-resistant spores. International Dairy Federation, Bulletin of the International Dairy Federation : No. 357, 3-27, 2000 **Abstract:** This monograph on research carried out on Bacillus sporothermodurans was originally presented at the International Dairy Federation Annual Sessions of 1998. After an introduction describing the problem of B. sporothermodurans contamination of UHT milk, a further 6 papers are included, covering: classification of milk isolates of B. sporothermodurans; isolation and methods of detection; pathogenicity/toxicology; heat resistance; UHT processes to inactivate heat-resistant sporeformers; and risk assessment/quality assurance.
Brucella	**A simulation model of brucellosis spread in British cattle under several testing regimes. England T, Kelly L, Jones RD, MacMillan A, Wooldridge M.** Prev Vet Med. 2004 Apr 30;63(1-2):63-73. Brucellosis is a widespread, economically devastating and highly infectious zoonosis. In cattle, infection predominantly is caused by Brucella abortus, and is usually detected in pregnant females through abortions. Great Britain (GB) has been declared free from brucellosis (officially brucellosis free (OBF)) since 1993 and as such is required by European Union (EU) regulations to test > or =20% of both beef and dairy cattle >24 months old routinely. Currently, however, GB serologically tests more cattle than required and the issue of reducing the level of testing has come under consideration. We developed a simulation model to determine the rate of spread of brucellosis under a variety of testing regimes. For dairy herds, we found that reducing the level of testing would have a major effect on the rate of spread of infection, should it be imported. For beef herds, reducing the level of testing would have much less effect. We also found that abortion notification is a very-important additional means of surveillance. As a result of our predictions, policy-makers decided not to reduce the level of testing and actively to promote abortion notification.
Campylobacter	**Savill M, Hudson A, Devane M, Garrett N, Gilpin B, Ball A. Elucidation of potential transmission routes of Campylobacter in New Zealand.** Water Sci Technol. 2003;47(3):33-8. Campylobacter is the most commonly reported notifiable disease in New Zealand. The cost of Campylobacter infections in the country during 1994 was estimated as dollar 61.7M although the true cost was probably higher. Investigation of the main environmental reservoirs and routes of transmission to humans is necessary to formulate the most appropriate intervention strategies. This project investigated the reservoirs of Campylobacter in a defined geographical area within New Zealand and compared strains isolated from humans and environmental sources within this area as a prelude to investigating the likely transmission routes to humans. Campylobacter jejuni was commonly found in faeces from dairy cows, beef cattle, sheep and ducks, chicken carcasses, sheep offal and surface waters and C. coli was commonly found in sheep faeces. Preliminary analysis of Penner types was suggestive of transmission to humans from dairy and beef cattle and possibly from sheep
	Environmental aspects of Campylobacter infections. Stelzer W, Jacob J, Schulze E. Zentralbl Mikrobiol. 1991;146(1):3-15. Epidemiological data indicate high incidence of campylobacteriosis. Improperly prepared poultry-products, unpasteurised milk as well as non-chlorinated drinking water were shown to be the main vehicles of Campylobacter transmission to man. There is a lack of knowledge concerning the role of various environments in transmission of Campylobacter. The review summarizes the present knowledge about occurrence and survival of Campylobacters in various environments (sewage, sludge, surface water, drinking water). In conclusion risk assessment for public health is discussed.
Coxiella burnetii	**Kloppert B, Wolter W, Zschock M, Kabisch D, Hamann HP, Frost JW. [Coxiella burnetii as zoonotic pathogen with special regard to food hygiene]** Dtsch Tierarztl Wochenschr. 2004 Aug;111(8):321-3 [Article in German] In Hesse, Germany, bulk milk of farms producing raw milk cheese is examined by PCR for Coxiella burnetii yearly. In 2003 the pathogen has been detected unusually frequent. By means of two examples the hygienic measures are shown, which were initiated by the veterinary administration. To detect Coxiella burnetii means always the preoccupation with unsolved questions. It is particularly uncertain, whether there is a risk of oral infection for the human being. From the point of view of food hygiene, surveys are needed urgently to work out a risk assessment. Based on this a uniform risk management and a reasonable risk communication can be fixed.
Enterobacter sakazakii	**Joint FAO/WHO Workshop on *Enterobacter Sakazakii* and Other Microorganisms in Powdered Infant Formula** http://www.who.int/foodsafety/publications/micro/feb2004/en/ **Source:** Food Safety Department, World Health Organization **Author:** Food and Agriculture Organization of the United Nations/World Health Organization **Summary:** This page links to the report and executive summary from this meeting held February 2-5, 2004 in Geneva. There is also a link to questions and answers regarding *Enterobacter sakazakii* in powdered infant formula. The report includes discussion of "Epidemiology and Public Health Aspects," "Hazard Identification," "Hazard Characterization," "Exposure Assessment," "Risk Characterization" for E. sakazakii and Salmonella enterica, "Risk Reduction Strategies for Formula-

Organism	International Risk Assessment
	fed Infants," and more. Appendices include "List of Background Papers," "Data Received in Response to the FAO/WHO Call for Data," and "Risk Assessment," which discusses a risk assessment model for comparing the effectiveness of control measures for E. sakazakii and S. enterica in powdered infant formula **Resource type:** report, tables, charts, executive summary, fact sheet **Publication Date:** 2004
	***Enterobacter sakazakii* in Powdered Infant Formula** http://www.who.int/foodsafety/fs_management/en/No_01_Esakazakii_Jan05_en.pdf **Source:** INFOSAN Information Note, No. 1/2005, 13 Jan. 2005/International Food Safety Authorities Network, World Health Organization **Author:** International Food Safety Authorities Network, World Health Organization **Summary:** Note to alert authorities to emerging issue of infections related to *E. sakazakii* in powdered infant formula, and to summarize current efforts toward determining the magnitude and resolution of the problem **Resource type:** notice **Publication Date:** January 13, 2005
	Hazards Associated with *Enterobacter sakazakii* in the Consumption of Dairy Foods by the General Population http://www.nzfsa.govt.nz/dairy/publications/information-papers/enterobacter-sak… **Source:** New Zealand Food Safety Authority **Summary:** Information for consumers about *Enterobacter sakazakii*. Contains links to other resources, including information regarding *E. sakazakii* in infant formula **Resource type:** fact sheet
	Health Professionals Letter on *Enterobacter sakazakii* Infections Associated with Use of Powdered (Dry) Infant Formulas in Neonatal Intensive Care Units http://www.cfsan.fda.gov/~dms/inf-ltr3.html **Source:** Office of Nutritional Products, Labeling and Dietary Supplements, Center for Food Safety and Applied Nutrition, Food and Drug Administration **Summary:** This letter describes the risk to newborns from *Enterobacter sukazakii* in milk-based powdered infant formulas. It is designed to communicate these risks to health care professionals, and provides steps to be taken to minimize the risk of infection from using this product **Resource type:** letter **Publication Date:** October 10, 2002
	Isolation and Enumeration of *Enterobacter sakazakii* from Dehydrated Powdered Infant Formula http://www.cfsan.fda.gov/~comm/mmesakaz.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Summary:** This page provides methodology for testing for *Enterobacter sakazakii* in dehydrated powdered infant formula **Resource type:** web page **Publication Date:** August 2002
	Joint FAO/WHO Workshop on *Enterobacter Sakazakii* and Other Microorganisms in Powdered Infant Formula http://www.who.int/foodsafety/publications/micro/feb2004/en/ **Source:** Food Safety Department, World Health Organization **Author:** Food and Agriculture Organization of the United Nations/World Health Organization **Summary:** This page links to the report and executive summary from this meeting held February 2-5, 2004 in Geneva. There is also a link to questions and answers regarding *Enterobacter sakazakii* in powdered infant formula. The report includes discussion of "Epidemiology and Public Health Aspects," "Hazard Identification," "Hazard Characterization," "Exposure Assessment," "Risk Characterization" for E. sakazakii and Salmonella enterica, "Risk Reduction Strategies for Formula-fed Infants," and more. Appendices include "List of Background Papers," "Data Received in Response to the FAO/WHO Call for Data," and "Risk Assessment," which discusses a risk assessment model for comparing the effectiveness of control measures for E. sakazakii and S. enterica in powdered infant formula **Resource type:** report, tables, charts, executive summary, fact sheet **Publication Date:** 2004
	Opinion of the Scientific Panel on Biological Hazards on the Request from the Commission Related to the Microbiological Risks in Baby Formulae and Follow-on Formulae http://www.efsa.eu.int/science/biohaz/biohaz_opinions/691_en.html **Source:** EFSA Journal, Vol. 113, 2004, p. 1-35/Panel on Biological Hazards, European Food Safety Authority **Author:** Scientific Panel on Biological Hazards, European Food Safety Authority **Summary:** Opinion adopted on September 9, 2004 regarding microbiological risks in infant formulae and follow-on formulae, with emphasis on *E. sakazakii*. Includes discussion of hazard

Organism	International Risk Assessment
	identification for *Salmonella*, *E. sakazakii*, and other micro-organisms, hazard characterization, exposure assessment, and control measures **Resource type:** report **Publication Date:** November 17, 2004
	Risk Profile of *Enterobacter sakazakii* in Powdered Infant Formula ftp://ftp.fao.org/codex/ccfh35/fh03_13e.pdf **Source:** Food and Agriculture Organization of the United Nations **Author:** United States of America and Canada, Codex Committee on Food Hygiene, Codex Alimentarius Commission **Summary:** Risk profile for *Enterobacter sakazakii* in powdered infant formula, presented at the Thirty-fifth Session of the Codex Committee on Food Hygiene, held Jan. 27-Feb. 1, 2003, at Orlando, FL, USA **Resource type:** report **Publication Date:** January 2003
	Risk Profile of *Enterobacter sakazakii*, an Emergent Pathogen Associated with Infant Milk Formula http://dx.doi.org/10.1016/S0924-2244(03)00155-9 **Source:** Trends in Food Science and Technology, Vol. 14, Issue 11, Nov. 2003, p. 443-454/ScienceDirect **Author:** Iversen, C.; Forsythe, S. **Summary:** Risk profile for *Enterobacter sakazakii* in infant formula milk powder. Includes discussion of hazard identification, exposure assessment, hazard characterization, risk management, and detection methods. The abstract is freely available online, but access to the full text requires subscription or purchase **Resource type:** report **Publication Date:** August 01, 2003
Enterococci	**Antibiotic resistance and virulence traits of enterococci isolated from Baylough, an Irish artisanal cheese. Gelsomino R, Huys G, D'Haene K, Vancanneyt M, Cogan TM, Franz CM, Swings J.** J Food Prot. 2004 Sep;67(9):1948-52. Eight representative Enterococcus strains from a collection of over 600 previously isolated from an Irish artisanal cheese were subjected to phenotypic and genotypic analysis of antibiotic resistance and virulence properties. Genes encoding resistance to tetracycline (tet(M) and tet(L)) and/or erythromycin (erm(B)) were detected in five strains. In addition, all strains contained two or more of the virulence genes tested (agg, gel, cyl, esp, ace, efaAfs, and efaAfm). Further investigation into the transferability and environmental dissemination of these resistance and virulence traits will allow risk assessment and safety evaluation of artisanal cheeses.
	Phenotypic and genetic diversity of enterococci isolated from Italian cheeses. Andrighetto C, Knijff E, Lombardi A, Torriani S, Vancanneyt M, Kersters K, Swings J, Dellaglio F., Journal of Dairy Research 68 (2): 303-316, 2001 **Abstract:** This study aimed to identify the enterococci present in different Italian cheeses at the species and intra-species level and investigate some technologically relevant characteristics of these strains, in order to improve understanding of the significance and role of these microorganisms in dairy products. 124 enterococcal strains, isolated from traditional Italian cow, goat and buffalo cheeses, were characterized using phenotypic features and RAPD-PCR. RAPD-PCR profiles obtained with 4 primers and 5 different amplification conditions were compared by numerical analysis, and allowed inter- and intraspecific differentiation of the isolates. Whole-cell protein analysis by SDS-PAGE was used as a reference method for species identification. The strains were identified as Enterococcus faecalis (82 strains), E. faecium (27 strains), E. durans (9 strains), E. gallinarum (4 strains) and E. hirae (2 strains). Species recognition by means of RAPD-PCR was in agreement with SDS-PAGE results, except for 8 strains of E. faecium that clustered in separated groups. Phenotypic identification based on carbohydrate fermentation profiles, using the rapid ID 32 STREP galleries, gave different results from SDS-PAGE in 12.1% of cases. The majority of strains had weak acidifying and proteolytic activities in milk. 1 E. faecium strain showed the vanA (vancomycin resistance) genotype while 4 strains showed a beta-haemolytic reaction on human blood. Several strains showed antagonistic activity towards indicator strains of Listeria innocua, Clostridium tyrobutyricum and Propionibacterium freudenreichii subsp. shermanii. Results showed considerable genetic and phenotypic diversity among enterococcus strains isolated from Italian cheeses. It is concluded that information gained in this study could be used as a basis for selection of safe and useful strains for starters or protective cultures in cheese production.
Listeria monocytogenes	**Risk Assessment of *Listeria monocytogenes* in Ready-to-Eat Foods** http://www.fao.org/es/esn/food/risk_mra_riskassessment_listeria_en.stm **Source:** Food and Nutrition Division, Economic and Social Department, Food and Agriculture Organization of the United Nations **Author:** Food and Agriculture Organization of the United Nations/World Health Organization **Summary:** Interpretative summary and technical report of the FAO/WHO risk assessment of

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	Listeria monocytogenes in RTE foods. The overall assessment included example risk assessments for the risk from *L. monocytogenes* in pasteurized milk, ice cream, fermented meats, and cold-smoked, vacuum-packed fish. The risk characterization includes discussion of the risk when different levels of *L. monocytogenes* are present in a food serving, the risks for susceptible population groups, and the effects of growth in foods on risk **Resource type:** report, summary **Publication Date:** 2004
	Comparison of the U.S. and FAO/WHO *Listeria monocytogenes* Risk Assessments http://www.jifsan.umd.edu/csl2003.htm **Source:** Joint Institute for Food Safety and Applied Nutrition **Author:** Buchanan, Robert L. **Summary:** This presentation describes both the FDA/FSIS and the FAO/WHO risk assessments for *Listeria monocytogenes*, and discusses differences between the two approaches. It was presented at the 2003 CSL/JIFSAN Joint Symposium - Food Safety and Nutrition: Risk Analysis **Resource type:** presentation **Publication Date:** June 12, 2003
	Survey of Listeria Monocytogenes in Ready to Eat Foods http://www.foodrisk.org/listeria_survey.htm **Source:** Food Safety Risk Analysis Clearinghouse **Author:** National Food Processors Association **Summary:** The National Food Processors Association (NFPA) conducted this study with the purpose of addressing uncertainties in risk assessment regarding the occurrence of L. Monocytogenes in ready to eat foods. NFPA collected and tested about 31,700 samples in eight different categories of ready to eat foods. Samples were collected and tested over a period of 14 -23 months from retail markets in Maryland and California Food Net sites. The product categories on the access database include fresh soft "Hispanic" style cheeses, bagged salads, blue veined and soft mold ripened cheeses, smoked seafood and seafood salad. Lunch meats and Deli salads will be included in the database in the near future. The downloadable access database includes information on the product, such as location packaged, geographic area, date listed, date of purchase, date of assay, if vacuum packaged, etc. Test information also includes presumptive positives, number of tubes that are turbid at the 1, 10 and 100 dilution, most probable number, presumptive colonies, cfu/g, etc. **Resource type:** database/dataset, database/documentation **Publication Date:** March 2003
	Quantitative Assessment of the Relative Risk to Public Health from Food-borne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods http://www.foodsafety.gov/~dms/lmr2-toc.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration/Food Safety and Inspection Service, U.S. Department of Agriculture/Centers for Disease Control and Prevention **Summary:** Assessment of the risks of serious illness and death from *Listeria monocytogenes* in 23 categories of ready-to-eat foods **Resource type:** risk assessment, tables, charts **Publication Date:** September 2003
	Program Information Manual: Retail Food Safety: Date Marking of Cheese http://www.cfsan.fda.gov/~ear/ret-chdt.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Author:** Beaulieu, Raymond D. **Summary:** This document is an interpretation of the need to date mark all cheeses as described in the Food Code Section 3-501.17. It specifies which cheeses are and are not exempt to the date marking provisions in the Food Code, based on their potential for supporting the growth of *L. monocytogenes* and other food-borne pathogens **Resource type:** policy document **Publication Date:** December 15, 1999
	Risk Profile: *Listeria monocytogenes* in Ice Cream http://www.nzfsa.govt.nz/science-technology/risk-profiles/lmono-in-ice-cream.pd… **Source:** New Zealand Food Safety Authority **Author:** Lake, Rob; Hudson, Andrew; Cressey, Peter/Institute of Environmental Science and Research Limited **Summary:** Profile of the risks associated with *Listeria monocytogenes* in ice cream in New Zealand. Includes each step of a qualitative risk assessment as well as other information that will be useful for risk management. Also includes an appendix entitled "Categories for Risk Profiles" **Resource type:** report, tables, charts **Publication Date:** October 2003
	Sanaa M, Coroller L, Cerf O.Risk assessment of listeriosis linked to the consumption of two soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. Risk Anal.

