RISK PROFILE:
LISTERIA MONOCYTOGENES
IN READY-TO-EAT SALADS

Prepared as part of a New Zealand Food Safety Authority
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by

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SUMMARY

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. Risk Profiles include elements of a qualitative risk assessment, as well as providing information relevant to risk management. Risk profiling may result in a range of activities e.g. immediate risk management action, a decision to conduct a quantitative risk assessment, or a programme to gather more data. Risk Profiles also provide information for ranking of food safety issues.

This Risk Profile concerns *Listeria monocytogenes* in ready-to-eat salads without dressings. This food type comprises largely lettuce and cabbage based salads, and excludes coleslaws or salads with non-vegetable ingredients.

The rate of reported invasive listeriosis in New Zealand is similar to that found in like countries. However, there is no epidemiological or surveillance evidence to link cases of *L. monocytogenes* infection in New Zealand with ready-to-eat salads.

Data on the prevalence of *L. monocytogenes* in New Zealand ready-to-eat salads are very limited, and somewhat dated given the apparently dynamic nature of the market. Prevalence data from overseas suggest that *L. monocytogenes* is likely to be a common (up to 10%) contaminant of salads and salad vegetables, albeit generally at very low numbers (<100 cfu/g). Data on the behaviour of *L. monocytogenes* in salads and salad vegetables suggests that under normal conditions of storage (4°C for 7 days) for this type of product, if growth does occur, then a 1-2 $\log_{10}$ increase is the most that could be expected.

The growth of *L. monocytogenes* on ready-to-eat salads will be affected by the interaction of several factors, which include time, temperature, ingredients and possibly the atmosphere. Although direct data on the prevalence of *L. monocytogenes* in ready-to-eat salads in New Zealand are not available, there are no human health surveillance data to suggest that this food/hazard combination currently represents a significant risk to human health. Based on discussions with a small number of companies manufacturing ready-to-eat salads in New Zealand, risk management measures including Food Safety Programmes and testing for *L. monocytogenes* are part of the production process.

Based on overseas risk assessments and outbreak analyses, ready-to-eat salads or vegetables are unlikely vehicles for *L. monocytogenes* infection in New Zealand, and other food vehicles appear to represent a more important route of exposure to this organism.

The risks from *L. monocytogenes* in ready-to-eat salads will be best managed by a combination of Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) to achieve a low prevalence of *L. monocytogenes* on raw product and to control any subsequent sources of contamination within the processing environment.

The data gaps identified in this Risk Profile are:

- Current prevalence of *L. monocytogenes* in ready-to-eat salads available in New Zealand;
- Quantitative data on levels of *L. monocytogenes* in ready-to-eat salads when contamination does occur;

- Information on the market size and market structure for ready-to-eat salads, including information on population levels of consumption.

1 INTRODUCTION

The purpose of a Risk Profile is to provide contextual and background information relevant to a food/hazard combination so that risk managers can make decisions and, if necessary, take further action. The place of a risk profile in the risk management process is described in "Food Administration in New Zealand: A Risk Management Framework for Food Safety" (Ministry of Health/Ministry of Agriculture and Forestry, 2000). Figure 1 outlines the risk management process.

Figure 1: Risk Management Framework

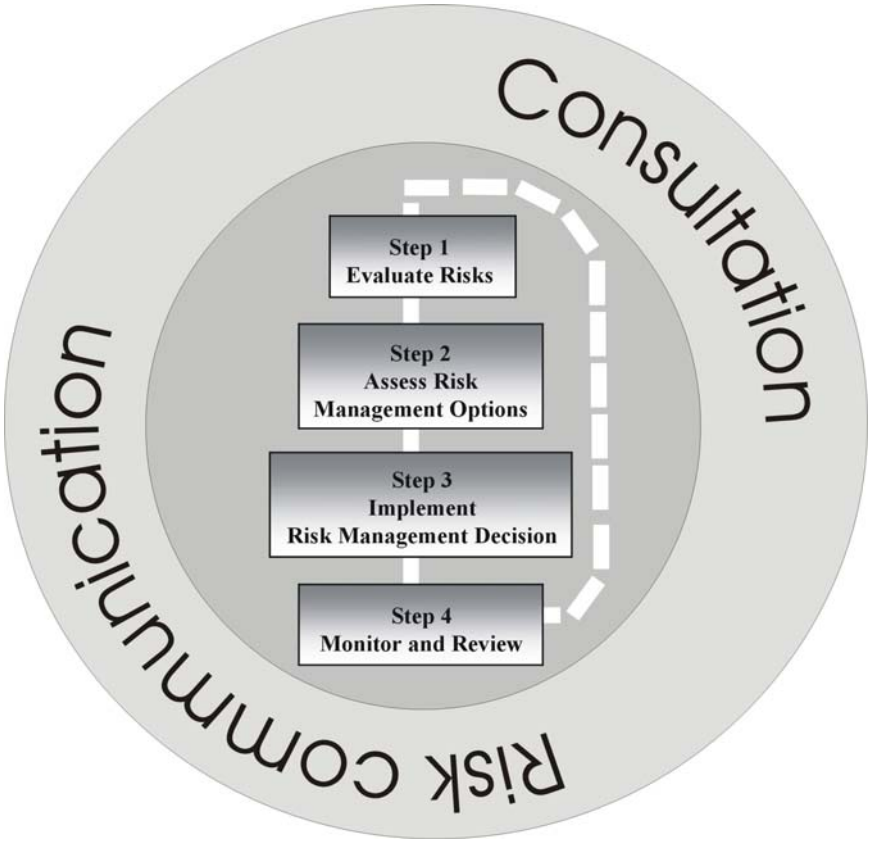


Figure reproduced from "Food Administration in New Zealand. A risk management framework for food safety" (Ministry of Health/Ministry of Agriculture and Forestry, 2000).

In more detail, the four step process is:

1. Risk evaluation

- identification of the food safety issue
- **establishment of a risk profile**
- ranking of the food safety issue for risk management
- establishment of risk assessment policy
- commissioning of a risk assessment
- consideration of the results of risk assessment

2. Risk management option assessment

- Identification of available risk management options
- Selection of preferred risk management option
- Final risk management decision

3. Implementation of the risk management decision

4. Monitoring and review.

The Risk Profile informs the overall process, and provides an input into ranking the food safety issue for risk management. Risk Profiles include elements of a qualitative risk assessment. However, in most cases a full exposure estimate will not be possible, due to data gaps, particularly regarding the level of hazard in individual foods. Consequently the risk characterisation part of a risk assessment will usually rely on surveillance data.

The Risk Profiles also provide information relevant to risk management. Based on a Risk Profile, decisions are made regarding whether to conduct a quantitative risk assessment, or take action, in the form of gathering more data, or immediate risk management activity.

This Risk Profile concerns *L. monocytogenes* in ready-to-eat salads (without dressings). This type of salad may contain a wide variety of ingredients, but this Risk Profile will focus on the vegetables, particularly the green leafy ones.

The sections in this Risk Profile are organised as much as possible as they would be for a conventional qualitative risk assessment, as defined by Codex (1999).

Hazard identification, including:

- A description of the organism
- A description of the food group

Hazard characterisation, including:

- A description of the adverse health effects caused by the organism.
- Dose-response information for the organism in humans, where available.

Exposure assessment, including:

- Data on the consumption of the food group by New Zealanders.
- Data on the occurrence of the hazard in the New Zealand food supply.
- Qualitative estimate of exposure to the organism (if possible).
- Overseas data relevant to dietary exposure to the organism

Risk characterisation:

- Information on the number of cases of adverse health effects resulting from exposure to the organism with particular reference to the food (based on surveillance data)
- Qualitative estimate of risk, including categorisation of the level of risk associated with the organism in the food (categories are described in Appendix 1).

Risk management information

- A description of the food industry sector, and relevant food safety controls.
- Information about risk management options.

Conclusions and recommendations for further action

2 HAZARD IDENTIFICATION: THE ORGANISM

The following information is taken from a data sheet prepared by ESR under a contract for the Ministry of Health. The data sheet is intended for use by regional public health units.

2.1 *Listeria monocytogenes*

2.1.1 The organism

The bacterium is Gram-positive, non-sporulating and rod-shaped. Six species of the genus *Listeria* have been recognised (ICMSF, 1996). Two are considered non-pathogenic; *L. innocua* and *L. murrayi*. (syn. *L. grayi*), while *L. seeligeri*, *L. ivanovii*, and *L. welshimeri* rarely cause human infection. This leaves *L. monocytogenes* as the most important species with respect to human health.

Two forms of disease caused by this organism are now recognised; a serious invasive disease and a non-invasive gastroenteritis. While the invasive form of disease is uncommon, the clinical consequences are often serious. The organism's ability to grow at refrigeration temperatures is significant as chilling is often used as a control measure in the food industry.

There are various typing schemes for *L. monocytogenes* (ICMSF, 1996) which include;

- Serotyping: distinguishes 13 serovars, of which three account for most of the human cases of invasive listeriosis, serotype 4b (most common), 1/2a and 1/2b;
- Phage-typing: can distinguish about 70% of isolates;
- Multilocus enzyme electrophoresis; and,
- Nucleic acid fingerprinting.

While these typing schemes are useful in epidemiological outbreak investigations, they are of limited use in distinguishing pathogenic from non-pathogenic strains (ICMSF, 1996).

2.1.2 Growth and survival

Growth:

Temperature: Optimum 37°C, range –0.4 to 45°C. Grows at refrigeration temperatures (4°C) (ICMSF, 1996).

pH: *Listeria* growth is strongly influenced by pH. Optimum 7.0, range 4.4-9.4 (ICMSF, 1996)

Atmosphere: Grows optimally under microaerophilic conditions but grows well both aerobically and anaerobically (anaerobic incubation has been shown to be more conducive to *Listeria* growth or survival than aerobic incubation). Can grow in food packaged under vacuum or nitrogen gas (AIFST, 2003). Growth of the organism was not retarded by a 5-10% CO_2 atmosphere and it can also grow in relatively high (e.g. 30%) CO_2, but growth is inhibited under 75% CO_2 (see survival below).

Water activity: Lower a_w limit for growth; 0.90 at 30°C in glycerol, 0.92 in NaCl and 0.92 in sucrose. The organism can grow in sodium chloride concentrations up to 10%, while some laboratories report growth at up to 12% NaCl (if pH is sufficiently high) (AIFST, 2003).

Survival:

Temperature: Survives freezing very well, but appears to depend on the serotype.

Atmosphere: Most literature reports on modified atmosphere packaging have studied *L. monocytogenes* and the data suggest that modified atmospheres containing approximately 75% CO_2 and no oxygen will inhibit this organism. (e.g. Hudson *et al*., 1994).

Viable but non-culturable (VNC) cells: There is some recent evidence that *L. monocytogenes* may become VNC.

2.1.3 Inactivation (Critical Control Points and Hurdles)

Note that in microbiological terms "D" refers to a 90% (or decimal or 1 log cycle) reduction in the number of organisms.

Temperature: Rapidly inactivated at temperatures above 70°C. D time at 50°C can be in the order of hours, at 60°C 5-10 minutes, 70°C approximately 10 seconds.

pH: Inactivated at pH values less than 4.4 at rates depending on the acidulant and temperature. Organic acids, such as acetic, are more effective than mineral acids (e.g. hydrochloric) at a given pH. Inactivation proceeds faster at higher temperatures.

Water activity (a_w): Although growth does not occur at less than a_w 0.90, the bacterium can survive for extended periods at lower a_w values (AIFST, 2003).

Preservatives: Due to halotolerant nature of the organism, it is able to survive for long periods in salted foods (AIFST, 2003). Inactivated on vegetables by lysozyme (100 mg/kg), 0.2% sodium benzoate at pH 5, 0.25-0.3% sodium propionate (pH 5, and less effective at lower temperatures), and 0.2-0.3% potassium sorbate (pH 5.0). The use of chlorine, chlorine dioxide, organic acids, and Salmide® reduced *L. monocytogenes* numbers on cabbage and lettuce by approximately 10 fold (Zhang and Farber, 1996).