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	2004 Apr;24(2):389-99. This article reports a quantitative risk assessment of human listeriosis linked to the consumption of soft cheeses made from raw milk. Risk assessment was based on data purposefully acquired inclusively over the period 2000-2001 for two French cheeses, namely: Camembert of Normandy and Brie of Meaux. Estimated Listeria monocytogenes concentration in raw milk was on average 0.8 and 0.3 cells/L, respectively, in Normandy and Brie regions. A Monte Carlo simulation was used to account for the time-temperature history of the milk and cheeses from farm to table. It was assumed that cell progeny did not spread within the solid cheese matrix (as they would be free to do in liquid broth). Interaction between pH and temperature was accounted for in the growth model. The simulated proportion of servings with no L. monocytogenes cell was 88% for Brie and 82% for Camembert. The 99th percentile of L. monocytogenes cell numbers in servings of 27 g of cheese was 131 for Brie and 77 for Camembert at the time of consumption, corresponding respectively to three and five cells of L. monocytogenes per gram. The expected number of severe listeriosis cases would be < or =10(-3) and < or =2.5 x 10(-3) per year for 17 million servings of Brie of Meaux and 480 million servings of Camembert of Normandy, respectively.
	Bunning VK, Crawford RG, Tierney JT, Peeler JT. Thermotolerance of heat-shocked Listeria monocytogenes in milk exposed to high-temperature, short-time pasteurization. Appl Environ Microbiol. 1992 Jun;58(6):2096-8. The effect of prior heat shock (48 degrees C for 15 min) on the thermotolerance of Listeria monocytogenes at the minimal high-temperature, short-time (71.7 degrees C for 15 s) parameters required by the Pasteurized Milk Ordinance was examined. The mean D71.7 degrees C value for heat-shocked L. monocytogenes was 4.6 +/- 0.5 s (control D = 3.0 +/- 1.0 s); the ratio of D to control D was 1.5. The increased thermotolerance of heat-shocked Listeria cells was not significant and appeared unlikely to have practical implications, in terms of risk assessment, for the safety of pasteurized milk.
	Farber JM, Ross WH, Harwig J. Health risk assessment of Listeria monocytogenes in Canada. Int J Food Microbiol. 1996 Jun;30(1-2):145-56. In this review, the major steps used in the formulation of a health risk assessment for Listeria monocytogenes in foods are discussed. Data is given on the numbers of human listeriosis cases reported in Canada along with the current Canadian regulatory policy on L. monocytogenes. Four major steps in the health risk assessment of this organism in foods, namely, hazard identification, hazard characterization, exposure assessment and risk characterization, were examined. For hazard characterization, since it is known that no direct human dose response data is available for L. monocytogenes, a flexible dose response model called the Weibull-Gamma model was evaluated. For the exposure assessment, pate and soft cheese, both high-risk foods in terms of listeriosis infection, were used as prototypes in some of the models that were used. Using disappearance data for cheese and 100 g as a typical serving, the data suggested an average of 102 servings per capita, per year in Canada. As a rough approximation, for L. monocytogenes, reference ID10 and ID90 dose levels of response for both normal and high risk populations were given as 10(7) and 10(9) for normal individuals, and 10(5) and 10(7) for high-risk people. The corresponding dose response models were graphically displayed. These models exhibited a higher degree of susceptibility and less host/pathogen heterogeneity for the higher risk group. The range of doses between the ID10 and ID90 reference values corresponded roughly to levels associated with cases of listeriosis. In the risk characterization stage, dose response data was combined with some predictive growth modelling data of L. monocytogenes on pate, assuming an initial exposure of a single cell for food stored at 4 degrees and 8 degrees C. Storage of pate at 4 degrees C for more than 35 days resulted in a rapidly increasing risk for the high risk population, while storage at 8 degrees C produced a similar risk after about 13 days. In addition, an equation, used to calculate the average probability of acquiring human listeriosis in Canada from soft and semi-soft cheese consumption, was formulated. Computations derived from this equation indicated a substantial level consistency between reported data and assumptions of the risk assessment model. An important part of risk characterization or possibly risk management is characterizing the economic and social consequences of estimated risks. The total annual estimated cost of listeriosis illnesses and deaths in Canada was estimated to be between 11.1 and 12.6 million dollars.
	Estimation of uncertainty and variability in bacterial growth using Bayesian inference. Application to Listeria monocytogenes. Pouillot R, Albert I, Cornu M, Denis JB. Int J Food Microbiol. 2003 Mar 15;81(2):87-104. The usefulness of risk assessment is limited by its ability or inability to model and evaluate risk uncertainty and variability separately. A key factor of variability and uncertainty in microbial risk assessment could be growth variability between strains and growth model parameter uncertainty. In this paper, we propose a Bayesian procedure for growth parameter estimation which makes it possible to separate these two components by means of hyperparameters. This model incorporates in a single step the logistic equation with delay as a primary growth model and the cardinal temperature equation as a secondary growth model. The estimation of Listeria monocytogenes growth parameters in milk using literature data is proposed as a detailed application. While this

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	model should be applied on genuine data, it is highlighted that the proposed approach may be convenient for estimating the variability and uncertainty of growth parameters separately, using a complete predictive microbiology model.
	Quantitative risk assessment of Listeria monocytogenes in ready-to-eat foods: the FAO/WHO approach. Rocourt J, BenEmbarek P, Toyofuku H, Schlundt J. FEMS Immunol Med Microbiol. 2003 Apr 1;35(3):263-7. Quantitative microbiological risk assessment is a very new and unique scientific approach able to link, for the first time, data from food (in the farm-to-fork continuum) and the various data on human disease to provide a clear estimation of the impact of contaminated food on human public health. The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have recently launched risk assessment studies of a number of pathogen-food commodity combinations (Salmonella in eggs and in broiler chickens, Listeria monocytogenes in ready-to-eat foods, Campylobacter in broiler chickens, Vibrio in seafood) to be used to lower the risk associated with these food-borne diseases and ensure fair practices in the international trade of food. The FAO/WHO Listeria risk assessment was undertaken in part to determine how previously developed risk assessments done at the national level could be adapted or expanded to address concerns related to L. monocytogenes in ready-to-eat foods at an international level. In addition, after initiation of the risk assessment, the risk assessors were asked by the Codex Committee on Food to consider three specific questions related to ready-to-eat foods in general, which are: (1). estimate the risk for consumers in different susceptible populations groups (elderly, infants, pregnant women and immunocompromised patients) relative to the general population; (2). estimate the risk for L. monocytogenes in foods that support growth and foods that do not support growth under specific storage and shelf-life conditions; (3). estimate the risk from L. monocytogenes in food when the number of organisms ranges from absence in 25 g to 1000 colonies forming units per gram or milliliter, or does not exceed specified levels at the point of consumption. To achieve these goals, new dose-response relationships and exposure assessments for ready-to-eat foods were developed. Preliminary data indicate that eliminating the higher dose levels at the time of consumption has a large impact on the number of predicted cases.
	Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. Bemrah N, Sanaa M, Cassin MH, Griffiths MW, Cerf O. Prev Vet Med. 1998 Dec 1;37(1-4):129-45. Microbial hazards have been identified in soft cheese made from raw milk. Quantification of the resulting risk for public health was attempted within the frame of the Codex Alimentarius Commission, 1995 approach to quantitative risk assessment, using Monte Carlo simulation software. Quantitative data could only be found for Listeria monocytogenes. The complete process of cheese making was modelled, from milking to consumption. Using data published on the different sources of milk contamination (environment and mastitis) and bacterial growth, distributions were assumed for parameters of the model. Equations of Farber, J.M., Ross, W.H., Harwing, J. (1996) for general and at-risk populations were used to link the ingested dose of L. monocytogenes to the occurrence of listeriosis. The probability of milk contamination was estimated to be 67% with concentration ranging from 0 to 33 CFU ml-1. The percentage of cheese with a predicted concentration of L. monocytogenes greater than 100 CFU g-1 was low (1.4%). The probability of consuming a contaminated cheese serving was 65.3%. Individual annual cumulative risk of listeriosis, in a population each consuming 50 servings of 31 g, ranged from 1.97 x 10(-9) to 6.4 x 10(-8) in a low-risk sub-population and 1.04 10(-6) to 7.19 10(-5) in a high-risk sub-population. The average number of expected cases of listeriosis per year was 57 for a high-risk sub-population and one for a low-risk healthy sub-population. When the frequency of environmental milk contamination was reduced in the model and L. monocytogenes mastitis was eliminated, the expected incidence of listeriosis decreased substantially; the average number of expected cases was reduced by a factor of 5. Thus the usefulness of simulation to demonstrate the efficiency of various management options could be demonstrated, even if results should be interpreted with care (as many assumptions had to be made on data and their distributions.
	Studies on the risk assessment of Listeria monocytogenes. Notermans S, Dufrenne J, Teunis P, Chackraborty T. J Food Prot. 1998 Feb;61(2):244-8. Humans are frequently exposed to Listeria monocytogenes, and high numbers may be ingested during consumption of certain types of food. However, epidemiological investigations show that listeriosis is a rare disease. Risk assessment studies using an animal mouse model indicate that almost all L. monocytogenes serovars present in food have clear virulent properties. The intravenous dose causing infection in 50% (IV ID50) of mice not previously exposed to L. monocytogenes (nonprotected mice) was 1.8 log(10) units. For mice previously exposed to L.monocytogenes (immunologically protected mice was >9.0 log10 5.6 log(10) units. The ID(50) of orally exposed nonprotected mice amounted to 6.5 log10 units, and no significant effects of type of food (water/milk) and storage time at 5 degrees C (milk) were observed. The oral ID50 of immunologically protected mice was >9.0 log10 units. Furthermore, there was approximately 1-2 log10 difference between the ID50 and the lethal dose causing death in 50% (LD50). The results

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	show that both the intestinal barrier and the specific immune defence mechanism are highly effective in preventing infection of mice orally exposed to L.monocytogenes. Delaying the immune defense had no effect on the protective activity of the intestinal barrier, indicating that these protective mechanisms independently. The risk assessment results obtained in the mouse model support the epidemiological finding that listeriosis is a rare disease in humans, despite frequent exposure to the organism.
	Predictive modelling of inactivation of Listeria spp. in bovine milk during high-temperature short-time pasteurization. Piyasena P, Liou S, McKellar RC. Int J Food Microbiol. 1998 Feb 17;39(3):167-73. A linear model was derived to describe the thermal inactivation of Listeria innocua in bovine whole milk in a high-temperature short-time pilot scale pasteurizer. Integrated lethal effect, or pasteurization effect (PE), was obtained by converting times at different temperatures in the various sections of the pasteurizer to the equivalent time at the reference temperature (72 degrees C). PE was then related by a simple linear function to the log10 of the % viable counts with a power transformation of the PE values to improve the linear fit. R2 values for the five L. innocua trials varied from 0.728 to 0.974. Validation of this model with Listeria monocytogenes confirmed that L. monocytogenes was more heat sensitive. Inter-trial variation was incorporated into the model using the @RISK simulation software. Output from simulations confirmed that pasteurization at the IDF standard conditions of 72 degrees C for 15 sec can ensure at least an 11-log reduction of L. monocytogenes. The results showed that L. innocua may be used as a model micro-organism to assess the thermal inactivation of L. monocytogenes, since its heat resistance is at least equal to or greater than that of the pathogenic species.
	Health risk assessment of Listeria monocytogenes in Canada. **Farber JM, Ross WH, Harwig J.** Int J Food Microbiol. 1996 Jun;30(1-2):145-56. In this review, the major steps used in the formulation of a health risk assessment for Listeria monocytogenes in foods are discussed. Data is given on the numbers of human listeriosis cases reported in Canada along with the current Canadian regulatory policy on L. monocytogenes. Four major steps in the health risk assessment of this organism in foods, namely, hazard identification, hazard characterization, exposure assessment and risk characterization, were examined. For hazard characterization, since it is known that no direct human dose response data is available for L.monocytogenes, a flexible dose response model called the Weibull-Gamma model was evaluated. For the exposure assessment, pate and soft cheese, both high-risk foods in terms of listeriosis infection, were used as prototypes in some of the models that were used. Using disappearance data for cheese and 100 g as a typical serving, the data suggested an average of 102 servings per capita, per year in Canada. As a rough approximation, for L. monocytogenes, reference ID10 and ID90 dose levels of response for both normal and high risk populations were given as 10(7) and 10(9) for normal individuals, and 10(5) and 10(7) for high-risk people. The corresponding dose response models were graphically displayed. These models exhibited a higher degree of susceptibility and less host/pathogen heterogeneity for the higher risk group. The range of doses between the ID10 and ID90 reference values corresponded roughly to levels associated with cases of listeriosis. In the risk characterization stage, dose response data was combined with some predictive growth modelling data of L. monocytogenes on pate, assuming an initial exposure of a single cell for food stored at 4 degrees and 8 degrees C. Storage of pate at 4 degrees C for more than 35 days resulted in a rapidly increasing risk for the high risk population, while storage at 8 degrees C produced a similar risk after about 13 days. In addition, an equation, used to calculate the average probability of acquiring human listeriosis in Canada from soft and semi-soft cheese consumption, was formulated. Computations derived from this equation indicated a substantial level consistency between reported data and assumptions of the risk assessment model. An important part of risk characterization or possibly risk management is characterizing the economic and social consequences of estimated risks. The total annual estimated cost of listeriosis illnesses and deaths in Canada was estimated to be between 11.1 and 12.6 million dollars.
	Risk assessment of L. monocytogenes in Swiss Emmental cheese. Aebi R, Muehlemann M, Buehlmann G, Schaellibaum M., AgrarForschung 10 (8) : 306-311, 2003 **Language of Text:** German **Language of Summary:** English, French **Abstract:** Risk assessment of Listeria monocytogenes in Swiss Emmental cheese is discussed. Origin and spread of L. monocytogenes contamination through the whole production chain from raw milk to the final product reaching the consumer were assessed; a contamination profile of production and distribution was developed. The main factor governing reduction in L. monocytogenes count during cheesemaking was heat treatment temp. L. monocytogenes contamination of the rind may be reduced by rind removal or specific treatments. Presence of L. monocytogenes in the retail product is mainly due to recontamination during packaging, distribution, etc. It is concluded that consumers may be exposed to L. monocytogenes counts of 1-10 per cheese portion, and that consumption of traditionally made Emmental cheese presents an extremely low hazard.

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Mycobacterium bovis	**Risk Profile: *Mycobacterium bovis* in Milk** http://www.nzfsa.govt.nz/science-technology/risk-profiles/mycobacterium-bovis-i… **Source:** New Zealand Food Safety Authority **Author:** Lake, Rob; Hudson, Andrew; Cressey, Peter/Institute of Environmental Science and Research Limited **Summary:** This risk profile includes elements of a qualitative risk assessment and other information that will be useful to risk managers. Includes an appendix entitled "Categories for Risk Profiles" **Resource type:** report, tables **Publication Date:** October 2002
Mycobacterium paratuberculosis	***Mycobacterium paratuberculosis* and Milk** http://www.ifst.org/hottop23.htm **Source:** Institute of Food Science and Technology **Summary:** This document provides an overview of *M. paratuberculosis*, including pathogenicity, potential sources for human infection, association with Crohn's disease, and heat resistance **Resource type:** report **Publication Date:** August 19, 1998
	***Mycobacterium paratuberculosis* -- Another Emerging Pathogen of the Human Gastrointestinal Tract?** http://www.wisc.edu/fri/briefs/paratb.htm **Source:** Food Research Institute, University of Wisconsin-Madison **Author:** Doyle, M. Ellin **Summary:** Review of information on the potential role of *Mycobacterium paratuberculosis* in causing Crohn's Disease. Includes discussion of possible vehicles of transmission **Resource type:** literature review **Publication Date:** April 1997
	Mycobacterium bovis versus Mycobacterium tuberculosis as a cause of acute cervical lymphadenitis without pulmonary disease. Fennelly GJ. Pediatr Infect Dis J. 2004 Jun;23(6):590-1. Bovine tuberculosis remains a common disease of cattle in countries such as Mexico. Children eating unpasteurised dairy products from Mexican cattle can develop Mycobacterium bovis cervical lymphadenitis. However, the bovine mycobacterium can be misdiagnosed as Mycobacterium tuberculosis based on standard laboratory testing. Accurate speciation is important for selection of the preferred antibiotic regimen for treatment of Mycobacterium bovis infection.
	Effects of prevalence and testing by enzyme-linked immunosorbent assay and fecal culture on the risk of introduction of Mycobacterium avium subsp. paratuberculosis-infected cows into dairy herds. Carpenter TE, Gardner IA, Collins MT, Whitlock RH. J Vet Diagn Invest. 2004 Jan;16(1):31-8. A stochastic simulation model was developed to assess the risk of introduction of Mycobacterium avium subsp. paratuberculosis infection into a dairy herd through purchase of female replacement cattle. The effects of infection prevalence in the source herd(s), number of females purchased, and testing by enzyme-linked immunosorbent assay (ELISA) alone or ELISA and fecal culture as risk mitigation strategies were evaluated. Decisions about negative test results were made on a lot and individual basis. A hypothetical dairy herd, free from M. a. paratuberculosis, which replaced 1 lot (10, 30, or 100) of cows per year, was considered. Probability distributions were specified for the sensitivities and specificities of ELISA and fecal culture, the proportion of infected herds and within-herd prevalence for randomly selected replacement source herds (high prevalence) and herds in level 2 (medium prevalence) and level 3 (low prevalence) of the Voluntary Johne's Disease Herd Status Program (VJDHSP). Simulation results predicted that 1-56% of the lots had at least 1 M. a. paratuberculosis-infected cow. Assuming that ELISA sensitivity was 25%, simulation results showed on a lot basis that between 0.4% and 18% and between 0.1% and 9% were predicted to have at least 1 infected cow not detected by ELISA and by a combination of ELISA and fecal culture, respectively. On an individual cow basis, between 0.1% and 8.3% of ELISA-negative cattle in ELISA-positive lots were estimated to be infected. In both the lot and individual analyses, the probability of nondetection increased with larger lot sizes and greater prevalence. Sensitivity analysis indicated that the effect of a lower ELISA sensitivity (10%) was a variable decrease in mean detection probabilities for all combinations of prevalence and lot size. The benefit of testing introduced cattle with ELISA alone or in combination with fecal culture was found to be minimal if cows were purchased from known, low-prevalence (level 3) herds. The value of testing by ELISA alone or in combination with fecal culture was greatest in high-prevalence herds for all lot sizes. Testing of random-source cattle, bought as herd replacements, can partially mitigate the risk of introduction of M. a. paratuberculosis but not as well as by using low-prevalence source herds (level-3 VJDHSP), with or without testing.
	Biosecurity on dairy operations: hazards and risks. Wells SJ. J Dairy Sci. 2000 Oct;83(10):2380-6.

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	The objective here was to present a model for considering biosecurity related to infectious diseases on US dairy operations using a risk assessment framework. With the example of an important dairy cattle pathogen (Mycobacterium paratuberculosis), I followed risk assessment steps to characterize risks related to the use of certain management practices and possible risk reduction within an infectious disease biosecurity program. Biosecurity practices focus on the prevention of introduction of these pathogens to the dairy, and estimates of the risks associated with introduction of different sources of cattle are presented. In addition, biosecurity practices also limit the transmission of these pathogens within an infected dairy operation, especially those focused on sick cow management, calving area management, and manure management. Recent information from the National Animal Health Monitoring System (NAHMS) Dairy 96 Study indicates that many of these practices have not been adopted on US dairy operations, indicating both risk of disease and opportunity for animal health improvement.
	Significance of Mycobacterium paratuberculosis in milk. Hammer P, Knappstein K, Hahn G., Bulletin of the International Dairy Federation : No. 330, 12-16, 1998 The presence of Mycobacterium paratuberculosis in milk and possible links with Crohn's disease in humans are discussed. Aspects covered include: taxonomy and biological characteristics; Johne's disease, caused by M. paratuberculosis in all types of domestic and wild ruminants and in some species of laboratory animals; Crohn's disease (incidence, possible causative factors, transmission); heat resistance of M. paratuberculosis, with respect to survival under pasteurization conditions; risk assessment on M. paratuberculosis in pasteurized milk according to Codex Alimentarius procedures; and future research requirements.
Pseudomonas	**Researchers Work to Arrest Spoilage Organisms' Progress** http://www.cheesemarketnews.com/articlearch_old/2002/01mar02/01mar02_04.html **Source:** Cheese Market News Article Archive, March 1, 2002 **Author:** Sander, Kate **Summary:** This article describes the work of Dr. Kathryn Boor and Dr. Martin Wiedmann on the tracking and identification of spoilage organisms in dairy products, including research on fingerprinting methods for Pseudomonas spp. and Listeria monocytogenes **Resource type:** release **Publication Date:** March 01, 2002
Staphylococcus	**Quantitative microbial risk assessment exemplified by Staphylococcus aureus in unripened cheese made from raw milk. Lindqvist R, Sylven S, Vagsholm I.** Int J Food Microbiol. 2002 Sep 15;78(1-2):155-70. This paper discusses some of the developments and problems in the field of quantitative microbial risk assessment, especially exposure assessment and probabilistic risk assessment models. To illustrate some of the topics, an initial risk assessment was presented, in which predictive microbiology and survey data were combined with probabilistic modelling to simulate the level of Staphylococcus aureus in unripened cheese made from raw milk at the time of consumption. Due to limited data and absence of dose-response models, a complete risk assessment was not possible. Instead, the final level of bacteria was used as a proxy for the potential enterotoxin level, and thus the potential for causing illness. The assessment endpoint selected for evaluation was the probability that a cheese contained at least 6 log cfu S. aureus g(-1) at the time of consumption; the probability of an unsatisfactory cheese, P(uc). The initial level of S. aureus, followed by storage temperature had the largest influence on P(uc) at the two pH-values investigated. P(uc) decreased with decreasing pH and was up to a factor of 30 lower in low pH cheeses due to a slower growth rate. Of the model assumptions examined, *i.e.* the proportion of enterotoxigenic strains, the level of S. aureus in non-detect cheeses, the temperature limit for toxin production, and the magnitude and variability of the threshold for an unsatisfactory cheese, it was the latter that had the greatest impact on P(uc). The uncertainty introduced by this assumption was in most cases less than a factor of 36, the same order of magnitude as the maximum variability due to pH. Several data gaps were identified and suggestions were made to improve the initial risk assessment, which is valid only to the extent that the limited data reflected the true conditions and that the assumptions made were valid. Despite the limitations, a quantitative approach was useful to gain insights and to evaluate several factors that influence the potential risk and to make some inferences with relevance to risk management. For instance, the possible effect of using starter cultures in the cheese making process to improve the safety of these products.
	Staphylococcus aureus in raw milk and human health risk. Zecconi A, Hahn G., Bulletin of the International Dairy Federation : No. 345, 15-18, 1999 Staphylococcus aureus in raw milk and the associated human health risks are discussed. Aspects considered include: S. aureus identification; S. aureus polymorphism; S. aureus enterotoxins; and risk assessment for S. aureus in raw milk cheese. It is concluded that control measures should be implemented to reduce the prevalence of S. aureus in dairy cattle and thus the risk of toxins in raw milk and its products. [This paper was presented at a conference entitled Quality and safety of raw milk and its impact on milk and milk products, held in Athens, Greece, in Sept. 1999.]

Organism	International Risk Assessment
Salmonella	**Salmonella and other Enterobacteriaceae in dairy-cow feed ingredients: antimicrobial resistance in western Oregon. Kidd RS, Rossignol AM, Gamroth MJ.** J Environ Health. 2002 Oct;65(3):7, 21. Several studies have suggested an association between the use of antimicrobial agents in animal feeds and an increased risk that humans will contract resistant strains of bacteria such as Salmonella species, Escherichia coli, and other enteric isolates. The authors of this study evaluated whether animal feeds might serve as sources of antimicrobial-resistant bacteria, especially bacteria that are pathogenic to humans. From July through August 1998, samples of feed ingredients were collected from a total of 50 feed piles located on 12 dairy farms in western Oregon. From a subset of 10 piles, repeated samples were collected over time until each pile was depleted. Analysis of the samples indicated that 42.0 percent of all 50 piles and 60.0 percent of the piles from which there was repeated sampling were presumptive positive for Salmonella. Sixty-two percent of 50 Enterobacteriaceae isolates showed ampicillin resistance, and 10.0 percent displayed tetracycline resistance. Other bacteria displayed varying degrees of resistance to ampicillin, streptomycin, tetracycline, or a combination of these antimicrobials. The extent of antimicrobial-resistant Enterobacteriaceae in feed ingredients observed in this study raises significant concerns about the potential for human health risks from food-producing animals such as dairy cows.
Yersinian	**Emerging food pathogens and bacterial toxins. Bielecki J.** Acta Microbiol Pol. 2003;52 Suppl:17-22. Many different food-borne diseases have been described. For example, Shigella bacteria, hepatitis A virus and Norwalk virus were shown as a unwashed hands microorganisms, but pathogen Campylobacter and Escherichia coli were named as raw and undercooked meat and poultry or raw milk and untreated water born bacteria. However, two of them: Listeria monocytogenes and Yersinia enterocolitica are known as growing at refrigerator temperatures. Essential virulence determinants of Listeria monocytogenes pathogenicity are well known as a bacterial toxins. Basic molecular mechanisms of pathogenicity depending from these toxins were presented. It was shown that other bacterial toxins may act as very danger food poisoning substances. This is why elimination of pathogenic microorganisms from foods is an obvious solution in some food processes, however this approach is not practical or even desirable in many processes. Thus, risk assessment and microbial monitoring will continue to play important roles in ensuring food safety. Some technological advances have the capability of delivering detection systems that can not only monitor pathogenic microorganisms, but also entire microbial populations in the food matrix.
General	Microorganisms **In** Dairy-Products **- Friends And Foes** **Author(s):** KEOGH BP **Publisher:** Dairy Industry Assn Australia, PO BOX 20, HIGHETT VICTORIA 3190, AUSTRALIA **Subject Category:** Agriculture, Dairy & Animal Science; Food Science & Technology **Source:** AUSTRALIAN JOURNAL OF DAIRY TECHNOLOGY 33 (2): 41-45 1978
	Microbial Pathogen Data Sheets http://www.nzfsa.govt.nz/science-technology/data-sheets/index.htm **Source:** New Zealand Food Safety Authority **Author:** Institute of Environmental Science and Research Limited **Summary:** Fact sheets on food-borne pathogens. Sections include "The Organism/Toxin," "Growth and Its Control," "The Illness," "Sources," "Outbreaks and Incidents," "Adequate Processing Guidelines," and others. Fact sheets may be available for *Bacillus cereus*, *Campylobacter*, *Clostridium botulinum*, *Clostridium perfringens*, *Cryptosporidium parvum*, enteric viruses, *E. coli* O157:H7, non-O157 STEC, *Giardia intestinalis*, hepatitis A virus, *Listeria monocytogenes*, *Mycobacterium bovis*, Norwalk-like viruses, *Salmonella* Typhi, non-typhoid *Salmonellae*, scombroid poisoning, *Shigella*, *Staphylococcus aureus*, *Toxoplasma gondii*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio vulnificus*, and *Yersinia enterocolitica* **Resource type:** fact sheets
	Microbiological risk analysis of milk and milk products in international trade. Kelly PM, Farm & Food 7 (3) : 23-28, 1997 **Abstract:** The Sanitary and Phytosanitary (SPS) Agreement arising from the latest initiatives of the World Trade Organisation (WTO) is a major challenge to food exporters and in particular to the Irish **dairy** industry. SPS compliance demands that food microbiological specifications are established on a scientific basis rather than on the current HACCP basis. Hazards analysis for milk and **dairy** products is explored in an attempt to explain the concept of risk analysis, procedures to be used, impact on the Irish **dairy** industry, and the work of the International **Dairy** Federation (IDF). Aspects considered include: definitions (hazard, risk, risk assessment); revised principles for establishment and application of microbiological criteria; WTO agreements on SPS and Technical Barriers to Trade (TBT); establishment of an expert group to study **microbiological risk assessment**; and examples illustrating the principles (Listeria monocytogenes (effective management strategies, criteria) and enterohaemorrhagic Escherichia coli (EHEC; effective management strategies, microbiological criteria)).