Radiation: Dose levels of 1 to 3 kGy, depending upon the type of fruit or vegetable, are sufficient to kill large numbers of most moulds, yeasts and bacteria naturally present on produce as taken from the field (WHO, 1998).

L. monocytogenes is more sensitive than other Gram positive bacteria to UV radiation.

2.1.4 Sources

Human: *L. monocytogenes* is carried asymptomatically in the faeces of 2-6% of the population. Person-to-person spread (other than mother to foetus) is not often recorded but has been recognised. Up to 30% of case contacts may carry the organism. *L. monocytogenes* is shed in high numbers ($\geq 10^4$/g) in the faeces of infected people.

Animals: *L. monocytogenes* can cause disease in animals, and veterinarians were originally considered to be an at risk group. *Listeria* can also be present in the faeces of healthy animals. The organism can cause listerial mastitis (Back *et al.,* 1993) in milk producing animals, but it can be excreted in milk of healthy cows (Vizcaino and Garcia, 1975) and goats (Løken *et al*., 1982) as well as mastitis infected animals. The organism can also be found on raw chicken and other raw meats. Improperly made silage can be a source of domestic animal infection.

Food: Should be considered as potentially present in all raw foods and ingredients. May be present in cooked foods as a result of post-cooking contamination. Risk posed is likely to be greatest in ready-to-eat cooked foods with long shelf lives on which *L. monocytogenes* can grow. Has been isolated from a wide variety of ready-to-eat and raw foods in NZ studies. In quantitative studies of food products low levels (<100 cfu/g) are typically detected, although it has been detected at numbers far in excess of this (Farber and Peterkin, 1991).

Environment: Is widespread in the environment including soil, vegetation, water and sewage. Has been isolated from toothbrushes and other domestic environments.

Transmission routes: One study estimates that one third of cases are foodborne. Other reports describe foodborne transmission as the primary source of human infections. Alternative routes include infections acquired in hospital and occupational exposure (e.g. veterinarians).

3 HAZARD IDENTIFICATION: THE FOOD

3.1 Relevant Characteristics of the Food: Ready-to-Eat Salads

This Risk Profile concerns ready-to-eat "green salads" from retail and catering premises that are primarily lettuce or cabbage based, but without mayonnaise or other dressings. This includes both pre-packed and open (i.e. unpackaged and generally self serve in retail or catering premises) salads, produced from ingredients grown on conventional or organic premises. Other vegetable ingredients may be mixed into green salads, such as onions, carrots, tomatoes and peppers. Potential ingredients such as cooked rice, ham and cheese are not included as they have been or may be covered by other Risk Profiles. Salad mixes that contain just grated cabbage, carrot and onion (i.e. dry coleslaw and ranchslaw mixes) without dressings, are included.

Those salads that contain mayonnaise and other dressings added before purchase are not included. The effect of salad dressings and mayonnaise added to the salad by the consumer is considered as a separate issue in Section 3.4.

The soil-plant environment is considered to be a natural niche for *L. monocytogenes*, with the organism surviving well in soil for periods exceeding a month, and contamination of produce at the point of harvest would be expected (Dowe *et al*., 1997). Subsequent washing and sanitising of vegetables is only partially effective in the removal of pathogens and other bacteria from produce, so occasional contamination of the final product appears to be inevitable. Quantitative data indicate that, when present, *L. monocytogenes* usually occurs at a concentration below 100 cfu/g, although a few samples contain the organism at numbers in excess of this figure (AIFST, 2003).

3.2 Fate of *L. monocytogenes* in Ready-to-Eat Salad Vegetables

The behavior of *L. monocytogenes* in salad vegetables is highly variable. The data presented in the tables below is further considered in the context of shelf life in Section 5.3.4.

Since there is a great deal of information available on the behaviour of the bacterium, the information is broken down into three areas; growth (Table 1), survival (Table 2) and inactivation (Table 3) on salad vegetables.

3.2.1 Growth

Table 1: Growth of *L. monocytogenes* on salad vegetables

Food	Atmosphere	Temperature (^oC)	Growth	Reference
Salad (lettuce carrot, cabbage)	Ambient initially (CO_2 steadily increased, O_2 absent after 60h)	4	Approx 1 log_{10} in 300h (12.5 days)	García-Gimeno *et al*., 1996
Butterhead lettuce	Heat sealed pouch >18% O_2: 3% CO_2	10	After 4 days; 1.5 log After 7 days; 1.5 log	Carlin and Nguyen-The, 1994
Broad-leaved endive			After 4 days; 0.5 log After 7 days; 1.5 log	
Curly-leaved endive			After 4 days; 0.5 log After 7 days; <1 log	
Endive (*Cichorium endivia*)	Ambient initially, CO_2 increased, O_2 decreased	3 6 10	< 1 log_{10} in 10 days 1 log_{10} in 10 days 2 log_{10} in 10 days	Nguyen-The *et al*., 1996
Endive leaves	Ambient initially	3 6 10	1 log_{10} in 14 days 1-2 log_{10} in 14 days 2-3.5 log_{10} in 7 days	Carlin *et al*., 1995
Asparagus	15% O_2:6% CO_2:79% N_2	4 15	Lag 7 days, then 1.5-2 log_{10} in the following 14 days 3-4 log_{10} in 6 days	Berrang *et al*., 1989
	Air	4 15	Lag 7 days, then 1.5-2 log_{10} in the following 14 days 3-4 log_{10} in 6 days	
Broccoli	11% O_2:10% CO_2:79% N_2	4 15	Lag 7 days, then <1 log_{10} in the following 14 days 3-4 log_{10} in 6 days	
	Air	4 15	Lag 7 days, then <1 log_{10} in the following 14 days 3-4 log_{10} in 6 days	
Cauliflower	18% O_2:3% CO_2:79% N_2	4 15	Lag 7 days, then <1 log_{10} in the following 14 days 3-4 log_{10} in 6 days	
	Air	4 15	Lag 7 days, then <1 log_{10} in the following 14 days 3-4 log_{10} in 6 days	
Celery	NS	4	Almost 2 log_{10} in 42 days	Breer and Baumgartner, 1992

Food	Atmosphere	Temperature (°C)	Growth	Reference
Cabbage (white) Cabbage (red)		4 4	Less than 1 log_{10} in 42 days Slight initial rise then decline	
Chicory endive	Ambient initially	6.5	2 log_{10} in 7 days	Aytac and Gorris, 1994
Iceberg lettuce (*Lactuca sativa*) leaves	Ambient initially	15	1 log_{10} after 2 days then plateau	Li *et al*., 2002
Iceberg lettuce	Ambient 2-3% O_2:2-3% CO_2:94-96% N_2	7 7	Lag approx. 3 days then grew 1-2 log_{10} in the next 4 days	Jacxsens *et al*., 1999
Iceberg lettuce	Ambient	1 10	No growth 1 log_{10} CFU/g after 14 days	Delaquis *et al*., 2002
Raw shredded cabbage	Not stated	5	4 log_{10} after 25 days then plateau	Beuchat *et al*., 1986
Shredded cabbage	Ambient 70% CO_2:30% N_2	5 25 5 25	Grew 2 log_{10} over 2 weeks then declined Initial growth then decline Grew 2 log_{10} over 13 days Slight initial growth then decline	Kallander *et al*., 1991
Shredded lettuce	Ambient	5 and 12	Results varied between trials at both temperatures. Growth, survival or growth and decline were recorded.	Steinbruegge *et al.,* 1988
Shredded lettuce	Ambient and 3% O_2:97% N_2	5 10	Chlorine treated; grew 1 log_{10} in 11 days Grew 3-4 log_{10} in 10 days, chlorine treated or untreated	Beuchat and Brackett, 1990a
Whole tomatoes	Ambient and 3% O_2:97% N_2	10 21	Grew 1 log_{10} in 20 days, better in air than under MA. Grew 1 log_{10} in 2 days	Beuchat and Brackett, 1991
Shredded carrots	Ambient 3% O_2:97% N_2	5 15	Grew 2 log_{10} in 24 days, but spoilage had occurred after 7 days, when 1 log_{10} growth had occurred Grew >5-6 log_{10} in 7 days.	Beuchat and Brackett, 1990b
Caesar salad (fresh cut)	Not stated	4 10	Grew slightly (<0.5 log_{10}) in 9 days Grew 2.5 log_{10} in 9 days	Farber *et al.,* 1998
Coleslaw, (cabbage and carrots, no	Not stated	4 10	Grew slightly (<0.5 log_{10}) in 9 days Grew 2.5 log_{10} in 9	Farber *et al.,* 1998

Food	Atmosphere	Temperature (^oC)	Growth	Reference
dressing)			days	
Shredded white cabbage (data for *L. innocua)*	Ambient initially (different O_2 permeability packaging)	11	No growth after 14 days, but 4-5 $\log_{10}$ growth from day 14 to 21	Omary *et al*., 1993
Cut Salad	NS	4	Grew 1.5 fold in 6 days	Breer and Baumgartner, 1992

3.2.2 Survival

Table 2: Survival of *L. monocytogenes* on salad vegetables

Food	Atmosphere	Temperature (^oC)	Survival period	Reference
Iceberg lettuce (*Lactuca sativa*) leaves	Ambient initially	5	18 days	Li *et al*., 2002
Chicory endive	Moderate vacuum (400 Mb)	6.5	7 days	Aytac and Gorris, 1994
Chicory endive	Ambient 2-3% O_2:2-3% CO_2:94-96% N_2	7 7	6 days	Jacxsens *et al*., 1999
Shredded lettuce	Ambient and 3% O_2:97% N_2	5	15 days	Beuchat and Brackett, 1990a
Shredded carrots	Ambient 4.9% CO_2:2.1% O_2:93% N_2	4	Initial 1 $\log_{10}$ decline after 2 days then static for 13 days	Kakiomenou *et al*., 1998
Shredded lettuce	Ambient 4.9% CO_2:2.1% O_2:93% N_2	4	Initial 1 $\log_{10}$ decline after 5 days then static for 13 days	Kakiomenou *et al*., 1998

NS = Not stated

3.2.3 Inactivation

Table 3: Inactivation of *L. monocytogenes* on salad vegetables

Food	Atmosphere	Temperature (^oC)	Inactivation	Reference
Mung bean sprouts	Ambient initially Moderate vacuum (400 mB)	6.5	< 1 $\log_{10}$ in 3 days then plateau 1.5 $\log_{10}$ in 5 days then plateau	Aytac and Gorris, 1994
Carrots	NS	4	Around 1 $\log_{10}$ in 42 days	Breer and Baumgartner, 1992
Grated carrots	Ambient 2-3% O_2:2-3% CO_2:94-96% N_2	7 7	Around 1 $\log_{10}$ in 7 days	Jacxsens *et al*., 1999

Food	Atmosphere	Temperature (^oC)	Inactivation	Reference
Chopped carrots	Not stated	4 10	Declined 2 log_{10} in 9 days Declined 2 log_{10} in 9 days	Farber *et al.,* 1998
Chopped tomatoes	Ambient 3% O_2:97% N_2	10 21	Declined 1-2 log_{10} in 10 days Declined 2-4 log_{10} in 8 days	Beuchat and Brackett, 1991
Lamb's lettuce	Heat sealed pouch >18% O_2: 3% CO_2	10	No growth or death during first 4 days. Declined 1 log in 7 days	Carlin and Nguyen-The, 1994

Some salad components appear to be listericidal, such as lamb's lettuce, mung beans, carrots and chopped tomatoes. A study of the effect of chopped carrots on *L. monocytogenes* (Farber *et al.*, 1990) found that the population decreased over 9 days. Data on the effect of shredded carrots are contradictory as in one study growth occurred (Beuchat and Brackett, 1990b) and in another the pathogen was inactivated (Jacxsens *et al*., 1999). However, Beuchat and Brackett (1999b) also found that a broth culture medium containing 1% raw carrot juice substantially inhibited growth of the bacterium. Oxygen appeared necessary for the anti-listerial activity. This effect may be explained by research documented in the WHO review (1998). Production of 6-methoxymellein and 6-hydroxymellein by carrot cells infected by fungi or upon partial hydrolysis is known to occur. These phytoalexins inhibit a wide range of spoilage and pathogenic bacteria. The AIFST (2003) suggest that carrot juice may be used as a control on the organism in pre-prepared salads.