Other relevant information

Cheese	**Food Safety and Cheese: Institute of Food Science and Technology Position Statement** http://drinc.ucdavis.edu/dfoods5.htm **Source:** Dairy Research and Information Center, University of California, Davis **Author:** Professional Food Microbiology Group, Institute of Food Science and Technology **Summary:** This page gives a description of bacterial illnesses associated with cheese contamination and their effects on humans, possible pathways for cheeses to become hosts of these bacteria, and measures to ensure the production of safe cheese **Resource type:** statement **Publication Date:** November 15, 1996
	Food Safety and Cheese http://www.ifst.org/hottop15.htm **Source:** Institute of Food Science and Technology **Summary:** This report describes the hazards to human health associated with cheese. It includes discussion of food-borne outbreaks associated with cheese, the microbiological safety of cheese, control measures, and consumer awareness of health risks associated with cheese **Resource type:** report **Publication Date:** April 16, 1998
	Program Information Manual: Retail Food Safety: Date Marking of Cheese http://www.cfsan.fda.gov/~ear/ret-chdt.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Author:** Beaulieu, Raymond D. **Summary:** This document is an interpretation of the need to date mark all cheeses as described in the Food Code Section 3-501.17. It specifies which cheeses are and are not exempt to the date marking provisions in the Food Code, based on their potential for supporting the growth of *L. monocytogenes* and other food-borne pathogens **Resource type:** policy document **Publication Date:** December 15, 1999
Data	***Escherichia coli* O157 on U.S. Dairy Operations** http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/Dairy02/Dairy02Ecoli.pdf **Source:** National Animal Health Monitoring System Program Unit, National Center for Animal Health Surveillance, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture **Summary:** Brief report on the presence of *E. coli* O157 on U.S. dairy operations, from the Dairy 2002 study **Resource type:** report, charts **Publication Date:** December 2003
	***Salmonella* and *Listeria* in Bulk Tank Milk on U.S. Dairies** http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/Dairy02/Dairy02bulktank.pdf **Source:** National Animal Health Monitoring System Program Unit, National Center for Animal Health Surveillance, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture **Summary:** Report from the National Animal Health Monitoring System Dairy 2002 study on the prevalence of *Salmonella* and *Listeria* in bulk tank milk on U.S. dairy operations **Resource type:** report, charts **Publication Date:** December 2003
	***Salmonella* and *Campylobacter* on U.S. Dairy Operations** http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/dairy/Dairy02/Dairy02SalCampy.pdf **Source:** National Animal Health Monitoring System Program Unit, National Center for Animal Health Surveillance, Centers for Epidemiology and Animal Health, Veterinary Services, Animal and Plant Health Inspection Service, U.S. Department of Agriculture **Summary:** Report on results from Dairy 2002 and previous studies on *Salmonella* and *Campylobacter* in cow feces from dairy operations **Resource type:** report, chart, table **Publication Date:** December 2003

	Home Delivery of Perishable Foods Project http://www.health.vic.gov.au/foodsafety/research/microbiological.htm **Source:** Food Safety Unit, Public Health Group, Rural and Regional Health and Aged Care Services Division, Department of Human Services, Victorian State Government **Author:** Microbiological Diagnostic Unit, Public Health Laboratory, University of Melbourne **Summary:** Report of a study on the potential for pathogen growth in home delivered foods. The study included determination of delivery times and temperature profiles for home delivered foods, challenge tests for the growth of *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Salmonella* on **cheese**, sliced meat, savoury pastry, fruit, and **milk**, comparison of challenge test results with predictive models, and application of predictive models to the home delivery data. Appendices include "Temperature measurement trials - comparison of core vs. surface temperature measurements," "Temperature profiles for Home deliveries," and "Challenge test results" **Resource type:** report, tables, charts **Publication Date:** November 2003
	Microbe growth in custard and cream products http://www.health.vic.gov.au/foodsafety/research/microbiological.htm **Source:** Food Safety, Victoria Government Health Information
Goat milk ***Enterobacter, Staphylococcus, Campylobacter,* EHEC, *Salmonella, Mycobacterium***	**Microbiological quality of raw goat's and ewe's bulk-tank milk in Switzerland. Muehlherr JE, Zweifel C, Corti S, Blanco JE, Stephan R.** J Dairy Sci. 2003 Dec;86(12):3849-56. A total of 407 samples of bulk-tank milk (344 of goat's milk and 63 of ewe's milk) collected from 403 different farms throughout Switzerland, was examined. The number of farms investigated in this study represents 8% of the country's dairy-goat and 15% of its dairy-sheep farms. Standard plate counts and Enterobacteriaceae counts were performed on each sample. Furthermore, the prevalence of Staphylococcus aureus, Campylobacter spp., Shiga toxin-producing Escherichia coli, Salmonella spp., and Mycobacterium avium spp. paratuberculosis was studied. The median standard plate count for bulk-tank milk from small ruminants was 4.70 log cfu/ml (4.69 log cfu/ml for goat's milk and 4.78 log cfu/ml for ewe's milk), with a minimum of 2.00 log cfu/ml and a maximum of 8.64 log cfu/ml. Enterobacteriaceae were detected in 212 (61.6%) goat's milk and 45 (71.4%) ewe's milk samples, whereas S. aureus was detected in 109 (31.7%) samples of goat's milk and 21 (33.3%) samples of ewe's milk. Campylobacter spp. and Salmonella spp. were not isolated from any of the samples. However, 16.3% of the goat's milk and 12.7% of the ewe's milk samples were polymerase chain reaction (PCR)-positive for Shiga toxin-producing E. coli. Seventy-nine (23.0%) goat's tank-milk and 15 (23.8%) ewe's tank-milk samples were PCR-positive for insertion sequence 900, providing presumptive evidence for the presence of M. avium ssp. paratuberculosis. These results form the basis for determining the microbiological quality standards for goat's and ewe's milk. Moreover, the data presented form part of the risk assessment program for raw milk from small ruminants in Switzerland.
Milk powder	**Drying risk assessment strategies.** Markowski AS, Mujumdar AS, Drying Technology 22 (1-2) : 395-412, 2004 **Abstract:** Drying risks assessment strategies are discussed with particular reference to the methodology involved and the various tools used at risk assessment. The methodology suggested is to apply qualitative risks assessment with risks estimation used for qualitative ranking of recommendations. The methodology follows, to a large extent, the general procedure for risks assessment of machinery and the application of the modified 'what if' hazard method with the risks estimation by a multi-layer risk matrix technique. A case study of risks assessment for the typical 2-stage spray-fluid bed dryer to produce milk powder is presented. Analysis indicated how an acceptable risk level may be achieved by the introduction of risks reduction options.
Farm	**Pathogens and manure management systems: a review. Bicudo JR, Goyal SM.** Environ Technol. 2003 Jan;24(1):115-30. There has been an increasing concern about the effects of pathogens that are present in animal manure on humanand animal health. In recent years, outbreaks of food-borne diseases associated with the consumption of animal products have received much attention from the media in North America and Europe, leading to increased consumer concerns about the safety of their food supply.

	The health risks associated with animal operations depend on various factors. The most important ones appear to be related to the animal species being reared and the concentration of pathogenic microorganisms in animal manure. The ability of the pathogens to survive for long periods and through treatment to remain infective in the environment until ingested by human or animal host is an added concern. On the other hand, the role of livestock in most waterborne bacterial outbreaks has often been difficult to clarify since both humans and various wildlife species can shed the same microorganisms and thereby serve as sources of infection. This paper summarizes existing information on the main microbial pathogens present in livestock wastes, and discusses the impact of livestock wastes and agricultural drainage on microbiological quality of water, as well as available management and treatment technologies to minimize the prevalence of pathogens in animal wastes. Despite the fact that most disease outbreaks have been associated with food poisoning by cross-contamination during meat or milk processing and during finished product storage this review shows that a number of best management practices and technical solutions have been developed in the last few years that can be effective tools in minimizing the spread of pathogens from livestock operations in the environment.
FMD and BSE/TSE	**Statement of the EFSA Scientific Expert Working Group on BSE/TSE of the BIOHAZ Panel on the Health Risks of the Consumption of Milk and Milk Derived Products from Goats** http://www.efsa.eu.int/science/biohaz/biohaz_documents/catindex_en.html **Source:** Panel on Biological Hazards, European Food Safety Authority **Author:** EFSA Scientific Expert Working Group on BSE/TSE, Scientific Panel on Biological Hazards, European Food Safety Authority **Summary:** Statement regarding the likelihood of milk and milk products from goats to present a risk of TSE contamination **Resource type:** statement **Publication Date:** November 24, 2004
	Risks of spreading foot and mouth disease through milk and dairy products. **Donaldson AI.** Rev Sci Tech. 1997 Apr;16(1):117-24. A review of epidemics of foot and mouth disease (FMD) has highlighted the important role which raw (untreated) milk can play in the spread of the disease in a country which is normally free of FMD and whose cattle are not routinely vaccinated. The greatest hazard is likely to be in the early stages of an outbreak, before disease control measures have been implemented. The spread of FMD through milk can be prevented by the effective application of control measures combined with 'codes of practice' for the treatment of potentially infected milk. The author considers the probable mechanisms of transmission of FMD by milk and dairy products. These mechanisms are based on the quantities of virus excreted in milk, the survival of the virus under various management and manufacturing conditions and the minimum doses required to initiate infection in susceptible animals by different routes. The key points for consideration when making a risk assessment of the importation of milk and dairy products are also discussed.
	Safety of milk and milk derivatives in relation to BSE: the lactoferrin example. **Vetrugno V.** Biometals. 2004 Jun;17(3):353-6. Bovine Spongiform Encephalopathy (BSE) belongs to Transmissible Spongiform Encephalopathies (TSEs) or Prion diseases. BSE is a feed borne infection of cattle. Epidemiological and laboratory data suggest that the BSE infectious agent is responsible for the variant form of Creutzfeldt-Jakob Disease (vCJD) and that the oral route is the most plausible way of infection. Therefore there is concern that the BSE agent can be transmitted to humans by biological materials (*i.e.* meat products, blood, milk) from susceptible BSE animal species (mostly cows but possibly, sheep and goats). Lactoferrin (LF) can be produced by purification from large volumes of cow's milk or whey. Therefore, a potential BSE risk for milk and milk products needs to be evaluated by risk assessment. The Committee for proprietary Medicinal Products--CPMP of the European Commission and the WHO have categorized risk tissues from TSE susceptible ruminant species in different classes in relation to the BSE risk for medicinal products. Milk, colostrum, and tissues of the mammary gland have been classified in the category of no detectable infectivity. A secondary contamination of milk can be virtually excluded (*i.e.* milk is taken from living animals).

	In the light of current scientific knowledge and irrespective of the geographical origin, milk and milk derivatives (*e.g.* lactoferrin, lactose) are unlikely to present any risk of TSE contamination provided that milk is sourced from healthy animals in the same conditions as milk collected from human consumption. So the risk of milk and milk derivatives in relation to BSE is negligible.
	[BSE: milk and risk potential?] [Article in German] **Heeschen WH.** Dtsch Tierarztl Wochenschr. 2002 Aug;109(8):350-3 A potential BSE risk for milk and milk products has to be evaluated by means of risk analysis, especially risk assessment. The 3rd element of risk assessment--hazard exposition--is of decisive significance. In 1997, the Scientific Steering Committee of the European Commission has categorized risk materials in 4 classes. Colostrum, milk and tissues of the mammary gland have been classified in category 4, *i.e.* "infectivity not detected". A secondary contamination of the milk can be excluded (living animals). However, the term "not detected" refers also to the low sensitivity of the mouse test, which has to be taken into consideration. Therefore, in 2000 investigations started in Great Britain to test milk fractions, especially the fraction of somatic cells, for the possible occurrence of prions, using newly developed and highly sensitive methods. Results can not be expected before 2003 at the earliest. In case prions would be detected, their biological activity has to be demonstrated in order to develop an appropriate risk assessment for the consumer. Investigations in Great Britain in the early nineties of the last century with suckling cows under practical conditions have shown no indications of a BSE transfer via the milk to the calves. Therefore, the statement of national and international organizations is still valid, that milk can be regarded safe according to the present state of scientific knowledge.
General approach in RA	**Practical approaches to risk assessment. Brooke-Taylor S.** Biomed Environ Sci. 2001 Jun;14(1-2):14-20. The importance of using risk assessment in developing food regulations is growing with the globalization of our food supply. The World Trade Organization has entrenched the principles of science-based risk assessment in the Agreement on Sanitary and Phytosanitary Measures. The relevant international organization for food standards, the Codex Alimentarius Commission, recognises risk analysis, and its component parts risk assessment, risk management and risk communication, as the basis for scientific decision-making. Risk assessment comprises two activities: hazard evaluation; and exposure estimation. A hazard may be chemical, microbiological or nutritional in origin. The practical application of risk assessment in Australia is illustrated in this presentation by four examples involving: (1) food additives, (2) microbiological safety of imported raw milk cheeses, (3) genetically modified foods and (4) imported food inspection.
	ILSI Europe Risk Analysis in Microbiology Task Force.Recontamination as a source of pathogens in processed foods. Reij MW, Den Aantrekker ED; Int J Food Microbiol. 2004 Feb 15;91(1):1-11. Food products that have been submitted to an adequate heat-treatment during processing are free of vegetative pathogens and, depending on the treatments, of sporeformers and are generally regarded as safe. Processed products such as pate, ice cream, infant formulae and others have nevertheless been responsible for food-borne illnesses. Thorough epidemiological investigations of several of these outbreaks have demonstrated that the presence of vegetative pathogens such as Salmonella spp. or Listeria monocytogenes in the consumed products was frequently due to post-process recontamination. The majority of studies on pathogens in foods are devoted to investigations on their presence in raw materials or on their growth and behaviour in the finished products. Reference to recontamination is, however, only made in relatively few publications and very little is published on the sources and routes of these pathogens into products after the final lethal processing step. The investigation of an outbreak, including epidemiological studies and typing of strains, is very useful to trace the origin and source of the hazard. Published data demonstrate that the presence of pathogens in the vicinity of unprotected product in processing lines represents a significant risk of recontamination. Microbiological Risk Assessment studies can be conducted as part of governmental activities determining appropriate protection levels for populations. Although recontamination has been identified as a relevant cause of food incidences, it is often not considered in such studies.

	This paper advocates that an effort should be made to develop our knowledge and information on recontamination further and start using it systematically in the exposure assessment part of Microbiological Risk Assessment studies.
GMO	**Biosafety assessment of the application of genetically modified Lactococcus lactis spp. in the production of fermented milk products.** Klijn N, Weerkamp AH, de Vos WM. Systematic and Applied Microbiology 18 (4) : 486-492, 1996 **Abstract:** Safety assessment of the use of genetically modified Lactococcus lactis in dairy products (cheese, fermented milks, fermented dairy products) is discussed. Aspects considered include: the introduction of genetically modified microorganisms in the food industry; clearance of genetically modified microorganisms for use in foods; principles of biosafety assessment (definition of risk, risk assessment, biological containment); biosafety assessment of genetically modified L. lactis in fermented dairy products (retrospective studies, survival in specific ecosystems (fermentation in milk and in cheesemaking), gene transfer (transfer of pAMbeta1 between Lactococcus spp.)); and hazard identification and normalization.
HACCP & QA	**Application of hazard analysis and critical control point system in the dairy industry. Kassem M, Salem E, Ahwal AM, Saddik M, Gomaa NF.** East Mediterr Health J. 2002 Jan;8(1):114-28 This study aimed to assess the hygiene quality of some packaged milk (pasteurized or sterilized) and dairy products before and after application of a hazard analysis and critical control point (HACCP) system at a milk and dairy products company in Cairo, Egypt. The steps taken to put HACCP in place are described and the process was monitored to assess its impact. Assessment of the hygiene quality of the milk and dairy products before and after HACCP showed an improvement in quality and an overall improvement in the conditions at the company.
	Implementing a quality assurance program using a risk assessment tool on dairy operations. Sischo WM, Kiernan NE, Burns CM, Byler LI. J Dairy Sci. 1997 Apr;80(4):777-87. Concerns and perceptions about antibiotic residues in milk prompted the dairy industry to develop a voluntary program to support rational antibiotic use on dairy farms. One deficiency of this program is the inability of producers to identify easily the weaknesses in antibiotic management in order to develop control plans. To overcome this deficiency, an educational approach was designed. The program centred on an on-farm risk assessment tool used by the producer and an industry educator to determine the current risk for residue violation. The risk assessment tool was tested by 25 field personnel working with northeastern milk receivers and 250 producers in seven states. The participants in the study identified a lack of adequate treatment records as being the highest risk factor for antibiotic residues, followed by deficiencies in understanding how to use antibiotics and poor relationships between veterinarians and their clients. When field representatives utilized the risk assessment tool, for most producers, risk of antibiotic residue decreased by approximately 19%. In particular, more farms kept written records or more complete records. Finally, producers with reported histories of antibiotic residues were less likely to implement management changes to reduce the risk of antibiotic residue.
Import	**Risk assessment on the importation of milk and milk products (excluding cheese) from countries not free from foot and mouth disease. Heng NH, Wilson DW.** Rev Sci Tech. 1993 Dec;12(4):1135-46. The authors discuss the risk assessment conducted by the Australian Quarantine and Inspection Service (AQIS) on the importation of milk and milk products (excluding cheese) from countries not free from foot and mouth disease (FMD). This assessment was undertaken in response to requests from countries wishing to export dairy products for sale on the Australian market. AQIS conducted a public consultation on the proposal, in line with Australian Government policy on transparency and accountability in the quarantine decision-making process. The authors examine the procedures involved in the investigation of the likely presence of FMD virus in milk of vaccinated and non-vaccinated cows, and of the heat treatment parameters effective in the inactivation of the virus. The data provide a useful aid in the assessment of the risk factors associated with the importation of milk and milk products, and in the development of quarantine conditions for importation.

Microbiological criteria	**Scientific Criteria to Ensure Safe Food** http://www.nap.edu/catalog/10690.html **Source:** National Academies Press **Author:** Committee on the Review of the Use of Scientific Criteria and Performance Standards for Safe Food, National Research Council **Summary:** This report presents recommendations for improving the food safety system in the U.S. It includes recommendations related to specific government agencies and for specific food product types (meat and poultry products, seafood, produce, and dairy products). There is also discussion of public health surveillance and food safety tools. Appendices include "Current and Proposed Definitions of Key Food Safety Terms," "Sanitation Performance Standards," "Food and Drug Administration and Environmental Protection Agency Guidance Levels in Seafoods," "Food Defect Action Levels in Produce," "International Microbiological Criteria," "International Microbiological Criteria for Dairy Products," "U.S. Department of Agriculture-Agricultural Marketing Service Standards for Milk and Dairy Products," and "Biographical Sketches of Committee and Subcommittee Members" **Resource type:** book, charts, tables **Publication Date:** 2003
Milk microbiological quality	**Grade "A" Pasteurized Milk Ordinance *2001 Revision*** http://www.cfsan.fda.gov/~ear/pmo01toc.html **Source:** Center for Food Safety and Applied Nutrition, Food and Drug Administration **Summary:** Recommended sanitary standards for Grade "A" raw milk for pasteurization and Grade "A" pasteurized milk and milk products for adoption by states, counties, and municipalities **Resource type:** standards **Publication Date:** May 15, 2002
Raw milk	**[Health risk due to the consumption of raw milk commercialized without due authorization]** [Article in Portuguese] **Badini KB, Nader Filho A, do Amaral LA, Germano PM.** Rev Saude Publica. 1996 Dec;30(6):549-52. Sixty raw milk samples commercialized without due authorization in the counties of Botucatu and S. Manuel, State of S. Paulo (Brazil), were submitted to mesophilic micro-organism and coagulase-positive Staphylococcus and most probable number of total coliform and fecal coliform counts. Forty-one (68.3%) and 50 (83.3%) of the samples were found, respectively to contain mesophilic microorganisms and total coliforms above the maximum limits established by the Health Ministry for type C pasteurized milk. Thirty (50.0%) and 11 (18.3%) of the samples were found, respectively, to the contaminated by coagulase-positive Staphylococcus and fecal coliforms. Only 5 (8.3%) samples were found to comply with the required legal standards. The results showed the unsatisfactory hygienic and sanitary conditions of the raw milk and suggest the existence of great risk to the health of the consumers, especially when the product is taken without being boiled.
Resources	**Australian Journal of Dairy Technology** http://www.diaa.asn.au/Index.html **Source:** Dairy Industry Association of Australia, Inc. **Summary:** This journal is the official journal of the Dairy Industry Association of Australia, Inc. **Resource type:** publication **Dairy Industry Association of Australia** http://www.diaa.asn.au/ **Summary:** Professional organization for the Australian dairy industry **Resource type:** website **International Dairy Federation** http://www.fil-idf.org/ **Source:** International Dairy Federation **Summary:** Organization for the dairy sector that works to promote milk and milk products worldwide.

	IDF is also the source of draft standards for milk products for adoption as Codex standards **Resource type:** website **International Dairy Journal** http://www.elsevier.com/gej-ng/29/81/27/32/show/Products/FOOD/jnl_index.htt **Source:** Food Science and Technology Program, Elsevier Science **Summary:** This journal publishes information on dairy science and technology, including microbiology, enzymology, biotechnology and bioengineering, dairy engineering and new developments in processing, raw material quality, milk assembly, analytical, nutritional, environmental, and legal subjects and more **Resource type:** publication

Appendix 7

Chemical Risk Assessment Framework

Evidence-based risk assessments underpin the development of food standards for chemicals. The risk assessment framework used to develop food standards for Australia and New Zealand are broadly based on the principles and procedures recommended by the international food standards setting body, the Codex Alimentarius Commission (CAC, 2005). The steps used by FSANZ to identify and quantify risks associated with chemicals in food are described briefly below and in the FSANZ framework document (ANZFA, 1996a).

Hazard identification and characterisation

The first two steps in a risk assessment process are hazard identification and characterisation. Chemical hazards are identified through standard toxicity tests performed according to internationally accepted protocols such as those published by the Organisation for Economic Cooperation and Development (OECD, 1993). Hazard characterisation considers the dose-response relationship for particular hazards and, if possible, establishes an intake level considered to be safe for the vast majority of the population.

Chemicals intentionally used in food production

FSANZ uses a cautious approach when assessing the safety of chemicals intentionally added to food. For food additives and agricultural and veterinary chemicals, there is generally sufficient data available to identify and characterise hazards and to establish a safe level of human exposure to these chemicals, as determined by the Office of Chemical Safety for the APVMA. Various international bodies, such as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Joint FAO/WHO Meeting on Pesticide Residues (JMPR), have also established safe levels of exposure for these chemicals. The acceptable daily intake (ADI) intake is the amount of the chemical, which may be safely consumed by a human over a lifetime without appreciable risk. The ADI is usually derived from experiments in animals in which a no-observed effect level (NOEL) is determined. Generally the NOEL for the most sensitive animal species is then divided by a safety factor, usually 100, to arrive at the ADI.

Chemicals unintentionally present in food

For many chemicals unintentionally present in food such as contaminants, there is a paucity of reliable data on which to identify and characterise hazards and thus to establish a safe level of human exposure. The reference value used to indicate the safe level of intake of a contaminant is the so-called ‘tolerable intake’, which can be calculated on a daily, weekly or monthly basis. Reference values, which define an acceptable level of exposure to a contaminant, are established internationally by JECFA. The tolerable intake (TI) is generally referred to as ‘provisional’ since there is often a lack of data on the consequences of human exposure at low levels, and new data may result in a change to the tolerable level. For contaminants that may accumulate in the body over time such as lead, cadmium and mercury, the provisional tolerable weekly intake (PTWI) or monthly intake (PTMI) is used as a reference value in order to minimise the significance of daily variations in intake. For contaminants that do not accumulate in the body, such as arsenic, the provisional tolerable daily intake (PTDI) can be used.

Exposure evaluation

Estimation of exposure to chemicals in food depends on the knowledge of the level of the substance in food, coupled with knowledge of the amount of each food consumed, though there is a degree of uncertainty associated with both of these parameters. With respect to food contaminants the level of contamination of food is influenced by a variety of factors such as geographic and climatic conditions, agricultural practices, local industrial activity and food preparation and storage conditions.

The level of exposure to a substance in food, as consumed, can be determined from food surveillance data when available. Different methods of dietary modelling combine data on the levels of substances in food with food consumption data in different ways to provide estimates of the daily or weekly dietary exposure to a particular substance from food commodities for all sections of the population for which food consumption data are available.

Australian Total Diet Study

FSANZ monitors the food supply to ensure that existing food regulatory measures provide adequate protection to consumer health and safety. The Australian Total Diet Study (ATDS) is part of that monitoring.

The ATDS, formerly known as the Australian Market Basket Survey, is a comprehensive assessment of consumers' dietary exposure (intake) to pesticide residues, contaminants and other substances. The survey is conducted approximately every two years.

The survey estimates the level of dietary exposure of the Australian population through the testing of food representative of the total diet. In order to achieve more accurate dietary exposure, the foods examined in the ATDS are prepared to a 'table ready' state before they are analysed. As a consequence, both raw, processed and cooked foods are examined from both domestic and international sources.

FSANZ coordinate the survey while the States and the Northern Territory purchase and prepare the food samples.

Dietary modelling

Dietary exposure assessments are conducted using dietary modelling techniques that combine food consumption data with food chemical concentration data to estimate the exposure to the food chemical from the diet. The assessment of the dietary exposure is conducted using FSANZ's dietary modelling computer program, DIAMOND.

Dietary exposure = food chemical concentration x food consumption

Exposures are estimated by combining usual patterns of food consumption, as derived from national nutrition survey (NNS) data, with both current and proposed levels of use of the food chemicals in the foods.

Risk characterisation

Risk characterisation brings together information on the hazard characterisation and on level of exposure to the substance in food for various population groups in order to characterise the risk for various population groups. This might be expressed in terms of a margin-of-safety between an ADI or TI level and the known level of human exposure via the whole diet.

Appendix 8

Regulatory Framework for Agricultural and Veterinary Chemicals

Agricultural and veterinary chemical regulation

The Australian Pesticides and Veterinary Medicines Authority (APVMA) is responsible for regulating the manufacture, import and supply of all Agvet chemicals onto the Australian market.

As of August 2005, Standard 1.4.2 had MRLs for 244 chemicals in Schedule 1 – Maximum Residue Limits and 7 chemicals listed in Schedule 2 – Extraneous Residue Limits, in association with dairy products (Appendix 3). The list includes veterinary medicines used for prophylaxis and growth promotion, and agricultural chemicals used as crop and grain protection agents.

Manufacturers of agricultural and veterinary chemical products must have their manufacturing premises licensed by the APVMA to produce specified chemicals. They must also comply with Codes of Good Manufacturing Practice (GMP). No veterinary or agricultural chemical product (including imported products) can be legally supplied in Australia without being registered by the APVMA. All products must be supplied with an APVMA approved label.

Veterinary and agricultural chemical products that are not registered by the APVMA can only be supplied in accordance with specific APVMA permits, such as for the purposes of conducting experimental trials. The only other instance where unregistered chemical products can be used is by a veterinary practitioner who may prescribe to an animal under his or her care.

No hormones for dairy cattle are included in the MRL Standard of the Code. This is consistent with dairy industry practices, which has seen the use of hormone treatments for growth promotant purposes banned since the 1960's. The Commonwealth Government's National Residue Survey program tests for hormonal growth promotants in beef cattle and sheep edible and non-edible (i.e. urine and faeces) matrices.

Maximum residue limits

Maximum residue limits (MRLs) for agricultural and veterinary chemicals 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. MRLs relevant to dairy produce are listed in Appendix 3.

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 by the APVMA.

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 particular foods and Schedule 2 lists all extraneous agricultural chemical limits in particular 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 and no detectable residue of any metabolites of that chemical in food (whether or not that the particular food is listed in the schedules).

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 GAP. In reality, human health is rarely an issue since even at the MRL the level of dietary intake is well below the ADI established from animal studies.

In regard to MRLs in milk, the APVMA and JECFA both recommend MRLs based on residues on an individual animal basis. This method is adopted world-wide and is documented in Codex policy both in the EU and the USA. Hence, the APVMA's recommendations to FSANZ for milk MRLs have individual cows as the basis for its recommendations. This is the case for all milk MRLs.

Stockfeed MRLs

Stockfeed is also subject to treatment with crop and grain protection agents. Sources of residues may result from applications made during the growth of the crop and also post-harvest, for protection mainly against fungal and insect infestation (covered in Section 3.2.2). The APVMA have established guidelines for MRLs based on livestock dietary exposure and internationally accepted methodology (APVMA 2002). Stockfeed legislation in some States directly includes reference to the APVMA MRL Standard (Table 1 and Table 4) as the legislative control for stockfeed legislation.

Animal feed controls are currently under review in Australia with the aim of developing an enhanced national capability framework.

Appendix 9

Maximum Residue Limits

Residue limits for agricultural and veterinary chemicals approved for use in dairy products used in food as of March 2005 listed in the Code Standard 1.4.2, Schedules 1 and 2. Maximum residue limits (MRLs) are expressed in milligrams of the chemical per kilogram of the food (mg/kg).

The portion of the commodity to which the MRL applies (and which is analysed) is the whole commodity. When an MRL for cattle milk or milks is qualified by "(in the fat)" the compound is regarded as fat-soluble, and the MRL applies to the fat portion of the milk. In the case of a derived or a manufactured milk product with a fat content of 2% or more, the MRL also applies to the fat portion. For a milk product with a fat content less than 2%, the MRL applied should be 1/50 that specified for "milk (in the fat)", and should apply to the whole product (as defined in the Code Section 1.4.2 schedule 4).

Note that "cattle milk" refers to bovine milk and "milks" refers to all mammalian milk.

- *: an asterix denotes that the maximum residue limit or the extraneous residue limit is set at or about the limit of determination.
- T: a 'T' denotes that the maximum residue limit or the extraneous residue limit is a temporary residue limit or extraneous residue limit.
- E: an 'E' denotes extraneous residue limit

** A recent review of Endosulphan has resulted in changes in the MRL Standard for Endosulfan found in milk together with changes to livestock feeding restraints and label approvals.