3.2.4 Shelf life

Refrigerated storage of ready-to-eat salads is essential to increasing the shelf life. However, refrigeration temperatures do not prevent the growth of *L. monocytogenes*, and so the length of the shelf life is important in any potential risk from this pathogen.

For ready-to-eat vegetables, the Australian Institute of Food Science and Technology (AIFST) (2003) state that strict temperature control is an important factor in preserving the product. Temperature must be maintained at $\leq 4^oC$ during processing, transportation and storage prior to consumption, to minimise growth of spoilage and pathogenic bacteria. A shelf life of seven days is recommended for such vegetables, reduced to six days for green peppers due to their higher bacterial loading. Seven days is also recommended as a shelf life for vegetables prepared ready-to-use in health care food services (Odumeru *et al*., 1997).

In Spain, ready-to-eat vegetable salads are normally given a 7-14 day shelf life, depending on the types of vegetables included (García-Gimeno and Zurera-Cosano, 1997). This study sought to obtain a more objective measure of shelf life in a mixed salad and provided evidence to indicate that a lactic acid bacteria concentration of 10^6 CFU/g indicates the start of spoilage in a mixture of 10% red cabbage, 75% lettuce and 15% carrot. Samples were tested from 0 hours through to 300 hours. At 4^oC the predicted shelf life was 204 hours (8.5 days). This corresponded well with the sensorial indication of spoilage. A 144 hours or 6 day shelf life had been applied by the manufacturer. At 10^oC the shelf life reduced to 84 hours, and at 15^oC it was 40 hours.

In a study of commercial mixed salads by the same authors, there was less than a 1 $\log_{10}$ increase in the organism after 300 hours of storage at 4°C in a salad mix inoculated with 10^3 cfu/g *L. monocytogenes* and packed under air (García-Gimeno *et al*., 1996). The pH of the samples remained close to 6 throughout the 300 hours and was not a limiting factor for growth. Samples stored at 4°C had normal air composition when packaged and reached 28% CO_2 at the end of the storage period. Oxygen was no longer detected at 60 hours. The increase of the CO_2 concentration inside the package was due to vegetable respiration and microbial metabolism, and greater CO_2 increases can be found in cut vegetables. The permeability of the plastic film therefore plays an important role in gaseous exchange and in modifying the atmosphere (García-Gimeno *et al.,* 1996). The authors concluded that modified atmosphere packing of vegetables affords the possibility of greater growth of *L. monocytogenes* because of the extended shelf life of the product.

Experiments with controlled atmosphere storage of lettuce showed that when the temperature was 24°C, no gas mixture could be employed that would allow storage for more than 10 days. However, at 1.5°C all gas mixes allowed storage up to 20 days, and with 2.5% O_2 and 2.5% CO_2 (balance N_2) storage up to 40 days could be achieved (Singh *et al*., 1972). A shelf life extension was also demonstrated for asparagus, cauliflower and broccoli in modified atmospheres (Berrang *et al*., 1989). Controlled atmospheres may therefore extend shelf lives of these foods but the atmospheric tolerance of the organism may allow *L. monocytogenes* to grow at refrigeration temperatures. The AIFST (2003) states that the survival and growth of *L. monocytogenes* on vegetables under controlled atmospheric conditions could be due to the low level of oxygen present in these packages (2-5%).

3.3 Effect of Vegetable Decontamination on *L. monocytogenes*

A review published by the WHO in 1998 (WHO, 1998) on surface decontamination of fruits and vegetables eaten raw is available from:
http://www.who.int/foodsafety/publications/fs_management/surfac_decon/en/.

L. monocytogenes was stated to be generally more resistant to disinfectants than *Salmonella*, pathogenic *Escherichia coli* and *Shigella*. Available information on the effect of a number of disinfecting agents on *L. monocytogenes* was summarised:

Chlorine:

At 200 ppm chlorine reduces the count of *L. monocytogenes* on brussel sprouts, shredded lettuce and cabbage by about 1-2 $\log_{10}$ units. However, simply dipping inoculated sprouts in sterile water reduced *L. monocytogenes* on sprouts by 1 $\log_{10}$ unit. The action of chlorine appears to occur during the first 30 seconds of exposure, so longer periods did not affect the reduction. However, the effectiveness of chlorine is increased if the temperature of the treatment solution is higher than the temperature of the fruit or vegetable.

Chlorine dioxide:

This disinfectant has gained popularity because its efficacy is less affected by pH and organic matter, and it does not react with ammonia to form chloramines. Less is known about the effectiveness of this chemical, although *L. monocytogenes* on lettuce was reduced by a maximum of 1.1 and 0.8 $\log_{10}$ units at 4°C and 22°C respectively.

Trisodium phosphate (TSP):

L. monocytogenes appears to be resistant to TSP and experiments have shown little reduction in numbers when using this chemical. Solutions of greater than 10% TSP damage the sensory qualities of lettuce.

Quaternary ammonium compounds (Quats):

These chemicals are primarily used for environmental cleaning in processing plants, and are not widely used directly on produce. They are more effective than chlorine against *L. monocytogenes*.

Organic acids:

Lactic and acetic acids, either alone, or in combination with chlorine, were effective in reducing *L. monocytogenes* numbers on shredded lettuce.

The review concludes:

- "Heavily contaminated fruits and vegetables should be subjected to a double wash treatment. Success in removing soil or faecal matter, and the contaminants therein, is more likely to be achieved by first washing in potable water and then washing or rinsing in water containing a disinfectant,
- The temperature of wash-water should be higher than that of the fruits or vegetables in order to minimise uptake of microorganisms by tissues,
- The lethal effect of chlorine occurs within the first few seconds of treatment. The population of microorganisms decreases as the concentration of chlorine increases to about 300 ppm, above which effectiveness is not proportional to increased concentration,
- Leaving fruits and vegetables wet after disinfecting or washing can negate any beneficial effect of treatment,
- Organic acids (e.g. acetic, lactic, citric and peroxyacetic acids) have good potential as disinfectants for fruits and vegetables, but conditions under which they are most effective have not been defined, and
- Prevention of contamination, at all points from the field to the plate, through application of good agricultural practices (GAP, GMP and HACCP programmes) is preferred to application of chemical disinfectants after contamination has occurred".

The recommendation that wash water should be at a higher temperature than the vegetable is based on research carried out by Bartz and Showalter (1981) cited in the WHO review (1998). This study immersed warm tomatoes (26°C to 40°C) for 10 minutes in suspensions of bacteria at 20-22°C, resulting in infiltration of stem tissues. An association was observed

between uptake of bacteria when the water temperature was less than the tomato temperature. When the water temperature was higher, infiltration was reduced.

This phenonomen was also observed with *S*. Montevideo and tomatoes (Zhuang *et al*., 1995). A significantly greater uptake ($P<0.05$) of the pathogen occurred when the water temperature was 15°C cooler than the tomatoes (tomatoes at 25°C dipped in 10°C suspension), compared to temperature differentials of 0°C (i.e suspension at 25°C) and +12°C (i.e. suspension at 37°C).

The WHO review is more optimistic of disinfection reductions than a study by Zhang and Farber (1996). They reported that none of a range of disinfectants and conditions produced more than a 1.8 $\log_{10}$ reduction of *L. monocytogenes* on lettuce and cabbage, and it was concluded that only a ten-fold reduction could be generally expected regardless of the disinfectant used. Studies by Li *et al*., (2002) dipped cut iceberg lettuce into 20°C and 50°C chlorine solutions (20mg l^{-1}) and water for 90 seconds. The presence of chlorine had no significant effect when compared to water on its own. All treatments resulted in a decrease in *L. monocytogenes* populations of approximately 1 $\log_{10}$ to 1.2 $\log_{10}$.

However, this study also demonstrated that during subsequent storage, treated lettuce can exhibit enhanced *L. monocytogenes* growth when compared to untreated leaves. Growth was more rapid on lettuces treated at 50°C compared to 20°C. This was possibly due to the reduction in competitive organisms and presence of released tissue fluid (providing nutrients) and residual water from the treatments. The overall conclusions were that all treatments initially reduced populations, but mild heat treatment (i.e. 50°C) of cut lettuce leaves enhanced growth during subsequent storage at 5°C or 15°C. This means that although 50°C heat treatment results in prolonged shelf life (delaying brown discolouration by inhibiting enzyme activity) it also facilitates *L. monocytogenes* growth.

Further work on storage has confirmed that washing over 45°C enhances *L. monocytogenes* growth. Delaquis *et al*., (2002) inoculated cut iceberg lettuce with *L. monocytogenes* either before or after a three minute cold (4°C) or warm (47°C) wash in water containing 100 mg l^{-1} chlorine. Washed lettuces were stored at either 1°C or 10°C in oxygen permeable packaging for up to 14 days. Washing at 4°C generally resulted in a decline in *L. monocytogenes* counts during subsequent storage at either temperature, while washing at 47°C generally lead to an increase in *L. monocytogenes* counts in lettuce stored at either temperature. The authors also monitored background microflora and concluded the effect seen was not due to removal of competing organisms.

Lin *et al*., (2002b) tested a variety of disinfectants for their efficacy in treating lettuce leaves and concluded that treatment with 2% H_20_2 at 50°C for 60 seconds followed by a water wash was effective and retained the sensory quality of the lettuce. Treatment in water alone lowered the number of inoculated *L. monocytogenes* by approximately 1.5 $\log_{10}$, while hydrogen peroxide treatment produced an approximate 3 $1\log_{10}$ reduction. However, it was recognised that the hydrogen peroxide treatment did not have regulatory approval.

Attention has been drawn to the possibility that the presence of waxy layers, differences in surface topography and the presence of abrasions may all reduce the potential efficacy of any sanitising treatments that might be employed (Burnett and Beuchat, 2000).

In summary, it appears that wash water should be warmer than the vegetables to limit uptake of bacteria and enhance the effect of any chlorine. However, a wash water temperature of above 45°C may enhance the growth of any remaining *L. monocytogenes* during subsequent storage. Studies indicate that the action of chlorine is only marginally better than water on its own (both treatments result in approximately 1 to 2 $_{log}$ reductions). Exposure times to treatment solutions need not be any longer than 30 seconds, and it is important to thoroughly dry the fruit or vegetable before storage under refrigerated conditions.

3.4 Effect of Dressings on *L. monocytogenes* in Salads

Consumers do not always consume the salads covered by this Risk Profile in the form in which they were purchased. For example, it is common for the manufacturer of pre-packed salads to include a sachet of dressing within the package. Alternatively the consumer may add a dressing of their choice to the salad ingredients before consumption. The following section describes the anti-microbial effect of mayonnaises, dressings and sauces. These effects are likely to require more time than usually occurs between dressing application by consumers and consumption, but may apply in some circumstances.

Acidity is an important factor in determining the growth and survival of pathogens in mayonnaises, dressings and sauces. A total formula pH value of less than 4.4 can have a listericidal effect. The organism was inactivated, decreasing by $\geq$ 8 $\log_{10}$ cfu/g in less than 72 hours, in full formula mayonnaise (pH 3.3, 1.8% acetic acid) when held at 26.6°C. The same effect took longer in cholesterol-free formulations (120 hours) and reduced calorie formula (192 hours). The only difference between the cholesterol free and reduced calorie formulas, where different inactivation rates were recorded, was the higher concentration of egg white in the cholesterol-free product. The effect was attributed to the egg white containing lysozyme which synergistically interacts with acetic acid and affects pH so as to enhance the anti-listerial activity compared to the reduced calorie formula (Erickson and Jenkins, 1991). Other components in dressings such as salt, sugar, inhibitory flavourings such as garlic and onions, and preservatives can have an interactive effect with the acidity to further inhibit the growth of pathogens (Smittle, 2000). If a salad dressing has an acidic component of mostly acetic acid at pH 3.0 or less, a listericidal effect can be observed even at refrigeration temperatures. However, this effect is lost if the pH is higher, the product relies on refrigeration for preservation, and the product contains ingredients which may be contaminated such as cheese or other dairy products (Smittle, 2000).

3.5 Effect of Competitive Organisms

The growth of *L monocytogenes* is influenced by the normal flora of vegetables. A Risk Profile written by the European Commission Scientific Committee on Food (2002) on the microbiological contamination of fruits and vegetables eaten raw, states: "Vegetables normally carry a non-pathogenic epiphytic microflora, pathogens may contaminate the plants via a number of routes, e.g. organic fertilisers, sewage sludge, wild and domestic animal droppings and irrigation water. In addition, where the vegetables are further prepared ready for eating; such as cutting, slicing, skinning and shredding, natural protective barriers of the plant are removed."

For example, in relation to microflora loading, a study of vegetables and ready-to-eat salads from an Indian market (Pingulkar *et al.*, 2001) found that the growth of *L. monocytogenes* on whole tomatoes was inhibited by the overgrowth of native microorganisms.

This effect was also illustrated by inconsistencies in the ability of *L. monocytogenes* to grow on shredded lettuce (Steinbruegge *et al.*, 1988). In some experiments growth occurred, in others the organism survived and in others it grew and then rapidly declined in numbers. A likely cause of these observations is differences in the organisms present on the cabbage between different experiments.

As foods such as lettuce are stored they may be fermented by the organisms present to produce lactic acid with a resultant drop in pH. This in turn may result in inactivation of the pathogen. These processes are well known in fermented cabbage products such as sauerkraut and kimchi. While ready-to-eat salads would not be expected to be eaten after full fermentation, partial fermentation may have occurred and so reduced the number of *L. monocytogenes* present. Such inactivation will occur more rapidly at high temperatures, but this will shorten the shelf life of the product and the palatability of the salad. Taking this principle further, Vescoco *et al.,* (1996) suggest applying anti-microbial-producing lactic acid bacteria as a biopreservative to ready-to-use vegetables.

Both *Enterobacter* and lactic acid bacteria isolated from lettuce have been shown to reduce numbers of *L. innocua* in model media (Francis and O'Beirne, 1998). The inhibition of *L. monocytogenes* by organisms on vegetables is therefore a combination of both the quantity and quality of commensal bacteria present. In another paper these authors demonstrated that *L. innocua* grew to higher numbers on lettuce that had been dipped in antimicrobial agents or packed under 100% N_2. This may have been due to changes to the flora that might otherwise inhibit the organism (Francis and O'Beirne, 1997).

Mesophilic bacteria isolated from spinach have been shown to exert an inhibitory affect on the growth of *L. monocytogenes* (Babic *et al.*, 1997). In particular a *Pseudomonas* isolate produced a strong inhibitory affect. Growth of *L. monocytogenes* on macerated spinach reached lower final numbers than for autoclaved macerate, reflecting the activity of the microflora, but there also appeared to be an inhibitory affect other than that produced by the microflora.

3.6 Overall Conclusions on Survival and Growth of *L. monocytogenes* in Ready-to-eat Salad Vegetables

The fate of *L. monocytogenes* in ready-to-eat salads is an important consideration as the food receives no treatment which will result in inactivation of the organism. Chemical disinfection appears to reduce the number of *L. monocytogenes* present by only 1-2 $\log_{10}$ and, while having some value, this is likely to be insufficient to guarantee removal of the pathogen.

An important concern when discussing growth in this food is the shelf life of the product and the likely storage temperatures. The information would suggest that a shelf life of 7 days when refrigerated at 4°C is the norm, and this is reinforced by the scientific data presented. Examination of Table 1 would indicate that growth, where it occurs, will be limited, in most cases, to less than a 2 $\log_{10}$ increase within 7 days. At refrigeration temperatures growth may

occur, but the amount of growth is small. The use of modified atmosphere packaging may allow greater growth of *L. monocytogenes* as the shelf life is increased so giving a longer period for the pathogen to grow.

Growth will be influenced by physicochemical properties of the food (such as pH) and, as is apparent from some of the papers cited above, differences in the normal microflora of the vegetables. Inhibition of pathogens by isolates from minimally processed vegetables has been observed, with most of the inhibiting species being either *Pseudomonas* or *Aeromonas* (Schuenzel and Harrison, 2002). Given the variability in growth of *L. monocytogenes* noted in some experiments it may be that the quantity and composition of the initial microflora are key determinants in the potential growth of *L. monocytogenes* on these foods.

Some vegetables appear to produce anti-listerial substances, and so the composition of salad mixes may also influence the ability of *L. monocytogenes* to survive in them. Similarly, the presence of salad dressing will influence markedly the potential growth of the organism. The listericidal effects of low pH dressings will, however, be minimised when the product is refrigerated as the bactericidal affect occurs more rapidly at higher temperatures.

3.7 The Food Supply in New Zealand

Worldwide 'refrigerated salads' have been reported to be one of the nine fastest growing consumer food categories with sales growth of 13% in 1999-2000 and 11% in 2000-2001 (http://acnielsen.com/pubs/ci/2002/q2/features/growth.htm). The drivers for this growth were reported to be convenience, health/safety, and new product innovation.

3.7.1 Production

Little information is available on the size of the New Zealand ready-to-eat salad market. A number of companies market ready-to-eat salads on either a national or a regional basis. Major national brands include Fresh Express (Pam's), Leader, Pacific Gourmet and KrispKut. A 2003 vegetable industry (VegFed) fact sheet (http://www.vegetables.co.nz/about/4_stat.cfm) indicates that the area in vegetable production is 50,000 ha, with 2,800 commercial growers employing over 25,000 people (N.B. these figures are for total vegetable production). It is not clear what proportion of this figure is for salad vegetables.

Ready-to-eat salads are generally prepared and delivered to supermarkets and other retail outlets by companies specialising in their production. Discussions with three of these companies in Christchurch and Dunedin indicated the following practices:

- Incoming ingredients are inspected for freshness and quality;
- Separate areas for raw, washed, cut and finished product are used, with one way flow of material during production;
- Washing ingredients in a chlorine based sanitiser is common (50-200 ppm);
- Modified atmosphere packaging is used in some instances but is not standard;
- Microbiological testing (including for *L. monocytogenes*) is standard although the frequency varies from six monthly to fortnightly;
- Microbiological testing is driven, at least partly, by the Approved Supplier Programme (see Section 7.1.4);

- Shelf lives were based on microbiological testing, and were 5-7 days from date of production depending on the salad;
- Food Safety Programmes had been implemented or were in the process of being audited.

Burwood Pacific, who manage food safety issues for the Progressive supermarket group rely on manufacturers to provide shelf life information. Generally salads have a four day expiry from date of production. Displays in delicatessen counters have two day shelf lives.

Salad ingredients are not major export commodities for New Zealand. The bulk of the $500m fresh vegetable export value is in onions and buttercup squash (see: http://www.hortresearch.co.nz/files/2004/facts-figs-2003.pdf). Fresh vegetables made up 1% of total exports of organic produce.

Pre-cooling and relative humidity control of vegetables prior to packaging is important in prolonging shelf life. Commercial packaging notes containing information on post harvest temperatures, post harvest humidity, packaging method, storage temperatures and length of time stored under these conditions are available on mesclun, lettuce, parsley and herbs, cabbages, capsicums, carrots, celery, tomatoes and cucumbers at the following website; http://www.peakfresh.com/index1.htm. Although these packaging notes are for commercial bulk packaging, not retail ready-to-eat product, dry packaging without washing is recommended for lettuce and mesclun.

3.7.2 Imported foods

No descriptors were found in the imported food statistics provided by Statistics New Zealand that could relate to ready-to-eat salads. The value of fresh vegetable imports appear to be approximately $30m (see: http://www.hortresearch.co.nz/files/2004/facts-figs-2003.pdf) but these have not been identified.

4 HAZARD CHARACTERISATION: ADVERSE HEALTH EFFECTS

There are two types of disease associated with infection by *L. monocytogenes*; invasive and non-invasive. The invasive disease is called listeriosis and normally occurs in people with weakened immune systems. The non-invasive disease is usually called febrile gastroenteritis i.e. gastroenteritis associated with mild 'flu-like' symptoms, and can occur in healthy people if large numbers of *L. monocytogenes* cells are consumed.

4.1 (Invasive) Listeriosis

To cause this disease, ingested *L. monocytogenes* cells penetrate the intestinal tissue and become exposed to phagocytic cells of the immune system. A portion of the *L. monocytogenes* cells survive and multiply within the host phagocytes. They then move throughout the host via blood or the lymphatic system.

The populations most at risk from this disease are the elderly, the immunocompromised, and the perinatal. Perinatal infections occur primarily as a result of transplacental transmission to the foetus following infection of the mother. The perinatal group includes foetuses or neonates, and infection can occur before or after birth. The symptoms experienced by the mother are usually only a mild fever.

Incubation: 1-90 days, mean 30 days.

Symptoms: Include 'flu'-like symptoms (e.g. fever, headache), diarrhoea and vomiting. In perinatal cases, clinical outcomes for the foetus or newborn include general septicaemia, intrauterine death, premature birth, stillbirth. In non-perinatal cases, symptoms commonly include bacteraemia and meningitis.

Long term effects: In one outbreak neurological problems (cranial nerve palsies) developed in 30% of the survivors of meningitis. Pre-term infants may suffer from excess fluid in the brain and partial paralysis.

Treatment: L. monocytogenes is susceptible to a number of antibiotics, but penicillin and ampicillin optionally with an aminoglycoside (e.g. gentamicin) is considered to be the combination of choice.

4.2 (Non Invasive) Febrile Gastroenteritis

The non-invasive form of listeriosis was recognised during the 1990s.

Incubation: 11 hours to 7 days, median 18 hours.

Symptoms: Diarrhoea, fever, muscle pain, headache, and less frequently with abdominal cramps and vomiting. Attack rate reported to be upwards of 74%.

4.3 Dose Response

4.3.1 Listeriosis

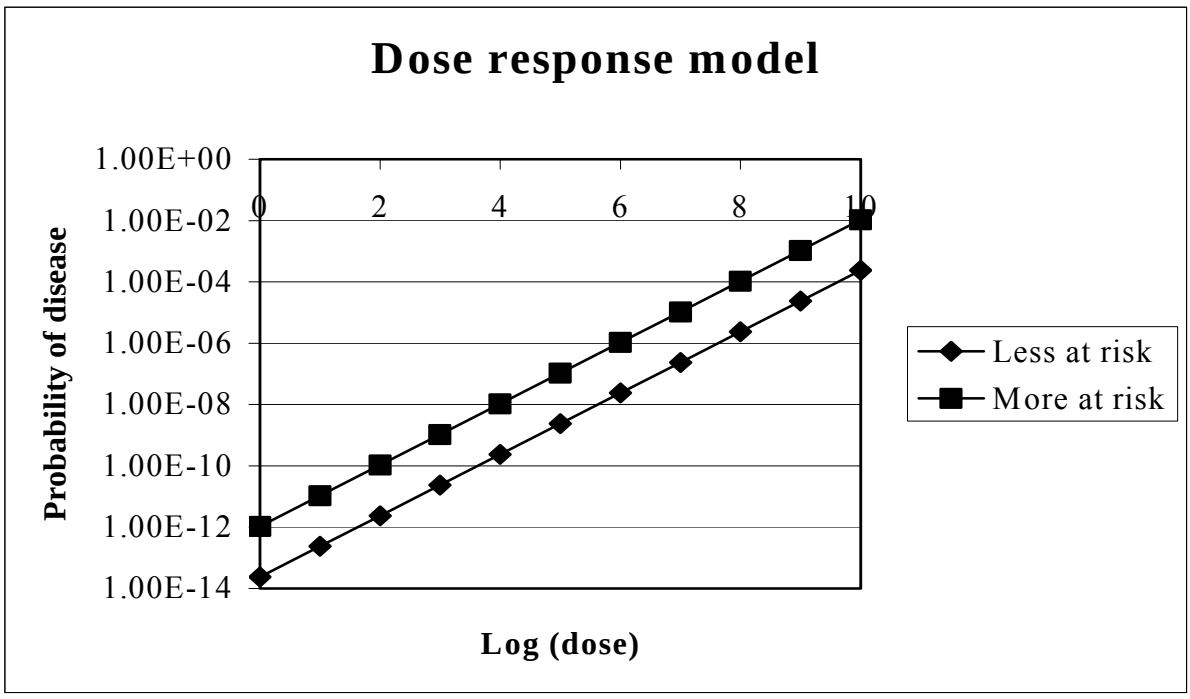
It is becoming increasingly realised that the only completely safe dose of *L. monocytogenes* is zero, even in healthy people. However the probability of invasive disease following exposure to even moderate levels of cells is very low.