ABAMECTIN SUM OF AVERMECTIN B 1A, AVERMECTIN B 1B AND D-8,9 ISOMER OF AVERMECTIN B 1A	
CATTLE MILK	0.02

ACETAMIPRID *COMMODITIES OF PLANT ORIGIN*: ACETAMIPRID *COMMODITIES OF ANIMAL ORIGIN*: SUM OF ACETAMIPRID AND N-DIMETHYL ACETAMIPRID ((E)-N^1-[(6-CHLORO-3-PYRIDYL)METHYL]-N^2-CYANOACETAMIDINE), EXPRESSED AS ACETAMIPRID	
MILKS	*0.01

ACIFLUORFEN ACIFLUORFEN	
MILKS	*0.01

ALDICARB SUM OF ALDICARB, ITS SULFOXIDE AND ITS SULFONE, EXPRESSED AS ALDICARB	
MILKS	*0.01

ALDOXYCARB SUM OF ALDOXYCARB AND ITS SULFONE, EXPRESSED AS ALDOXYCARB	
MILKS	*0.02

ALIPHATIC ALCOHOL ETHOXYLATES ALIPHATIC ALCOHOL ETHOXYLATES	
CATTLE MILK	1

AMETRYN AMETRYN	
MILKS	*0.05

AMITRAZ SUM OF AMITRAZ AND N-(2,4-DIMETHYLPHENYL)-N'-METHYLFORMAMIDINE, EXPRESSED AS AMITRAZ	
MILKS	0.1

AMITROLE AMITROLE	
MILKS	*0.01

AMOXYCILLIN INHIBITORY SUBSTANCE, IDENTIFIED AS AMOXYCILLIN	
CATTLE MILK	*0.01
SHEEP MILK	*0.01

AMPICILLIN INHIBITORY SUBSTANCE, IDENTIFIED AS AMPICILLIN	
CATTLE MILK	*0.01

ASULAM ASULAM	
MILKS	*0.1

ATRAZINE ATRAZINE	
MILKS	T*0.01

AVOPARCIN AVOPARCIN	
MILKS	*0.01

AZINPHOS-METHYL AZINPHOS-METHYL	
MILKS	*0.05

AZOXYSTROBIN AZOXYSTROBIN	
MILKS	0.005

BACITRACIN INHIBITORY SUBSTANCE, IDENTIFIED AS BACITRACIN	
MILKS	*0.5

BENDIOCARB COMMODITIES OF PLANT ORIGIN: UNCONJUGATED BENDIOCARB; COMMODITIES OF ANIMAL ORIGIN: SUM OF CONJUGATED AND UNCONJUGATED BENDIOCARB, 2,2-DIMETHYL-1,3-BENZODIOXOL-4-OL AND N-HYDROXYMETHYLBENDIOCARB, EXPRESSED AS BENDIOCARB	
MILKS	0.1

BENFLURALIN BENFLURALIN	
MILKS	T*0.01

BENTAZONE BENTAZONE	
MILKS	*0.05

BENZYL G PENICILLIN INHIBITORY SUBSTANCE, IDENTIFIED AS BENZYL G PENICILLIN	
MILKS	*0.0015

BIFENAZATE SUM OF BIFENAZATE AND BIFENAZATE DIAZENE (DIAZENECARBOLXYLIC ACID, 2-(4-METHOXY-[1,1'-BIPHENYL-3-YL] 1-METHYLETHYL ESTER), EXPRESSED AS BIFENAZATE	
MILKS	*0.01

BIFENTHRIN BIFENTHRIN	
MILKS	0.5

BITERTANOL BITERTANOL	
MILKS	0.2

BROMACIL BROMACIL	
MILKS	*0.04
BROMOXYNIL BROMOXYNIL	
MILKS	*0.02
BUPROFEZIN BUPROFEZIN	
MILKS	*0.01
BUTAFENACIL BUTAFENACIL	
MILKS	*0.01
BUTROXYDIM BUTROXYDIM	
MILKS	*0.01
CAPTAN CAPTAN	
MILKS	*0.01
CARBARYL CARBARYL	
MILKS	T*0.05
CARBENDAZIM SUM OF CARBENDAZIM AND 2-AMINOBENZIMIDAZOLE, EXPRESSED AS CARBENDAZIM	
MILKS	*0.1
CARBETAMIDE CARBETAMIDE	
MILKS	*0.1
CARBOFURAN SUM OF CARBOFURAN AND 3-HYDROXYCARBOFURAN, EXPRESSED AS CARBOFURAN	
MILKS	*0.05
CARFENTRAZONE-ETHYL CARFENTRAZONE-ETHYL	
MILKS	*0.025
CEFTIOFUR DESFUROYLCEFTIOFUR	
CATTLE MILK	0.1
CEFUROXIME INHIBITORY SUBSTANCE, IDENTIFIED AS CEFUROXIME	
CATTLE MILK	*0.1

CEPHALONIUM INHIBITORY SUBSTANCE, IDENTIFIED AS CEPHALONIUM	
CATTLE MILK	*0.02
CEPHAPIRIN CEPHAPIRIN AND DES-ACETYLCEPHAPIRIN, EXPRESSED AS CEPHAPIRIN	
CATTLE MILK	*0.01
CHLORFENAPYR CHLORFENAPYR	
MILKS	*0.01
CHLORFENVINPHOS CHLORFENVINPHOS, SUM OF E AND Z ISOMERS	
CATTLE MILK (IN THE FAT)	T0.2
CHLORFLUAZURON CHLORFLUAZURON	
CATTLE MILK	0.1
CHLORHEXIDINE CHLORHEXIDINE	
MILKS	0.05
CHLORMEQUAT CHLORMEQUAT CATION	
MILKS	*0.1
CHLORPYRIFOS CHLORPYRIFOS	
MILKS (IN THE FAT)	T0.2
CHLORPYRIFOS-METHYL CHLORPYRIFOS-METHYL	
MILKS (IN THE FAT)	*0.05
CHLORSULFURON CHLORSULFURON	
MILKS	*0.05
CHLORTHAL-DIMETHYL CHLORTHAL-DIMETHYL	
MILKS	*0.05
CLAVULANIC ACID CLAVULANIC ACID	
CATTLE MILK	*0.01
CLODINAFOP-PROPARGYL CLODINAFOP-PROPARGYL	
MILKS	*0.05

CLODINAFOP ACID (R)-2-[4-(5-CHLORO-3-FLUORO-2-PYRIDINYLOXY) PHENOXY] PROPANOIC ACID	
MILKS	*0.1
CLOPYRALID CLOPYRALID	
MILKS	0.05
CLOQUINTOCET-MEXYL CLOQUINTOCET-MEXYL	
MILKS	*0.05
CLOQUINTOCET ACID 5-CHLORO-8-QUINOLINOXYACETIC ACID	
MILKS	*0.1
CLORSULON CLORSULON	
CATTLE MILK	1.5
CLOXACILLIN INHIBITORY SUBSTANCE, IDENTIFIED AS CLOXACILLIN	
CATTLE MILK	*0.01
COUMAPHOS SUM OF COUMAPHOS AND ITS OXYGEN ANALOGUE, EXPRESSED AS COUMAPHOS	
MILKS (IN THE FAT)	0.1
CYCLANILIDE SUM OF CYCLANILIDE AND ITS METHYL ESTER, EXPRESSED AS CYCLANILIDE	
MILKS	0.05
CYFLUTHRIN CYFLUTHRIN, SUM OF ISOMERS	
MILKS	0.1
CYHALOTHRIN CYHALOTHRIN, SUM OF ISOMERS	
MILKS (IN THE FAT)	0.5
CYPERMETHRIN CYPERMETHRIN, SUM OF ISOMERS	
MILKS (IN THE FAT)	1
CYPROCONAZOLE CYPROCONAZOLE, SUM OF ISOMERS	
MILKS	*0.01
CYPRODINIL CYPRODINIL	
MILKS	*0.01

CYROMAZINE CYROMAZINE	
MILKS	*0.01
2,4-D 2, 4-D	
MILKS	*0.05
DAMINOZIDE DAMINOZIDE	
MILKS	*0.05
2,4-DB 2, 4-DB	
MILKS	*0.05
DELTAMETHRIN DELTAMETHRIN	
CATTLE MILK (IN THE FAT)	0.5
GOAT MILK (IN THE FAT)	0.2
SHEEP MILK (IN THE FAT)	0.2
DEXAMETHASONE AND DEXAMETHASONE TRIMETHYLACETATE DEXAMETHASONE	
CATTLE MILK	*0.05
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	
MILKS	*0.02
DIAZINON DIAZINON	
MILKS (IN THE FAT)	0.5
DICAMBA DICAMBA	
MILKS	0.1
DICHLORVOS DICHLORVOS	
MILKS	0.02
DICLOFOP-METHYL DICLOFOP-METHYL	
MILKS	*0.05
DIFENOCONAZOLE DIFENOCONAZOLE	
MILKS	*0.01
DIFLUBENZURON DIFLUBENZURON	
CATTLE MILK	0.05

SHEEP MILK	0.05
DIFLUFENICAN DIFLUFENICAN	
MILKS	0.01
DIMETHIPIN DIMETHIPIN	
MILKS	*0.01
DIMETHOATE SUM OF DIMETHOATE AND OMETHOATE, EXPRESSED AS DIMETHOATE *SEE ALSO* OMETHOATE	
MILKS	*0.05
DIMETHOMORPH SUM OF E AND Z ISOMERS OF DIMETHOMORPH	
MILKS	*0.01
DIQUAT DIQUAT CATION	
MILKS	*0.01
DISULFOTON SUM OF DISULFOTON AND DEMETON-S AND THEIR SULFOXIDES AND SULFONES, EXPRESSED AS DISULFOTON	
MILKS	0.01
DITHIOCARBAMATES TOTAL DITHIOCARBAMATES, DETERMINED AS CARBON DISULPHIDE EVOLVED DURING ACID DIGESTION AND EXPRESSED AS MILLIGRAMS OF CARBON DISULPHIDE PER KILOGRAM OF FOOD	
MILKS	*0.2
DIURON SUM OF DIURON AND 3,4- DICHLOROANILINE, EXPRESSED AS DIURON	
CATTLE MILK	0.1
DORAMECTIN DORAMECTIN	
CATTLE MILK	T0.06
2,2-DPA 2,2-DICHLOROPROPIONIC ACID	
MILKS	*0.1
EMAMECTIN EMAMECTIN B1A, PLUS ITS 8,9-Z ISOMER AND EMAMECTIN B1B, PLUS ITS 8,9-Z ISOMER	
MILKS	*0.0005

ENDOSULFAN** SUM OF A- AND B- ENDOSULFAN AND ENDOSULFAN SULPHATE	
MILKS (IN THE FAT)	T0.5
EPRINOMECTIN EPRINOMECTIN B1A	
CATTLE MILK	0.03
EPTC EPTC	
MILKS	*0.1
ERYTHROMYCIN INHIBITORY SUBSTANCE, IDENTIFIED AS ERYTHROMYCIN	
MILKS	*0.04
ESFENVALERATE *SEE* FENVALERATE	
ETHAMETSULFURON METHYL ETHAMETSULFURON METHYL	
MILKS	*0.02
ETHEPHON ETHEPHON	
MILKS	0.1
ETHION ETHION	
MILKS (IN THE FAT)	0.5
ETHOFUMESATE ETHOFUMESATE	
MILKS (IN THE FAT)	0.2
FENAMIPHOS SUM OF FENAMIPHOS, ITS SULFOXIDE AND SULFONE, EXPRESSED AS FENAMIPHOS	
MILKS	*0.005
FENBENDAZOLE FENBENDAZOLE	
MILKS	0.1
FENHEXAMID FENHEXAMID	
MILKS	*0.01
FENITROTHION FENITROTHION	
MILKS (IN THE FAT)	T*0.05

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	
MILKS	0.02
FENTHION SUM OF FENTHION, ITS OXYGEN ANALOGUE, AND THEIR SULFOXIDES AND SULFONES, EXPRESSED AS FENTHION	
MILKS	T0.2
FENVALERATE FENVALERATE, SUM OF ISOMERS	
MILKS (IN THE FAT)	0.2
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)	
MILKS	0.01
FLAMPROP-METHYL FLAMPROP-METHYL	
MILKS	*0.01
FLAVOPHOSPHOLIPOL FLAVOPHOSPHOLIPOL	
CATTLE MILK	T*0.01
FLUAZIFOP-BUTYL FLUAZIFOP-BUTYL	
MILKS	0.1
FLUCYTHRINATE FLUCYTHRINATE	
MILKS	*0.05
FLUDIOXONIL FLUDIOXONIL	
MILKS	*0.01
FLUMETHRIN FLUMETHRIN, SUM OF ISOMERS	
MILKS	T0.05

FLUMETSULAM FLUMETSULAM	
MILKS	*0.1
FLUQUINCONAZOLE FLUQUINCONAZOLE	
MILKS	0.1
FLUROXYPYR FLUROXYPYR	
MILKS	0.1
FLUTOLANIL *COMMODITIES OF PLANT ORIGIN*: FLUTOLANIL *COMMODITIES OF ANIMAL ORIGIN*: FLUTOLANIL AND METABOLITES HYDROLYSED TO 2-TRIFLUOROMETHYL-BENZOIC ACID AND EXPRESSED AS FLUTOLANIL	
MILKS	*0.05
FLUTRIAFOL FLUTRIAFOL	
MILKS	*0.05
GLUFOSINATE AND GLUFOSINATE-AMMONIUM SUM OF GLUFOSINATE-AMMONIUM, N-ACETYL GLUFOSINATE AND 3-[HYDROXY(METHYL)-PHOSPHINOL] PROPIONIC ACID, EXPRESSED AS GLUFOSINATE (FREE ACID)	
MILKS	*0.05
GLYPHOSATE GLYPHOSATE	
MILKS	*0.1
HALOSULFURON-METHYL HALOSULFURON-METHYL	
MILKS	T*0.01
HALOXYFOP SUM OF HALOXYFOP, ITS ESTERS AND CONJUGATES, EXPRESSED AS HALOXYFOP	
MILKS	0.02
HEXAZINONE HEXAZINONE	
MILKS	*0.05
IMAZAMOX IMAZAMOX	
MILKS	*0.05
IMAZAPIC SUM OF IMAZAPIC AND ITS HYDROXYMETHYL DERIVATIVE	
MILKS	*0.01

IMAZAPYR IMAZAPYR	
MILKS	*0.01

IMAZETHAPYR IMAZETHAPYR	
MILKS	*0.1

IMIDACLOPRID SUM OF IMIDACLOPRID AND METABOLITES CONTAINING THE 6-CHLOROPYRIDINYMETHYLENEMOIETY, EXPRESSED AS IMIDACLOPRID	
MILKS	0.05

IMIDOCARB (DIPROPIONATE SALT) IMIDOCARB	
CATTLE MILK	0.2

INDOXACARB INDOXACARB	
MILKS	0.05

IODOSULFURON METHYL IODOSULFURON METHYL	
MILKS	*0.01

IPRODIONE IPRODIONE	
MILKS	*0.1

ISOXAFLUTOLE THE SUM OF ISOXAFLUTOLE, 2-CYCLOPROPYLCARCONYL-3-(2-METHYLSULFONYL-4-TRIFLUOROMETHYLPHENYL)-3-OXOPROPANENITRILE AND 2-METHYLSULFONYL-4-TRIFLUOROMETHYLBENZOIC ACID EXPRESSED AS ISOXAFLUTOLE	
MILKS	T*0.05

IVERMECTIN H_2B_{1A}	
CATTLE MILK	0.05

KETOPROFEN KETOPROFEN	
CATTLE MILK	*0.05

KRESOXIM-METHYL *COMMODITIES OF PLANT ORIGIN*: KRESOXIM-METHYL *COMMODITIES OF ANIMAL ORIGIN*: SUM OF A-(P-HYDROXY-O-TOLYLOXY)-O-TOLYL (METHOXYIMINO) ACETIC ACID AND (E)-METHOXYIMINO[A-(O-TOLYLOXY)-O-TOLYL]ACETIC ACID, EXPRESSED AS KRESOXIM-METHYL	
MILKS	*0.001