The FAO/WHO risk assessment used a dose response model described by:

$P_{\text{health outcome}}=1-\exp^{-R*N}$

where R is a variable that defines the dose/response relationship and N is the number of cells consumed. The values of R vary depending on population group (to reflect different susceptibilities) but are around the 10^{-12}-10^{-14} level. The model is a single hit model which means that there is a probability of illness associated with each cell consumed. It is therefore total consumption of cells that dictates risk; there is no "infectious dose", and there is no difference to risk if a small number of cells are eaten frequently or many cells eaten at the same time as long as the total eaten is the same. Figure 2 shows dose response curves for at risk and not at risk groups.

Figure 2: Dose response models at median values for R for invasive disease caused by *L. monocytogenes**



* Information provided by Dr. Tom Ross, University of Tasmania, and is that used in the FAO/WHO *Listeria* quantitative risk assessment.

The FDA/FSIS modelled value of R accounts for variation of virulence in the types of *L. monocytogenes* extant in the population. It is known that certain serotypes of *L.*

monocytogenes appear to be associated with human disease, but there is no certainty that any one isolate will be pathogenic to humans just because it belongs to a particular serotype. A recent study has grouped *L. monocytogenes* into three distinct lineages (Jeffers *et al*., 2001), and there did appear to be some differences between the contributions that the lineages made to human disease. However, these lineages are not based on serotyping. The conservative approach is to treat all isolates as potentially capable of causing disease, but modelling of variability will be a more accurate reflection of real life.

4.3.2 Febrile gastroenteritis

Dose response data for febrile gastroenteritis are limited. In a New Zealand outbreak involving ham, 21 of 24 (87.5%) people consuming the food contaminated with 1.8×10^7 *L. monocytogenes* cells/g became ill with symptoms of febrile gastroenteritis (Sim *et al*., 2002). Assuming approximately 100g of ham was eaten by each person at the meal, then the dose ingested to produce this response was of the order of 10^9 cfu. In the outbreak described by Dalton *et al.* (1997) an attack rate of 75% was recorded where the median number of cells consumed was estimated as being as high as 2.9×10^{11} cfu. In other outbreaks it is difficult to estimate dose responses as portion sizes are not detailed or the number of cells present not accurately known. However, of all of the other outbreaks, the lowest number in food that has been shown to cause febrile non-invasive listeriosis is 1.9×10^5 cfu g^{-1} (Miettinen *et al*. 1999), although the serving sizes were not detailed. In this incident, all five people eating the contaminated fish became ill with gastroenteritis, nausea, abdominal cramps and diarrhoea. Therefore consumption of more than, perhaps, 10^7 cells appears to be sufficient to cause *L. monocytogenes* febrile gastroenteritis at a high infection rate in some circumstances. It is possible that foods contaminated with lower numbers of *L. monocytogenes* may also cause febrile non-invasive gastrointestinal disease, and because this organism is not routinely screened for in clinical laboratories, many cases of non-invasive listeriosis may evade detection.

4.4 High Risk Groups in the New Zealand Population

Although there is increasing evidence that healthy individuals can become infected by *L. monocytogenes*, there are some high risk groups in the population (Sutherland and Porritt, 1997). The well categorised risk groups for listeriosis include pregnant women and their foetuses, neonates, the elderly, and adults with a compromised immune system e.g. renal transplant patients, patients on corticosteroid treatment, and HIV/AIDS patients. The following sections provide information on the New Zealand population of these groups.

4.4.1 Perinatal population

Live births data for the 2003 calendar year were 56,130 (http://www.stats.govt.nz/).

Births were spread evenly throughout the year, but were strongly weighted towards the Northern areas of New Zealand. This total compares well with the results of the 2001 Census, which reported 55,130 New Zealanders under the age of one year on Census night. Of these 51.3% were male and 48.7% female. This represents 1.4% of the total New Zealand population.

Based on a figure of approximately 56,000 live births per annum and the number of perinatal cases of listeriosis in 2003 (6), this equates to an incidence of approximately 11 cases/100,000/year in the perinatal population.

4.4.2 Elderly population

According to the 2001 Census of New Zealand, 615,580 New Zealanders were aged 60 years or over. This is 16.0% of the total population. The aged population is 45.2% male and 54.8% female. The population 80 years and over is 112,090 (2.6% of the population) and is made up of 34.3% males and 65.7% females (http://www.stats.govt.nz/).

4.4.3 Immune compromised

AIDS: At the end of June 2003, 788 people in New Zealand were notified with AIDS. At the same date 1,974 people in New Zealand were found to be infected with HIV (http://www.moh.govt.nz/aids.html). This represents 0.05% of the total New Zealand population.

Cancer: The most recently available statistics on the incidence of cancer and cancer mortality in New Zealand are from the 1998 year. In that year, 16,531 new cases of cancer were registered (311.9 cases per 100,000 population), made up of 8,842 males (357.0 cases per 100,000) and 7,689 females (279.6 cases per 100,000). During the same period mortality due to cancer was 7,582 (131.9 cases per 100,000) made up of 3911 males (152.4 per 100,000) and 3671 females (117.6 per 100,000) (http://www.nzhis.govt.nz/stats/cancerstats.html). It is uncertain what proportion of the New Zealand population are suffering from cancer at any particular time.

Recipients of organ or tissue donations: The NZHIS publication "Selected morbidity data for publicly funded hospitals 1997/98" lists only two patients under the category "V42 Organ or tissue replacement by transplant" and only five patients under the category "V43 Organ or tissue replacement by other means". A similar document covering private hospital morbidity during 1995 reported 57 corneal transplants, 21 cases of transplantation of muscle and tendon of the hand, but no major organ transplants (http://www.nzhis.govt.nz).

However, this is an obvious underestimate as, presumably, a number of renal, heart and other transplants take place in New Zealand. Some information on major organ transplants can be obtained from diverse sources of information. An Australian summary indicates that the kidney is the most common organ transplanted, followed by liver, lung or heart-lung, heart and pancreas (http://www.abs.gov.au/ausstats).

In 2002, 117 kidney transplants were performed in New Zealand bringing the total number of surviving New Zealand kidney transplant recipients to 1114 (http://www.anzdata.org.au). In 2001, 36 liver transplants were performed at the Auckland liver transplant unit. The unit reported outcome statistics for 109 liver transplant recipients, but it is unclear whether this is the total surviving New Zealand population (http://www.nzliver.org/outcomes). The New Zealand Organ Donation website gives the following numbers for transplants performed in 2003; kidney (excluding living donor transplants) 66, liver 38, heart 22, lungs 14, pancreas 6 (http://www.donor.co.nz). It appears likely that the total New Zealand population of

surviving major organ transplant recipients is less than 2000 people (0.05% of the total population).

4.5 Serotypes Isolated from Human Cases and also from Ready-to-eat Salads

In New Zealand the clinical isolates of *L. monocytogenes* for the period 1999 to 2003 are approximately evenly split between serotypes 1/2 and 4 (Pat Short, ESR Enteric Reference Laboratory, Kenepuru Science Centre, pers. comm., December 2003).

In overseas studies, the same serotypes have been found in salad vegetables.

Isolates from salads in Spain comprised serotype 4b (6 isolates), 1/2a (6 isolates), 1/2b (4 isolates) and 1/2c (one isolate) (de Simón *et al*., 1998), while in the UK 11 isolates belonged to 1/2 (6), 3 (1) and 4b (4) (Velani and Roberts, 1991) and in the USA most isolates were serotype 1a (Heisck *et al*., 1989).

Italian ready-to-eat vegetable samples yielded 17 (51.5%) serotype 1/2a isolates, 6 (18.2%) 1/2b, 3 (9.1%) 1/2c, 6 (18.2%) 4b and 1 (3.0%) 4e. Over the same time period clinical isolates were 21 (27.2%) 1/2a, 9 (11.7%) 1/2b, 4 (5.2%) 1/2c, 1(1.3%) 3b, 2 (2.6%) 4ab, and 40 (52.0%) 4b (Gianfranceschi *et al*., 2003). The predominance of serotype 4b isolates among clinical isolates was not therefore found in those from vegetables, but qualitatively the serotypes isolated from both sources were similar.

Schlech *et al*. (1983) detected *L. monocytogenes* serotype 4b in both coleslaw and human listeriosis cases during an investigation of a listeriosis outbreak. Further investigation revealed that the cabbage used for preparing the coleslaw might have been contaminated with *L. monocytogenes* from sheep manure at the farm.

5 EXPOSURE ASSESSMENT

5.1 The Hazard in the New Zealand Food Supply: *Listeria* in Ready-to-Eat Salads

Little published New Zealand information is available on the prevalence and numbers of *L. monocytogenes* on the type of ready-to-eat salads included in this Risk Profile.

Crop and Food Research tested 104 samples of coleslaw or cabbage salad (with or without added mayonnaise) from Auckland Central retail outlets between October 1998 and May 2000. One (0.96%) mayonnaise-containing sample was positive for *L. monocytogenes* (Graham Fletcher, Crop and Food Research, personal communication).

Graham and Dawson (2002) reported on the analyses of 291 hydroponically grown sprouts, herbs and leafy vegetables. *L. monocytogenes* was not isolated.

5.2 Food Consumption: Ready-to-eat Salads

5.2.1 Total salad consumption

Analysis of data from the 1997 National Nutrition Survey (NNS) (Russell *et al*., 1999) gives an estimate for the total per capita consumption of raw vegetable salads by New Zealanders aged 15 years and over of 32.3 g/day. This estimate included salads variously described as; coleslaw, lettuce, tomato, Caesar, carrot, cauliflower, Greek, green, spinach or Waldorf. The estimate excludes non-vegetable salads and obviously cooked salad types such as rice, pasta, potato and bean. Lettuce salad was the most commonly consumed salad type (47% of total salad servings), followed by coleslaw (32%), and green (18%).

Salads were more frequently consumed by females than males across all age groups. This is consistent with a UK Department of Health survey in which females reported consuming salads more frequently than males (http://www.doh.gov.uk/public/hse01.htm). In New Zealand, overall 15.5% of female respondents reported consuming salads, while 13.9% of men reported salad consumption. In the UK 27% of males and 34% of females reported consuming salads in the previous 24 hour period, however, the UK survey would include all salads and not just the restricted subset being considered in the current risk profile.

The 1997 NNS data indicates that those in the age range 25-64 are more likely to consume salads than either younger or older age groups. This is consistent with the results of the UK survey.

There were no clear demographic trends in amounts of salads consumed, for example, in some age groups men consumed greater quantities of salad than females, while in other age groups the trend was reversed.

None of the overseas food consumption studies which have previously been used as reference points for New Zealand food consumption figures use the descriptor ‘salads’ and, instead, classify foods in terms of ‘vegetables’ or subclasses of vegetables.

5.2.2 Ready-to-eat salad consumption

No information was found to determine the proportion of salads consumed that could be classified as 'ready-to-eat'. The dietary records included in the 24-hour recall portion of the 1997 National Nutrition Survey do not classify food according to source.

Statistics New Zealand's Household Economic Survey concluded that New Zealanders report spending 23% of their total food expenditure on 'meals away from home, ready-to-eat meals' (http://www.stats.govt.nz/domino/external/web/prod_serv.nsf/htmldocs/Consumer+Spending). While foods in this category are likely to be more expensive than foods prepared in the home, if this figure is applied in a pro rata manner to raw vegetable salad consumption, then New Zealanders would be consuming approximately 7 g/day of ready-to-eat salads.

Overseas information on consumption patterns for ready-to-eat salads also appears to be sparse. A USDA report stated that "per capita consumption of bagged salad increased from 0.9 pounds in 1994 to 2.0 pounds in 1999" (USDA, 2001). This equates to approximately 3 g/person/day. However, it is uncertain whether the definition of 'bagged salad' is sufficiently close to the definition being used in the current risk profile for any analogies to be drawn. The UK National Food Survey 1994-1998 reported an average consumption of salads eaten outside the home of 3.9 g/person/day, see website; (www.defra.gov.uk/esg/Work_htm/publications/cf/nfs/for_nfs98/section4.pdf). However, this study also does not appear to match the definition employed in the current Risk Profile.

A risk assessment for *L. monocytogenes* (FDA and FSIS, 2003) carried out by the Food Safety Inspection Service (FSIS) of the US Department of Agriculture (USDA) determined risks for 'vegetables' and 'deli salads' (http://www.cfsan.fda.gov/~dms/lmr2-5.html). Based on the number of servings and the median serving it can be determined that the median level of 'vegetables' consumption is approximately 22g/person/day. The vegetable category encompasses a diverse set of products that are typically consumed without cooking, this includes raw salads but not salad dressings and most closely represents the definition of ready-to-eat salad in this Risk Profile. 'Deli salad' consumption in the US is approximately 12 g/person/day. The description of deli salads includes meat, seafood, egg and pasta salads, vegetable and fruit salads with salad dressing and salad portion of sandwiches. This category is distinctly different to the category being considered in the current Risk Profile.

5.3 Qualitative Estimate of Exposure

5.3.1 Number of servings of ready-to-eat salad and serving size

5.3.1.1 Total population

From the NNS, 688 individual dietary records were deemed to represent consumption of a serving of raw vegetable salad. If it is assumed that approximately 20% of these servings will fit the ready-to-eat salad definition used in the current risk profile and using a total survey population of 4636 and a total New Zealand population of 4,054,200 (at 31 March 2004) (http://www.stats.govt.nz/):

Annual number of servings (total population) = 688 x 0.2 x 4,054,200/4636 x 365
= 4.4×10^7 servings

5.3.1.2 Elderly population

From the NNS, 155 individual dietary records were deemed to represent consumption of a serving of raw vegetable salad for an individual aged 60 years or more. A total of 1087 people aged 60 years or more completed dietary recall questionnaires as part of the NNS. According to the 2001 Census 615,580 New Zealanders were aged 60 years or more.

Annual number of servings (elderly population) = 155 x 0.2 x 615,580 /1087 x 365
= 6.4×10^6 servings

5.3.1.3 Perinatal population

The assumptions made by the FDA/FSIS to calculate the perinatal population were used to calculate the number of perinatal servings for pregnant women in the New Zealand population. This approach has recently (September 2003) been altered (http://www.foodsafety.gov/~dms/lmr2-toc.html). This was done by multiplying the number of servings for the intermediate population (see below) by the annual pregnancy rate and by 0.25 (3/12) to estimate the number of pregnant women in the last trimester – the period of greatest susceptibility for perinatal listeriosis. A pregnancy rate for New Zealand could not be located and the US figure of 2.77% was used, however, trial calculations for the New Zealand population (live births plus abortions x 1.33, to account for the difference between gestation period and year length, as a percentage of the intermediate age population) gave a similar figure.

Annual number of servings (perinatal population) = 3.6×10^7 x 0.0277 x 0.25
= 2.5×10^5 servings

5.3.1.4 Intermediate population

The annual number of servings of ready-to-eat salad consumed by the intermediate population is calculated by subtracting the value for the elderly from the total population (the perinatal population are assumed to be a very small number and therefore are not subtracted in the FSIS method).

Annual number of servings (intermediate population) = 3.7×10^7 servings

5.3.1.5 Serving Size

Based on the data in the NNS database the 50, 75, 95, and 99th percentile serving sizes for raw vegetable salads are given in Table 4.

Table 4: New Zealand and US serving sizes for raw vegetable salads

	Percentile serving sizes (g)			
	50th	**75th**	**95th**	**99th**
New Zealand (Russell *et al*., 1999)				
All raw vegetable salads	122	250	614	1670
US (FDA/FSIS (2003:page 35**)**				
Vegetables	28	55	123	220

For the purpose of this risk profile it has been assumed that serving sizes for ready-to-eat salads are likely to be the same or similar to serving sizes of raw vegetable salads in general. The vegetable category in the FDA/FSIS mostly closely resembles ready-to-eat salads in this risk profile and USA serving sizes are considerably lower than the New Zealand serving sizes.

5.3.2 Contamination frequency

There are no recent data that would allow the reliable assignment of an overall frequency of contamination to ready-to-eat salads in New Zealand (see section 5.1).

5.3.3 Predicted contamination level at retail

Insufficient New Zealand data on levels of *L. monocytogenes* in retail salads are available to allow comment to be made. The lack of qualitative and quantitative data therefore represents a significant data gap.

5.3.4 Growth rate during storage and most likely storage time

The likelihood of *L. monocytogenes* growth on ready-to-eat salads during storage will be strongly dependent on the components of the salad and whether salad dressings are present or not which may affect the pH value. Growth rates are also affected by time/temperature combinations. The internationally recommended shelf life given to a product is approximately 7 days (AIFST, 2003), although 7 to 14 days has been reported (García-Gimeno and Zurera-Cosano, 1997) depending on the components of the salad (see section 3.2.4). Modified atmosphere packaging has the potential to enhance growth of *L. monocytogenes*, through inhibition of other microbial populations.

5.3.5 Heat treatment

Not applicable to ready-to-eat salad products.

5.3.6 Exposure summary

There are insufficient current data to speculate on the likely exposure to *L. monocytogenes* due to consumption in New Zealand of the type of ready-to-eat salad covered by this Risk Profile.

5.4 Overseas Context

Surveys in the United Kingdom offer the most up to date and broad overview of the microbiological quality of ready-to-eat salads for retail sale. Open (unpackaged) salads were also sampled from catering premises. Dressed or seasoned salads were specifically excluded. Split into three main categories, the microbiological surveys cover;

- Bagged prepared ready-to-eat salad vegetables (Sagoo *et al*., 2003a),
- Open (unpackaged) ready-to-eat prepared salad vegetables (catering & retail) (Sagoo *et al*., 2003b), and
- Ready-to-eat organic vegetables from retail establishments (Sagoo *et al.*, 2001).

The results of the surveys are summarised in Table 5.

Table 5: Results of recent surveys in the UK; bagged, open (unpackaged) and organic ready-to-eat salads

Survey	No. of samples	Results
Bagged	3852	• 20 samples (0.5%) were microbiologically unsatisfactory (presence of *Salmonella* or *L. monocytogenes* at $>10^2$ cfu/g), • Total *Listeria* spp. (incl. *L .monocytogenes*), detected in 169 samples, in two samples present at $>10^2$cfu/g (0.05%)-one of these was *L. innocua*, the other see below, • *L. monocytogenes* detected in 88 (2.2%) samples, in one sample present at $>10^2$cfu/g (0.03%)- serotype 1/2. This result was deemed "unacceptable" for the salad in question at 660 cfu/g
Open	2950	• 87 samples (3%) were microbiologically unsatisfactory (presence of *E. coli* at 10^2-10^5 cfu/g), • Total *Listeria* spp. (incl. *L. monocytogenes*), detected in 125 samples, in one sample present at $>10^2$cfu/g (0.03%), and • *L. monocytogenes* detected in 88 samples (3.0%), in one sample present at $>10^2$cfu/g (0.03%)- serotype 4b.
Organic	3200	• 15 samples (0.5%) were microbiologically unsatisfactory, • 6 (0.2%) of these samples unsatisfactory. due to presence of *Listeria* spp., • 4 of the 6 samples had prevalence $>10^2$cfu/g. • Of these 4, 2 samples were in excess of 10^3cfu/g, these were; • *L. innocua* (watercress) and *L seeligeri* (radish) • *L. monocytogenes* NOT detected in any of the 3200 samples

The following information from the scientific literature concerns the prevalence of *L. monocytogenes* in processed salad ingredients, and salad containing ready-to-eat foods. The data have been summarised in Table 6. There is also a list of bacterial pathogens isolated from raw vegetables in the WHO review document (1998:p5-6) which includes isolations of *L. monocytogenes;*
http://www.who.int/foodsafety/publications/fs_management/surfac_decon/en/

Table 6: Overseas prevalence and quantitative data for *L. monocytogenes* in salads and salad vegetables

Country/Region	Food	No. samples tested	No. (%) positive for *L. monocytogenes*	Reference
Australia	Ready-to-eat salads and vegetables	54	1 (1.9)	Arnold and Coble, 1995
Australia	Minimally processed cut and packaged lettuce samples	120	3 (2.5)	Szabo *et al*., 2000
Canada	Lettuce Celery Radishes Tomatoes	0 30 10 20	0 0 0 0	Farber *et al*., 1989
Canada	Cabbage	92	2 (2.2)	Quoted in Beuchat, 1996
Canada	Salad mix Coleslaw mix Chopped lettuce (all stored at 4 or 10°C for up to 11 days)	39 35 39	9 (23.1), 4<100/g, 5 >100/g 1 (2.9), >100/g 5 (12.8), 4<100/g, 1>100/g	Odumeru *et al*., 1997
England	Prepacked salads	60	4 (6.7)	Sizmur and Walker, 1988
England	Salad sandwiches	16	1 (6.3) NB data for *Listeria* only. Present at <100/g.	Wilson, 1996
Hong Kong	Market salads	573	6 (1.0)	FEHD, 2002
India	Restaurant-bought salads	12	0	Pingulkar *et al*., 2001
Italy	Ready-to-eat vegetables	738	33 (4.5)	Gianfranceschi *et al*., 2003
Japan	Raw vegetables cut for salad	27	0 (2 positive for other *Listeria* species)	Kaneko *et al*., 1999a
Netherlands	Salad vegetables	25	11(44)	Quoted in Beuchat, 1996
Northern Ireland	Prepared salads	40	3 (7.5)	Harvey and Gilmour, 1993
Northern Ireland	Salads/vegetables	414	8 (2.0) *Listeria* spp. only	Wilson, 1995
Norway	Pre-cut salad	100	0	Johannessen *et al*., 2002
Portugal	Ready-to-eat vegetables	23	0	Guerra *et al*., 2001
Spain	Ready-to-use mixed vegetable salads	6	4 (66.7)	García-Gimeno *et al*., 1996
Spain	Prepared salads	146	15 (10.2). Data for 14 samples; 10< 100/g, 1 100-1,000/g, 3 >1,000/g	de Simón *et al*., 1998
Spain	*Ready-to-eat lettuce from restaurants	10	1 (10%)	Soriano *et al*., 2001
Switzerland	"Fresh" or "raw" salad Washed and cut salads	99 67	1 (1.0) 4 (6.0)	Breer and Baumgartner, 1992

Country/Region	Food	No. samples tested	No. (%) positive for *L. monocytogenes*	Reference
UK	Prepacked mixed salads Individual salad ingredients	42 108	8 (19.0) 2 (1.8) all samples < 200/g	Velani and Roberts, 1991
UK	Salads Crudités	2276 247	77 (2.8) NS (<1%) A salad sample contained 10^2-10^3/g.	Little *et al*., 1997
USA	Supermarket-bought without further cleaning: Radishes Cabbage Cucumbers Lettuce	 92 92 92 92	 (14.4) (1.1) (2.2) (1.1)	Heisick *et al*., 1989
USA	Vegetable salads	63	1 (1.6)	Lin *et al.*, 1996a
USA	Salad and salad bar items	13	0	Snyder, 1997
USA	Cauliflower Celery Cucumbers Green peppers Lettuce	10 12 2 2 12	0 0 0 0 0	Thunberg *et al*., 2002
USA-California - Maryland	Bagged salads Bagged salads Bagged salads	1501 1465	14 (0.9) 8 (0.6) Quantitative data 0.04-0.1/g 77.3% >0.1-1.0/g 4.5% >1-10/g 4.5% >10-10^2/g 9.1% >10^2-10^3/g 4.5%	Gombas *et al*., 2003

NS= Not stated

* all restaurants reported use of sodium hypochlorite to sanitise vegetables.