LASALOCID LASALOCID	
CATTLE MILK	*0.01

LEVAMISOLE LEVAMISOLE	
GOAT MILK	0.1
MILKS [EXCEPT GOAT MILK]	0.3

LINCOMYCIN INHIBITORY SUBSTANCE, IDENTIFIED AS LINCOMYCIN	
CATTLE MILK	*0.02
GOAT MILK	*0.1

LINURON SUM OF LINURON PLUS 3,4-DICHLOROANILINE, EXPRESSED AS LINURON	
MILKS	*0.05

LUFENURON LUFENURON	
MILKS	T0.2

MALDISON MALDISON	
MILKS (IN THE FAT)	1

MCPA MCPA	
MILKS	*0.05

MCPB MCPB	
MILKS	*0.05

MEBENDAZOLE MEBENDAZOLE	
MILKS	0.02

MECOPROP MECOPROP	
MILKS	*0.05

MEFENPYR-DIETHYL MEFENPYR-DIETHYL	
MILKS	*0.01

MELOXICAM MELOXICAM	
CATTLE MILK	0.005

MEPIQUAT MEPIQUAT	
MILKS	0.05

MESOSULFURON-METHYL MESOSULFURON-METHYL	
MILKS	*0.01

METALAXYL METALAXYL	
MILKS	T*0.05
METHAMIDOPHOS METHAMIDOPHOS *SEE ALSO* ACEPHATE	
MILKS	*0.01
METHIDATHION METHIDATHION	
MILKS (IN THE FAT)	0.5
METHOMYL SUM OF METHOMYL AND METHYL HYDROXYTHIOACETIMIDATE ('METHOMYL OXIME'), EXPRESSED AS METHOMYL *SEE ALSO* THIODICARB	
MILKS	0.05
METHOPRENE METHOPRENE, SUM OF CIS- AND TRANS-ISOMERS	
CATTLE MILK	0.1
METHOXYFENOZIDE METHOXYFENOZIDE	
MILKS	*0.01
METOLACHLOR METOLACHLOR	
MILKS	*0.05
METOSULAM METOSULAM	
MILKS	*0.01
METRIBUZIN METRIBUZIN	
MILKS	*0.05
METSULFURON-METHYL METSULFURON-METHYL	
MILKS	*0.1
MEVINPHOS MEVINPHOS	
MILKS	*0.05
MONENSIN MONENSIN	
CATTLE MILK	*0.01
MORANTEL MORANTEL	
MILKS	*0.1
MOXIDECTIN MOXIDECTIN	
CATTLE MILK (IN THE FAT)	2

NALED SUM OF NALED AND DICHLORVOS, EXPRESSED AS NALED	
MILKS	T*0.05
NEOMYCIN INHIBITORY SUBSTANCE, IDENTIFIED AS NEOMYCIN	
MILK	T1.5
NOVOBIOCIN NOVOBIOCIN	
CATTLE MILK	*0.1
OMETHOATE OMETHOATE *SEE ALSO* DIMETHOATE	
MILKS	*0.05
OXABETRINIL OXABETRINIL	
MILKS	*0.05
OXAMYL SUM OF OXAMYL AND 2-HYDROXYIMINO-N,N-DIMETHYL-2-(METHYLTHIO)-ACETAMIDE, EXPRESSED AS OXAMYL	
MILKS	*0.02
OXFENDAZOLE OXFENDAZOLE	
MILKS	0.1
OXYCLOZANIDE OXYCLOZANIDE	
MILKS	0.05
OXYDEMETON-METHYL SUM OF OXYDEMETON-METHYL AND DEMETON-S-METHYL SULPHONE, EXPRESSED AS OXYDEMETON-METHYL	
MILKS	*0.01
OXYFLUORFEN OXYFLUORFEN	
MILKS	*0.01
OXYTETRACYCLINE INHIBITORY SUBSTANCE, IDENTIFIED AS OXYTETRACYCLINE	
MILKS	0.1
PARAQUAT PARAQUAT CATION	
MILKS	*0.01
PARATHION-METHYL PARATHION-METHYL	
MILKS	T*0.05

PARBENDAZOLE PARBENDAZOLE	
MILKS	*0.1
PENDIMETHALIN PENDIMETHALIN	
MILK	*0.01
PERMETHRIN PERMETHRIN, SUM OF ISOMERS	
MILKS	0.05
PHENMEDIPHAM PHENMEDIPHAM	
MILKS	*0.1
PHENOTHRIN SUM OF PHENOTHRIN (+)CIS- AND (+)TRANS-ISOMERS	
MILKS	*0.05
PHORATE SUM OF PHORATE, ITS OXYGEN ANALOGUE, AND THEIR SULFOXIDES AND SULFONES, EXPRESSED AS PHORATE	
MILKS	*0.05
PHOSMET SUM OF PHOSMET AND ITS OXYGEN ANALOGUE, EXPRESSED AS PHOSMET	
MILKS (IN THE FAT)	0.2
PICLORAM PICLORAM	
MILKS	*0.05
PICOLINAFEN *COMMODITIES OF PLANT ORIGIN*: PICOLINAFEN *COMMODITIES OF ANIMAL ORIGIN*: SUM OF PICOLINAFEN AND 6-[3-TRIFLUOROMETHYL PHENOXY]-2-PYRIDINE CARBOXYLIC ACID	
MILKS	*0.01
PIPERONYL BUTOXIDE PIPERONYL BUTOXIDE	
CATTLE MILK	0.05
PIRIMICARB SUM OF PIRIMICARB, DIMETHYL-PIRIMICARB AND N-FORMYL-(METHYLAMINO) ANALOGUE (DIMETHYLFORMAMIDIO-PIRIMICARB), EXPRESSED AS PIRIMICARB	
MILKS	*0.1
PIRIMIPHOS-METHYL PIRIMIPHOS-METHYL	
MILKS	*0.05

PROCAINE PENICILLIN INHIBITORY SUBSTANCE, IDENTIFIED AS PROCAINE PENICILLIN	
MILKS	*0.0025
PROCYMIDONE PROCYMIDONE	
MILKS	0.02
PROFENOFOS PROFENOFOS	
CATTLE MILK	*0.01
PROMETRYN PROMETRYN	
CATTLE MILK	*0.05
PROPANIL PROPANIL	
MILKS	*0.01
PROPAQUIZAFOP PROPAQUIZAFOP AND ACID AND OXOPHENOXY METABOLITES, MEASURED AS 6-CHLORO-2-METHOXYQUINOXALINE, EXPRESSED AS PROPAQUIZAFOP	
MILKS	*0.01
PROPARGITE	
MILKS	*0.1
PROPICONAZOLE PROPICONAZOLE	
MILKS	*0.01
PROPYZAMIDE PROPYZAMIDE	
MILKS	*0.01
PYMETROZINE PYMETROZINE	
MILKS	*0.01
PYRIDATE SUM OF PYRIDATE AND METABOLITES CONTAINING 6 CHLORO-4-HYDROXYL-3-PHENYL PYRIDAZINE, EXPRESSED AS PYRIDATE	
MILKS	*0.2
PYRIMETHANIL PYRIMETHANIL	
MILKS	*0.01
PYRIPROXYFEN PYRIPROXYFEN	
MILKS	T*0.02

PYRITHIOBAC SODIUM PYRITHIOBAC SODIUM	
MILKS	*0.02

QUINOXYFEN QUINOXYFEN	
MILKS	0.01

QUIZALOFOP-ETHYL SUM OF QUIZALOFOP-ETHYL AND QUIZALOFOP ID ACID AND OTHER ESTERS, EXPRESSED AS QUIZALOFOP-ETHYL	
MILKS	0.1

QUIZALOFOP-P-TEFURYL SUM OF QUIZALOFOP-P-TEFURYL AND QUIZALOFOP ACID, EXPRESSED AS QUIZALOFOP-P-TEFURYL	
MILKS	0.1

SETHOXYDIM SUM OF SETHOXYDIM AND METABOLITES CONTAINING THE 5-(2-ETHYLTHIOPROPYL)CYCLOHEXENE-3-ONE AND 5-HYDROXYCYCLOHEXENE-3-ONE MOIETIES AND THEIR SULFOXIDES AND SULFOXIDES AND SULFONES, EXPRESSED AS SETHOXYDIM	
MILKS	*0.05

SIMAZINE SIMAZINE	
MILKS	*0.01

SPECTINOMYCIN INHIBITORY SUBSTANCE, IDENTIFIED AS SPECTINOMYCIN	
GOAT MILK	*2

SPINOSAD SUM OF SPINOSYN A AND SPINOSYN D	
MILKS	0.02

SPIROXAMINE *COMMODITIES OF PLANT ORIGIN*: SPIROXAMINE *COMMODITIES OF ANIMAL ORIGIN*: SPIROXAMINE CARBOXYLIC ACID, EXPRESSED AS SPIROXAMINE	
MILKS	0.05

STREPTOMYCIN AND DIHYDROSTREPTOMYCIN INHIBITORY SUBSTANCE, IDENTIFIED AS STREPTOMYCIN OR DIHYDROSTREPTOMYCIN	
MILKS	*0.2

SULFOSULFURON SUM OF SULFOSULFURON AND ITS METABOLITES WHICH CAN BE HYDROLYSED TO 2-(ETHYLSULFONYL)IMIDAZO[1,2-A]PYRIDINE, EXPRESSED AS SULFOSULFURON	
MILKS	*0.005

SULPHADIAZINE SULPHADIAZINE	
CATTLE MILK	0.1

SULPHADOXINE SULPHADOXINE	
CATTLE MILK	*0.1

SULPHATROXOZOLE SULPHATROXOZOLE	
CATTLE MILK	0.1

TEBUCONAZOLE TEBUCONAZOLE	
MILKS	0.05

TEBUFENOZIDE TEBUFENOZIDE	
MILKS	*0.01

TEBUTHIURON SUM OF TEBUTHIURON, AND HYDROXYDIMETHYLETHYL, N-DIMETHYL AND HYDROXY METHYLAMINE METABOLITES, EXPRESSED AS TEBUTHIURON	
MILKS	0.2

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	
MILKS	*0.02

TERBUFOS SUM OF TERBUFOS, ITS OXYGEN ANALOGUE AND THEIR SULFOXIDES AND SULFONES, EXPRESSED AS TERBUFOS	
CATTLE MILK	*0.01

TERBUTRYN TERBUTRYN	
MILKS	0.1

TETRACHLORVINPHOS TETRACHLORVINPHOS	
MILKS (IN THE FAT)	0.05

TETRACYCLINE INHIBITORY SUBSTANCE, IDENTIFIED AS TETRACYCLINE	
MILKS	*0.1

THIABENDAZOLE THIABENDAZOLE OR, IN THE CASE OF ANIMAL PRODUCTS, SUM OF THIABENDAZOLE AND 5-HYDROXYTHIABENDAZOLE, EXPRESSED AS THIABENDAZOLE	
MILKS	0.05
THIACLOPRID THIACLOPRID	
MILKS	*0.01
THIDIAZURON THIDIAZURON	
MILKS	*0.01
THIFENSULFURON THIFENSULFURON	
MILKS	0.01
THIODICARB SUM OF THIODICARB, METHOMYL AND METHOMYLOXIME, EXPRESSED AS THIODICARB *SEE* ALSO METHOMYL	
MILKS	*0.05
THIOMETON SUM OF THIOMETON, ITS SULFOXIDE AND SULFONE, EXPRESSED AS THIOMETON	
MILKS	*0.05
TILMICOSIN TILMICOSIN	
CATTLE MILK	T*0.025
TOLFENAMIC ACID TOLFENAMIC ACID	
CATTLE MILK	0.05
TRIADIMEFON SUM OF TRIADIMEFON AND TRIADIMENOL, EXPRESSED AS TRIADIMEFON *SEE ALSO* TRIADIMENOL	
MILKS	*0.1
TRIADIMENOL TRIADIMENOL *SEE ALSO* TRIADIMEFON	
MILKS	*0.01
TRIALLATE TRIALLATE	
MILKS	*0.1

TRIASULFURON TRIASULFURON	
MILKS	*0.01
TRIBENURON-METHYL TRIBENURON-METHYL	
MILKS	*0.01
TRICHLORFON TRICHLORFON	
MILKS	0.05
TRICLOPYR TRICLOPYR	
MILKS (IN THE FAT)	0.1
TRIFLOXYSTROBIN SUM OF TRIFLOXYSTROBIN AND ITS ACID METABOLITE ((E,E)-METHOXYIMINO-[2-[1-(3-TRIFLUOROMETHYLPHENYL)-ETHYLIDENEAMINOOXYMETHYL]PHENYL] ACETIC ACID), EXPRESSED AS TRIFLOXYSTROBIN EQUIVALENTS	
MILKS	*0.02
TRIFLOXYSULFURON SODIUM TRIFLOXYSULFURON	
MILKS	*0.01
TRIFLUMURON TRIFLUMURON	
MILKS	*0.05
TRIFLURALIN TRIFLURALIN	
MILKS	*0.05
TRITICONAZOLE TRITICONAZOLE	
MILKS	*0.01
TRIMETHOPRIM TRIMETHOPRIM	
CATTLE MILK	0.05
TYLOSIN TYLOSIN	
MILKS	*0.05
VIRGINIAMYCIN INHIBITORY SUBSTANCE, IDENTIFIED AS VIRGINIAMYCIN	
CATTLE MILK	0.1

Schedule 2 – Extraneous Residue Limits

ALDRIN AND DIELDRIN SUM OF HHDN AND HEOD	
MILKS (IN THE FAT)	E0.1
BHC **(OTHER THAN THE GAMMA ISOMER, LINDANE)** SUM OF ISOMERS OF 1,2,3,4,5,6-HEXACHLOROCYCLOHEXANE, OTHER THAN LINDANE	
MILKS (IN THE FAT)	E0.1
CHLORDANE SUM OF CIS- AND TRANS-CHLORDANE AND IN THE CASE OF ANIMAL PRODUCTS ALSO INCLUDES 'OXYCHLORDANE'	
MILKS (IN THE FAT)	E0.05
DDT SUM OF P,P '-DDT; O,P '-DDT; P,P '-DDE AND P,P '-TDE (DDD)	
MILKS (IN THE FAT)	E1.25
HCB HEXACHLOROBENZENE	
MILKS (IN THE FAT)	E0.5
HEPTACHLOR SUM OF HEPTACHLOR AND HEPTACHLOR EPOXIDE	
MILKS (IN THE FAT)	E0.15
LINDANE LINDANE	
MILKS (IN THE FAT)	E0.2

Appendix 10

Chemical residues measured in bovine dairy products (ADASC, 2004)

Maximum residue limits, maximum levels and extraneous levels for milk.