The information summarised above suggests that *L. monocytogenes* is present as a contaminant in salads or salad vegetables usually at prevalences less than 10%. This might be expected as the bacterium is a ubiquitous environmental contaminant and the foods are not subjected to a listericidal treatment. However, the quantitative data indicates that the bacterium is usually present at less than 100 cfu/g, and thus growth to high numbers before purchase is an unusual event.

6 RISK CHARACTERISATION

Listeriosis is a notifiable disease in New Zealand, and it is generally assumed that the severity of the disease means that there are no unreported cases. However, the non-invasive febrile gastroenteritis form of infection is not notifiable, and the only information on its incidence comes from an outbreak. Consequently this section is principally concerned with invasive listeriosis.

6.1 Adverse Health Effects in New Zealand

6.1.1 Incidence

Notification and mortality data from the EpiSurv database for listeriosis for the years 1990 to 2003 are given in Table 7. It is important to note that these cases are not associated with any specific transmission vehicle.

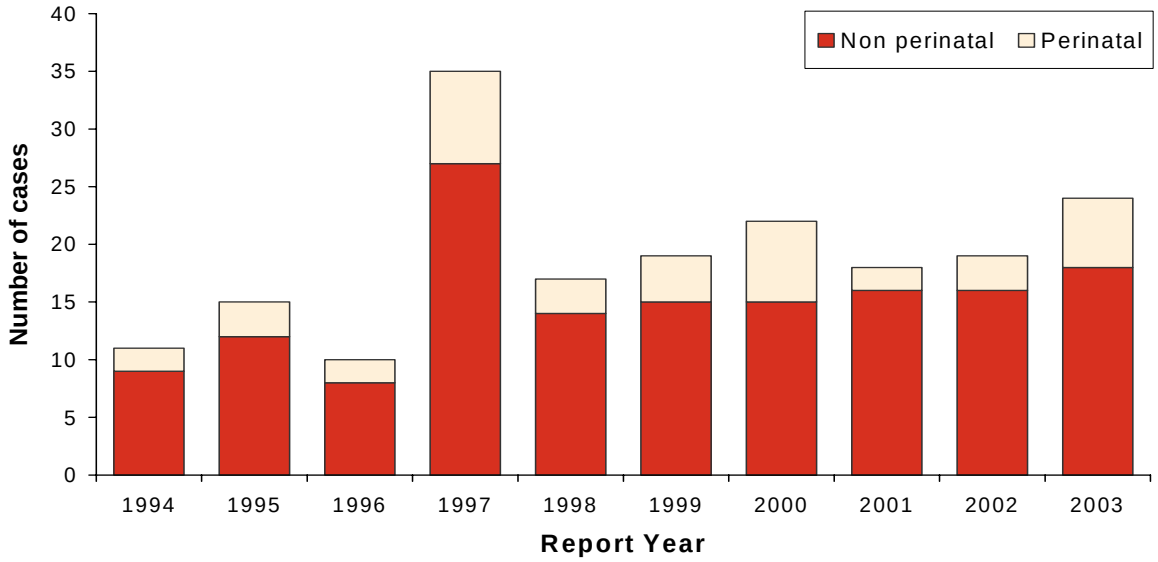
Table 7: Number of reported cases of invasive listeriosis and mortality from 1990 to 2003

Year	Listeriosis cases	Deaths (perinatal)	Deaths (non-perinatal)	Reference
1990	16	2	NA	Kieft *et al*., 2000
1991	26	1	NA	Kieft *et al*., 2000
1992	16	0	NA	Kieft *et al*., 2000
1993	11	2	NA	Kieft *et al*., 2000
1994	8	0	NA	Kieft *et al*., 2000
1995	13	1	0	Kieft *et al*., 2000
1996	10	1	0	Kieft *et al*., 2000
1997	35	6	2	Kieft *et al*., 2000
1998	17	0	0	Kieft *et al*., 2000
1999	19	2	1	Kieft *et al*., 2000
2000	22	4	2	Lopez *et al*., 2001
2001	18	1	1	Sneyd *et al*., 2002
2002	19	3	0	Sneyd and Baker, 2003
2003	24	2	2	ESR, 2004

NA = Not Available

Figure 3 shows a graphical representation of annual case numbers of listeriosis with the proportions of perinatal and non-perinatal cases identified.

Figure 3: Listeriosis notifications by year 1994 – 2003



Reproduced from ESR (2004)

6.1.2 Clinical consequences of *Listeria* infection

Listeriosis has a high proportion of serious outcomes i.e. hospitalisation and death. Hospitalisation and fatality rates for notified cases of listeriosis in New Zealand during the period 1997-2003 are given in Table 8. These outcomes are not always reported for each case, so percentages are expressed in terms of the number of cases for which outcomes are known.

Table 8: Outcome data for listeriosis in New Zealand, 1998 to 2003

Year	Hospitalised cases	Fatalities	Reference
1997	33/33 (100%)	8/35 (22.9%)	ESR, 1998
1998	16/16 (100%)	0/17 (0.0%)	Perks *et al.*, 1999
1999	18/19 (94.7%)	3/19 (15.8%)	Kieft *et al.*, 2000
2000	22/22 (100%)	6/22 (27.3%)	Lopez *et al.*, 2001
2001	17/18 (94.4%)	2/18 (11.1%)	Sneyd *et al.*, 2002
2002	13/13 (100%)	3/19 (15.8%)	Sneyd and Baker, 2003
2003	22/22 (100%)	4/24 (16.7%)	ESR, 2004

Estimates for the United States are similar to the New Zealand data, with 92% of cases hospitalised, and 20% of cases resulting in death (Mead *et al*., 1999).

6.1.3 Information from Ministry of Health's suspect foodborne illness investigation programme

The Ministry of Health's Suspect Foodborne Illness Investigation Programme provides investigative analyses to Public Health Units and provides a means of collating such investigations. The programme is funded by the Ministry of Health and provided by ESR. It contains information relating particular foods to episodes of suspected foodborne illness. This may be due to a genuine risk factor related to the symptoms presented, or preconceptions of the person experiencing the illness or the investigating officer. If the laboratory investigation identifies a known food pathogen in the suspect food at levels sufficient to cause illness and the symptoms known to be caused as a result of infection by the organism are consistent with the case details then the food may be identified as confirmed. Less compelling evidence may be provided in cases where a known pathogen is identified in faecal specimens associated with the suspected foodborne illness episode but not from the food samples provided (in some cases food samples may not have been provided, but a food may still be suspected).

While salads, including some that clearly fit the definition used in the current Risk Profile, are frequently implicated in suspect food poisoning cases, no records could be found of *L. monocytogenes* being isolated from salads associated with a suspect food poisoning or from clinical specimens in cases in which salads were implicated.

6.1.4 Outbreaks

Outbreaks of infection with *L. monocytogenes* in New Zealand are rare. From 1997 to 2003 only three have been reported to the national surveillance system. None of these outbreaks were linked to consumption of ready-to-eat salads. Two of the outbreaks were connected, and associated with consumption of corned beef and ham (Sim *et al.*, 2002; Whyte, 2000) while no food vehicle was identified in the other (Anonymous, 1998). An earlier small outbreak, in 1992, was linked to the consumption of smoked mussels (Brett *et al.*, 1998). The relatively long incubation period between exposure and the onset of symptoms, means that it can be extremely difficult to link listeriosis outbreaks to potential food sources.

6.2 Adverse Health Effects Overseas

6.2.1 Incidence

Comparisons of listeriosis rates between countries must be made cautiously, as reporting practices may differ. However, the data in Table 9 indicate that New Zealand's rate is similar to that of other developed countries.

Table 9: Comparison of listeriosis incidence between countries

Country	Period	Rate /100,000	Reference
New Zealand	**1999**	**0.5**	**Kieft *et al.*, 2000**
New Zealand	**2000**	**0.6**	**Lopez *et al.*, 2001**
New Zealand	**2001**	**0.5**	**Sneyd *et al.*, 2002**
New Zealand	**2002**	**0.5**	**Sneyd and Baker, 2003**
New Zealand	**2003**	**0.6**	**ESR, 2004**
Australia	2000	0.3	Lin *et al*., 2002c
Australia	2002	0.3	OzFoodNet Working Group, 2003
Canada	1990-1999	0.1-0.3	Health Canada, 2000
Denmark	2001	0.7	Dansk Zoonosecenter, 2002
Denmark	2002	0.5	Danish Zoonosis Centre, 2003
France	1997	0.4	De Valk *et al*., 1998
UK	1983-2001	Approx. 0.2 - 0.5	PHLS, 2002
USA	2000	0.4	Anonymous, 2001
USA	2002	0.3	Anonymous, 2003

6.2.2 Contributions to outbreaks and incidents

As shown by the data in Table 10, most cases of infection with *L. monocytogenes* are sporadic rather than part of outbreaks, and outbreaks caused by *L. monocytogenes* make up only a very small proportion of the total outbreaks reported.

Table 10: Contribution of *L. monocytogenes* to foodborne disease outbreaks and incidents overseas

Country	Year	No. (%) Outbreaks	No. (%) incidents or cases	Reference
Canada	1981	NS	1 (0.2) incidents 41 (<0.1) cases	Todd, 1992
USA	1989	1 (0.2)	2 (<0.1) cases	Bean *et al*., 1996
USA	1993-1997	3 (0.1)	100 (0.1) cases	Olsen *et al*., 2000

NS = Not stated

An analysis was conducted in England and Wales of outbreaks of foodborne infectious intestinal disease attributed to the consumption of salad vegetables and fruit (Long *et al.*, 2002). Over the years from 1992 to 2000, outbreaks attributed to these food vehicles accounted for between 1.8 and 10.8% of outbreaks that were considered foodborne. The aetiological agents involved were primarily salmonellae and norovirus. No outbreaks of listeriosis were recorded among the 85 outbreaks analysed.

A similar analysis of outbreaks of foodborne illness associated with fresh produce in the United States from 1973 to 1997 has been published (Sivapalasingam *et al.*, 2004). Although salads were the most commonly identified vehicle (76 of 190 outbreaks), *L. monocytogenes*

was not identified as the etiologic agent for any of the outbreaks where an agent was identified (103 of the 190 outbreaks).

Overseas outbreaks of listeriosis where ready-to-eat salad has been implicated as the vehicle of infection have been summarised in Table 11.

Table 11: Overseas outbreaks of listeriosis where ready-to-eat salad was the implicated vehicle

Country	Year	No. Cases	Food	Odds ratio	Reference
USA	1979	20, 5 deaths. 15 infections nosocomial	Raw celery, tomatoes and/or lettuce	Not given. Three meals were associated with disease, all contained the implicated vegetables	Ho *et al*., 1986
Canada	1981	41, 7 deaths	Coleslaw	2.31, P = 0.04	Schlech *et al*., 1983

CI= 95% confidence intervals. NS = Not stated.

In the Canadian outbreak all cases had consumed coleslaw, and the same serotype was isolated from the food and from cases (serotype 4b). A review of the sources of raw vegetables used to manufacture the coleslaw identified a farmer who grew cabbage and kept a flock of sheep. Two of the sheep had died of listeriosis during 1979 and 1981. Cabbage was grown in fields fertilised with both composted and raw manure from the flock of sheep (Schelch *et al*., 1983).

6.2.3 Case-control studies

Available information from case-control studies of listeriosis do not provide much information in relation to ready-to-eat salads. Two case-control studies in the US and France did not identify any relevant food categories as being associated with increased risk of listeriosis (Schwartz *et al*., 1989; de Valk *et al.*, 1998). Schuchat *et al.* (1992) identified an increased risk of listeriosis with 'deli counter food' in the US (multivariate model; odds ratio = 1.59, 95% confidence interval = 1.02-2.48), which may have included ready-to-eat salads. Jensen *et al*. (1994) found an increased risk of listeriosis associated with fruit and vegetable consumption for an outbreak in Denmark (odds ratio = 9.2, $p<0.1$), but found no association for sporadic cases or at an overall level.