Residue	MRL, ML or ERL for Milk		
	AUS[1]	EU[2]	Codex[3]
	(expressed as mg/Kg in milk unless stated otherwise)		
Antimicrobials			
β-lactams			
Penicillin G	0.0015	0.004	0.004
Cloxacillin	0.01	0.03	-
Ampicillin	0.01	0.004	-
Amoxicillin	0.01	0.004	-
Cephalosporins			
Ceftiofur	0.1	0.1	0.1
Cefuroxime	0.1	-	-
Cephalonium	0.02	0.02	-
Tetracyclines			
Tetracycline	0.1	0.1	-
Oxytetracycline	0.1	0.1	0.1
Chlortetracycline	-	0.1	-
Sulfonamides			
Sulfadiazine	0.1	0.1	-
Sulfadimidine	-	0.1	0.025
Sulfadoxine	0.1	0.1	-
Sulfatroxazole	0.1	0.1	-
Macrolides			
Erythromycin	0.04	0.04	-
Lincomycin	0.02	0.15	-
Oleandomycin	-	-	-
Tylosin	0.05	0.05	-
Tilmicosin	-	0.05	-
Aminoglycosides			
Streptomycin	0.2	0.2	0.2
Dihydrostreptomycin	0.2	0.2	0.2
Neomycin	T1.5	1.5	0.5[##]
Gentamicin	-	0.1	-
Antihelmintics			
Benzimidazoles			
Triclabendazole	-	-	-

Residue	MRL, ML or ERL for Milk		
	AUS[1]	EU[2]	Codex[3]
	(expressed as mg/Kg in milk unless stated otherwise)		
Albendazole	-	0.1	0.1
Fenbendazole	0.1	0.01	0.1
Oxfendazole	0.1	0.01	0.1
Febantel	-	0.01	0.1
Thiabendazole	0.05	0.1	0.1
Levamisole	0.3	-	-
Macrocyclic Lactones			
Ivermectin	0.05	-	0.01
Abamectin	0.02	0.005	0.005
Moxidectin	0.08	0.04	-
Eprinomectin	0.03	0.02	0.02
	MRL, ML or ERL for Milk (expressed as mg/kg in fat)		
Organochlorines			
Aldrin & Dieldrin	E 0.1 (in the fat)	0.006	0.006
BHC (α, β)	E 0.1 (in the fat)	-	-
Chlordane/Oxychlordane	E 0.05 (in the fat)	0.002	0.002
Lindane	-	0.001	-
DDT (sum of DDT, DDE, DDD)	E 1.25 (in the fat)	0.04	0.02
Heptachlor/Heptachlor Epoxide	E 0.15 (in the fat)	0.004	0.006
HCB	E 0.5 (in the fat)	0.01	-
Endosulfan (α,β,sulfate)	T0.5 (in the fat)	0.004	0.004
Organophosphates			
Bromophos-ethyl	-	-	-
Chlorpyriphos	T0.2 (in the fat)	0.01	0.02
Chlorpyriphos-methyl	T0.05 (in the fat)	0.01	0.01
Chlorfenvinphos	T0.2 (in the fat)	-	-
Coumaphos	0.1 (in the fat)	-	-
Dichlorvos	0.02	-	0.02
Diazinon	0.5 (in the fat)	0.01	0.02
Ethion	0.5 (in the fat)	-	-
Fenchlorphos	-	-	-
Fenitrothion	T0.05 (in the fat)	-	0.002
Fenthion	0.2	-	-
Malathion	1.0 (in the fat)	-	-
Parathion methyl	0.05	-	-
Pirimiphos methyl	0.05	0.05	0.05
Synthetic Pyrethroids			
Deltamethrin	0.5 (in the fat)	0.02	0.02
Flumethrin	0.05	0.03	0.05
Cypermethrin	1.0 (in the fat)	0.02	0.05

Residue	MRL, ML or ERL for Milk		
	AUS[1]	EU[2]	Codex[3]
	(expressed as mg/Kg in milk unless stated otherwise)		
Fenvalerate/Esfenvalerate	0.2 (in the fat)	0.02	0.1
Cyfluthrin	0.1	0.02	0.01
Cyhalothrin	0.5 (in the fat)	0.05	-
Permethrin	0.05	0.05	0.1
Aflatoxins			
Aflatoxin M1	*	0.05 (µg/kg in whole milk)	0.5 (µg/kg in whole milk)
Heavy Metals			
Arsenic	-	-	-
Cadmium	-	-	-
Lead	-	-	-
Mercury	-	-	-

Key: [1] Australian MRLs, MLs & ERLs from *Australia New Zealand Standards Code*(5)
[2]. EU MRLs are listed in the EMEA Maximum Residues Limits (11)
[3] Codex MRLs for veterinary drugs and pesticides are listed in the Food and Agriculture Organisation of the United Nations Codex Alimentarius Maximum Residue Limits (12)
T Temporary MRL
- No MRL/ML/ERL is specified
[*] ML for sum of all PCBs;
[#] ERLs only for Organochlorines
[##] JECFA has recently recommended an MRL of 1.5 mg/kg for milk and is under consideration by Codex (FSANZ Application A535).

Appendix 11

Registered Antimicrobial Agents

Registered antimicrobial agents for use in the Australian Dairy cattle industry (JETACAR, 1999). The shaded rows in the table indicate the groups of antimicrobial agents that belong to families used in human medicine, but only those antibiotics with a category[2] listing are used therapeutically for both Dairy cattle and humans.

Antimicrobial Agent (group)	Category[1]	Category[2]	Treatment (individual)	Treatment (in feed/water)
Penicillins amoxicillin procaine penicillin ampicillin cloxacillin	A	 C C C C B	Mastitis	-
Cephalosporins cetiofur cephalonium cefuroxime	C	 B	Respiratory disease, footrot, mastitis	-
Macrolides Erythromycin, tylosin, oleandomycin	C	 C	Various infections	-
Lincosamide lincomycin	C	B	Mastitis	-
Tetracyclines oxytetracycline, chlortetracycline	C		Various infections, including mastitis	-
Aminoglycosides neomycin, apramycin, streptomycin	C	 C	Various infections	-
Sulfonamides many agents	C	C	Various infections	-
Streptogramins Virginiamycin[4](	B		-	Lactic Acidosis
Polyethers (ionophores) monensin	B		Bloat prevention[3]	
Others novobiocin	C		Mastitis	

[1] Categories for antibiotic use in Dairy animals; the letter designation holds for all antibiotics in each respective group.
Category A: essential antibiotics for treatment or prevention of animal infections where there are few or no alternatives for many infections.
Category B: other alternatives are available but fewer than for category C.

Category C: a reasonable number of alternative agents in different classes are available to treat most infections.

[2] Categories for antibiotic use in humans (category description as above)

[3] Monensin is a rumen modifier and improves the efficiency of ruminant digestion and has a registered claim for improved milk production (i.e. it is not strictly a growth promotant).

[4.] Note that Virginiamycin has recently undergone revised labelling restrictions in dairy cattle (APVMA 2003b).

Appendix 12

Review of antibiotics and antimicrobial resistance in Australia

Significance of transfer of Antimicrobial Resistance from Animals to Humans

The extent of harm to human health from the transference of AMR bacteria from animals is uncertain. Many studies have found that the use of antibiotics in animals poses significant risks for human health, and some researchers contend that the potential risk of the transference is great for vulnerable populations. However, a small number of studies contend that the health risks of the transference are minimal.

A recent FAO/OIE and WHO workshop sought to determine the human health impacts of the transference of AMR from animal to humans (OIE, 2003a; OIE, 2003b). The workshop stated that the use of antibiotics in humans and animals alters the composition of micro-organism populations in the intestinal tract, thereby placing individuals at increased risk for infections that would otherwise not have occurred. The report also states that use of antibiotics in humans and animals can also lead to increases in treatment failures and in the severity of infection.

Review of Antimicrobial Resistance in Australia

To address issues surrounding AMR, the Government established the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) in 1999. This group prepared a report giving independent expert advice on the threat posed by AMR (JETACAR, 1999). An outcome of the JETACAR process was the formation of the Commonwealth Interdepartmental JETACAR Implementation Group (CIJIG) comprising technical experts and senior representatives from government agencies. CIJIG is responsible for implementing the recommendations of the JETACAR report (DoHA 2004).

In Recommendation 1 of its report (September 1999), the JETACAR recommended that Australia adopt a conservative approach to minimise the use of antibiotics in humans and animals and, to further this policy, that in-feed antibiotics used in food-producing animals for growth promotant purposes, or other routine uses where the duration and dose level are the same, or very similar, should not be used unless they are:

- of demonstrable efficacy in livestock production under Australian farming conditions;
- rarely or never used as systemic therapeutic agents in humans or animals, or are not considered critical therapy for human use; and
- not likely to impair the efficacy of any other prescribed therapeutic antibiotic or antibiotics for animal or human infections through the development of resistant strains of organisms.

In Recommendation 2 of their report, the JETACAR recommended that the National Registration Authority (NRA) review the use of antibiotic growth promotants currently registered in Australia that do not appear to fulfil the above criteria in terms of their impact on human and animal health, using a risk analysis approach and including a cost-benefit analysis. The JETACAR also recommended that the NRA review the prophylactic use of these antibiotics in animals and the possible public health impact of this use.

In addition, there are two other taskforces established to ensure effective implementation and to provide policy advice to CIJIG. These are the Australian Health Ministers Conference

(AHMC) JETACAR taskforce and the Primary Industries Standing Committee (PISC) JETACAR taskforce. FSANZ is represented on CIJIG and the AHMC JETACAR taskforce by the Chief Scientist.

The first annual report of the national Antimicrobial Resistance Central Coordinating Unit (CCU) should be available by the end of 2005 on the JETACAR Implementation website (DoHA 2004).

Expert Advisory Group on Antimicrobial Resistance

The Government through the National Health and Medical Research Council has also established the Expert Advisory Group on Antimicrobial Resistance (EAGAR) to provide advice to government and regulatory agencies on AMR and especially measures to reduce the risks it poses.

As part of any Application on antimicrobial agents used for veterinary purposes, EAGAR undertakes a risk assessment on the antimicrobial agent. This includes evaluation of the mode of action, use of related antimicrobial agents (both human and animal), proposed usage pattern, potential for cross-resistance to other animal and human agents, potential for co-selection for unrelated resistance in animal bacteria, importance of disease if transmitted to humans, the benefit of the agent to animal health and the impact of failure of antibiotic treatment in humans. Based on this process EAGAR informs the APVMA whether an antimicrobial agent represents an unacceptable risk to public health and safety when used for veterinary purposes.

Antibiotics under review

Through the activities of JETACAR and EAGAR, the registration of several antibiotics, have been withdrawn, or are under Review. The streptogramin, Virginiamycin was used prophylactically as a growth promotant in feed premix for various animals, including cattle. However, the labelling instructions have recently been revised for dairy cattle usage by the APVMA (APVMA 2003b), as it became evident that there was an unacceptable risk that use of Virginiamycin for undefined periods of time will induce AMR in *Enterococcus faecium* in some animals and poultry. Virginiamycin-containing products can now only be used specifically for use in cattle diets at times of increased risk of acidosis during adaptation to high grain diets, and cannot be used continuously for a period of more than one month, or for repeated treatment in the same lactation period in dairy cattle.

Avoparcin was used in Australian livestock feeds since 1978 for growth promotion and improved animal feed conversion efficiency. Specific concerns were raised regarding possible links between the emergence in Australia of Vancomycin Resistant Enterococci (VRE) in humans and the use of Avoparcin (a related antibiotic). A Special Review of Avoparcin was undertaken in 1998, however several studies revealed that there were no detectable residues of Avoparcin or its metabolites in cattle milk (i.e. < 0.01 mg/kg) and that the potential for human dietary exposure should be negligible. Although these studies concluded that Avoparcin residues were highly unlikely to enter the human food chain and to play a role in the emergence of VRE in human, the manufacturing companies withdrew Avoparcin from the market for commercial reasons (APVMA 2001).

The NRA, in accordance with Division 4, Part 2 of the Agricultural and Veterinary Chemicals Code Act, 1994, is also reconsidering the registration of products containing the active constituents Kitasamycin, oleandomycin and tylosin, and associated label approvals

(APVMA 2001). The basis for this action is that the NRA is no longer satisfied that the use of products containing these antibiotics would not be likely to have an effect that is harmful to human beings.

Appendix 13

Feeds and Feed Commodities for cattle

(includes lotfed, grazing and dairy cattle) (APVMA, 2002)

Note that the assumed maximum percentage of a commodity in the animal diet is presented, this is for modelling purposes in the determination of the MRL, and is not indicative of the percentage of the feed in the actual diet. For example, it is highly unlikely that grain would exceed 80% of the total diet, and cattle would only be fed at this level for a limited period of time.

A. Feeding percentages

Commodity	**Assumed maximum percentage of diet %dry matter intake**
pasture [1]	100
grain	100
pulses/legumes	100
fodder and forage	100
processed grain fractions [2]	40
molasses	40
fruit by-products	20
oilseeds	30
plant protein meals	30
other[3]	5

[1] Where pasture has been spot sprayed for weed control, it is assumed for MRL purposes and estimation of exposure, that the animal's diet is not expected to contain more than 20% of treated pasture.

2 If the MRL for a chemical in a processed grain fraction (as given by a separate entry in Table 1 of the MRL Standard) is greater than that seen in the primary cereal grain, then the maximum percentage for the processed grain fraction in the livestock diet is assumed to be 20-40%. When there are no separate MRLs for a chemical in processed grain fractions, then the maximum percentage that can be fed in a livestock diet is assumed to be 100%, because the residue situation becomes identical to feeding the raw grain.

B. Commodity Description – examples of feed in the different commodity groups listed in part A; it may be altered as feeding patterns change over time.

Grains
wheat, oats, barley, triticale, rice, maize/corn, millet, sorghum, rye
Processed grain fractions (excluding grain dust)
pollard, bran, millrun, wheat germ, brewers grain, malt combings, biscuits, bread, hominy, semolina
Pulses/legumes
succulent or mature dried seed and immature pods of leguminous plants
peas (eg field pea, chick pea, cow pea, pigeon pea), beans (eg adzuki, faba, kudzu, mung, navy, winged), lentils, soya beans, lupins
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
Molasses/sugar
raw or processed sugar, molasses
Fruit by-products (does not include cannery wastes)
citrus pulp, pineapple pulp, pome fruit pomace, grape marc, grape pomace
Pasture
grass and legume pastures and mixed grass/legume pastures
Fodder
hay, silage and straw of legumes, grasses and cereals, sugar cane tops
Forage (not including cotton forage)
cereal forage, oilseed forage, legume forage etc.
Fodder vegetables
field turnips, kale, beets

"Other"
Vegetables (not including vegetables grown specifically for grazing or fodder)
Vegetable by-products (e.g. potato peels)
Cannery waste and by-products
Oils/fats (e.g. vegetable oils, tallow)

Appendix 14

Therapeutic products used in goat production and registration status for use in goats (POINTON *ET AL.*, 2004)

Product Name	Active	Registered for goat?	Registered species
Alamycin	Oxytetracycline dihydrate	N	Cattle, pig, sheep
Amoxycillin	Amoxycillin	N	Cattle, pig, sheep, cat, dog
Bivatop	Oxytetracycline dihydrate	N	Cattle, pig, sheep
Cepravin LC	Cefuroxime	N	Cattle
Clavulox	Clavulanic acid, Amoxycillin	N	Cattle (intramammary), cat, dog
Cortisone	Cortisone	N	Cattle, horse, pig, sheep, cat, dog
Cydectin	Moxidectin	N	Sheep, cattle, deer
Flunixin	Flunixin	N	Cattle, horse, pig, dog
Gallimycin	Erythromycin	N	Cattle, pig, sheep
Glanvac 3	Clostridium etc vaccine	Y	
Glanvac 6	Clostridium etc vaccine	Y	
Illium Xylazil-20 Analgesic	Xylazine	Y	
Ivomec Epronex	Eprinomectin	N	Cattle, deer
Ivomec pour-on	Ivermectin	N	Cattle, deer
Ketol	B hydroxybutyrate	?	
Leotrox	Sulfatroxazole, trimethoprim	Y	
Levamisole		N (Except Nufarm drench resistance test kit)	Cattle, pig, poultry, sheep, bird, dog, cat
Mastalone	Oxytetracycline, neomycin	N	Cattle
Noromectin Pour-on	Ivermectin	N	Cattle, Dairy cattle
Orbenin LC	Cloxacillin	N	Cattle
Panacur 25	Fenbendazole	Y	
Parnell ketamine	Ketamine	Y	
Procaine penicillin	Procaine penicillin	N	Cattle, horse, pig, sheep, cat, dog
Scourban	Sulfadimidine, sulfadiazine	N	Cattle, horse, cat, dog
Sedaject acepromazine	Acepromazine	N	Cattle, horse, pig, sheep
Seponver	Selenium, closantel	N	Sheep
Special Formula 17900 Forte V	Neomycin, novobiocin, dihydrostreptomycin	N	Cattle
Tribactral	Sulfadiazine, trimethoprim	N	Cattle, horse, pig, sheep, cat, dog
Trisoprim 480 antibacterial injection	Sulfadiazine, trimethoprim	N	Cattle, horse, pig, sheep
Utoztme	Oxytetracycline hyddrochloride	Y	
White drenches/Benzimadazoles	Fenbendazole (3 products)	Y	
	Oxfendazole ((5 products, but not milking goats)	Y	
	Albendazole (4 products but not milking goats)	Y	