6.2.4 Risk assessments

A number of risk assessments have now been published concerning *L. monocytogenes.*

The United State's joint FDA/FSIS risk assessment was published in September 2003 (http://www.foodsafety.gov/~dms/lmr2-toc.html). A further risk assessment by the FAO/WHO (Codex 2002) is in draft form and can be found at; http://www.who.int/foodsafety/micro/jemra/assessment/listeria/en/ under the related documents link.

After the most recent round of revisions, the FAO/WHO (Codex 2002) model has combined aspects of the FDA/FSIS one and almost merged the two. However, since the latest version

of the Codex 2002 assessment is still in draft form, only the FDA/FSIS assessment will be discussed here. ftp://ftp.fao.org/codex/alinorm04/al04_13e.pdf

It should be noted that this is very much a North American risk assessment and so used an exposure assessment which is particular to that part of the world (even though data from all over the world were used to calculate prevalences in food). We might assume that the hazard characterization (essentially dose response) would be the same in New Zealand as North America, but the derived risk characterisation will be different because of the different exposure assessments.

The relative risks predicted for the various ready-to-eat food categories in the FDA/FSIS risk assessment are given in Table 12, for various at-risk population groups, and also as an overall ranking. One food, frankfurters, may or may not be reheated prior to consumption so is considered as two separate food categories. It is recognised that additional foods or cross-contamination may contribute further cases. Note that the rankings in this table have changed from those given in the draft risk assessment.

Table 12: Predicted relative risk rankings for listeriosis based on the North American sub-population using median estimates on a per serving basis

Food Categories[a]	Sub-Population			
	Intermediate Age[b]	Elderly[b]	Perinatal[b]	Total [b,c]
	Relative Rank (1- 23)			
SEAFOOD				
Smoked seafood	6	5	5	5b
Raw seafood	12	12	12	13d
Preserved fish	13	13	13	12d,e
Cooked ready-to-eat crustaceans	5	6	6	6b
FRUIT AND VEGETABLES				
Vegetables	18	18	18	18
Fruits	15	15	15	14e
DAIRY PRODUCTS				
Fresh soft cheese (e.g. queso fresco)	10	10	10	10
Soft ripened cheese, >50% moisture	17	17	17	17f
Soft unripened cheese, >50% moisture	8	8	8	8c
Semi-soft Cheese, 39-50% moisture	16	16	16	16f
Processed cheese	20	20	20	21g
Hard cheese <39% moisture	23	23	23	23
Fluid milk, pasteurised	9	9	9	9c
Fluid milk unpasteurised	4	4	4	4b
Ice cream and frozen dairy products	21	21	21	20g
Cultured Milk Products	22	22	22	22g
High Fat and Other Dairy Products	7	7	7	7
MEATS				
Reheated frankfurters	11	11	11	11
Non-reheated frankfurters	2	2	2	2a

Food Categories[a]	Sub-Population			
	Intermediate Age[b]	Elderly[b]	Perinatal[b]	Total [b,c]
Dry/semi dry fermented sausages	14	14	14	15d
Deli meats	1	1	1	1a
Pâté and meat spread	3	3	3	3
COMBINATION FOODS				
Deli salads	19	19	19	19

[a] Food categories are grouped by type of food but are not in any particular order.
[b] A ranking of 1 indicates the food category with the greatest predicted relative risk per serving of causing listeriosis and a ranking of 23 indicates the lowest predicted relative risk of causing listeriosis.
[c] Ranks with the same letter are not significantly different based on the Bonferroni Multiple Comparison Test (alpha = 0.05).
Source: FDA/FSIS 2003 (http://www.cfsan.fda.gov/~dms/lmr2-5.html)

It should be noted that the description of 'deli salads' used in the FDA/FSIS risk assessment is significantly different to the description of ready-to-eat salads used in this current Risk Profile. Foods in the deli-type salads category includes a wide variety of meat, seafood, egg and pasta salads, vegetable and fruit salads with salad dressing (e.g. coleslaw and potato salad), as well as the salad portion of sandwiches. In a change from the draft risk assessment carried out by FDA/FSIS in 2001, the vegetable and fruit salads made with dressing have been moved from the Vegetables and Fruits category to the deli-type salads category.

The Vegetable category appears to most closely resemble the ready-to-eat salad definition in this Risk Profile. Vegetables are defined in the risk assessment as a diverse set of products that are typically consumed without cooking. This includes raw as well as mixed vegetable salads containing raw vegetables but not salad dressing, e.g. spinach, carrots, tomatoes, celery, lettuce, onions.

The annual number of servings of vegetables is high, while the median serving size, contamination level and growth rate are low. The relative risk ranking for vegetables was therefore low at 18 (out of 23). The predicted number of cases of listeriosis per serving was 2.8×10^{-12} (the relative risk faced by an individual when a single serving is consumed). The predicted number of fatal infections per year from vegetables is also low at 0.2.

The relative risk ranking for the deli salad category given was also low at 19. Cases per serving 5.6×10^{-13} and predicted number of fatal infections per year lower than vegetables at >0.1.

A review of emerging hazards and issues associated with produce in the US did not identify *L. monocytogenes* as a significant hazard, although it did record the coleslaw outbreak (Tauxe *et al*., 1997).

6.3 Qualitative Estimate of Risk

The information summarised above leads to the conclusion that the transmission of *L. monocytogenes* by ready-to-eat salads has the potential to contribute to a proportion of invasive listeriosis cases in New Zealand. Evidence for this conclusion comes from:

- The consistent presence of *L. monocytogenes* in a proportion of samples of ready-to-eat salads in surveys from overseas;
- The ability of *L. monocytogenes* to survive on, and even grow on, ready-to-eat salads, under some circumstances.

Evidence suggesting that the risk of *L. monocytogenes* infection from ready-to-eat salads is likely to be low comes from:

- The lack of any confirmed outbreaks or sporadic cases of listeriosis due to consumption of ready-to-eat salads in New Zealand;
- The small number of outbreaks or sporadic cases of listeriosis linked to salad consumption overseas;
- The relatively low ranking (compared to other potential food vehicles for *L. monocytogenes*) given to vegetables in the FDA/FSIS risk assessment.

However, there is insufficient New Zealand information available to satisfactorily gauge the risk posed by this food/hazard combination.

6.4 Risk Categorisation

The rationale for categorisation of food/hazard combinations is presented in Appendix 1.

The invasive form of listeriosis causes a high (>5%) proportion of serious outcomes (hospitalisation, long term illness, and death). Although there are no data to identify the proportion of listeriosis transmitted by ready-to-eat salads compared to other food groups, any incidence will be in the lowest category because the overall incidence is below 1 per 100,000.

The non-invasive form of the disease is presumed to cause few serious outcomes, but data on incidence of this form are not available.

6.5 Summary

Food/hazard combination	Severity	Incidence	Trade importance	Other considerations
L. monocytogenes in ready-to-eat salads	1 (>5% serious outcomes)	4 (<1 per 100,000)	Not an issue for this food	Incidents attract adverse media attention

7 RISK MANAGEMENT INFORMATION

Contamination of ready-to-eat salads by *L. monocytogenes* is potentially derived from both the incoming raw material, particularly on produce grown in soil or in close proximity to the soil, and from the environment during processing. There are suggestions that some salad ingredients such as tomatoes and carrots are naturally listericidal (see section 3.2) although most are not. Prevention of contamination at all points of the food chain (with GAP, GMP and HACCP programmes) is preferable over the application of disinfectants (European Commission Scientific Committee on Food, 2002; WHO, 1998).

There is also considerable debate whether disinfection is an effective CCP for *L. monocytogenes*. The WHO review (1998) does point out however, that while disinfectants have variable effects on pathogen control, they are certainly useful for sanitising wash-water to prevent contamination of the produce. A study of vegetable processing plants in Japan found heavy bacterial contamination (APC >5.0 $\log_{10}$ cfu/cm^2) in samples from most equipment surfaces (although no *L. monocytogenes* was detected) (Kaneko *et al*., 1999b).

7.1 Relevant Food Controls in New Zealand

Because ready-to-eat salads are unable to undergo a listericidal step in their production, there are no Standards in the Food Standard Codes for ready-to-eat salads. The guidelines associated with the Code are the relevant documents in this instance.

7.1.1 Joint Australia New Zealand Food Standards Code (FSANZ)

On the 20 December 2002, the New Zealand Food Regulations 1984 were revoked, replaced or retained, principally to make way for the joint Food Standards Australia New Zealand (FSANZ) Code. Any regulations falling outside of the joint system (not covered by 'the code') are contained in the Food (Safety) Regulations 2002, (applicable only in New Zealand).

Under Chapter 1 of the Food Standards Code, Standard 1.6.1 (see website:http://www.foodstandards.gov.au/_srcfiles/Standard_1_6_1_Micro_v70.doc), "Microbiological Limits for Food" lists the maximum permissible levels of foodborne micro-organisms which pose a risk to human health. It is unlawful to exceed these limits. The attached Schedule to 1.6.1 is only relevant to those foods which undergo a listericidal step (such as a pasteurisation step) before consumption to achieve zero prevalence; because this is not possible for ready-to-eat salads, the associated guidelines must be referred to.

7.1.2 Microbiological Criteria -Guidelines

There are three sets of guidelines intended to assist with sampling protocols and interpretation of results. These three guidelines have no legislative standing, they are purely advisory and are not intended to set benchmarks of acceptability. Industry is encouraged to use the principles of HACCP to continually improve processes. The guides are intended to complement other risk management strategies undertaken by government and industry.

The guidelines are currently being revised so that only one document would need to be referred to in future, however for the time being, the following guidelines are in place;

i. Microbiological Reference Criteria for Food (Ministry of Health, 1995a),

The Microbiological Reference Criteria can be of prime importance in deciding if a food is unsound. Chapter 4 covers the general microbiological reference criteria for *L. monocytogenes.* The criteria in Chapter 4 do not apply to ready-to-eat salads because;

- they state that they do not apply to raw fruit or raw vegetables, and in addition
- the criteria does not apply to foods produced with good manufacturing practice and recommended for consumption within four days of manufacture.

Chapter 5 does give some general reference criteria for vegetable or fruit salads (excluding combination with meat) such as aerobic plate count at 35°C, Coagulase producing *Staphylococcus*, Faecal coliform and *Salmonella*, but not for *Listeria* spp.

ii. Microbiological guideline criteria (User Guide to Standard 1.6.1) (http://www.foodstandards.gov.au/assistanceforindustry/userguides/microbiologicallimit1410.cfm)

The User Guide does not specifically mention ready-to-eat salads, therefore the guidelines for ready-to-eat foods must be referred to.

iii. Guidelines for the microbiological examination of ready-to-eat foods (http://www.foodstandards.gov.au/mediareleasespublications/publications/guidelinesformicrobi1306.cfm).

Under the guidelines for ready-to-eat foods, levels of 100 cfu *L. monocytogenes* or more per gram indicate a failure in controls and are considered "potentially hazardous". Recall action may be initiated.

In New Zealand, recalls are coordinated by the NZFSA. At the time of writing, Section 15 "Recalls" in the Food Administration Manual (Ministry of Health, 1995b) is in use. However this recall procedure is under review. The procedure does not include microbial guidelines.

There have been no recalls due to *Listeria monocytogenes* in ready-to-eat salads at the time of writing. This may be because there is no obligation to carry out *Listeria* monitoring on ready-to-eat salads. In addition, the shelf life of the product is very likely to have expired before enumeration has been completed. Occasionally the retail customer may test ready-to-eat salad for *L. monocytogenes* at end product testing. Manufacturers can lose approved supplier status should end products not conform with customer end product specifications.

7.1.3 The Food Act 1981 and Food Safety Programmes (FSPs)

The Food Act 1981 was amended in 1996 to recognise appropriate Food Safety Programmes and allow exemptions from the Food Hygiene Regulations 1974. Section 8G of the Food Act 1981 gives the definition of an appropriate food safety programme (one which is eligible for an exemption) (http://www.legislation.govt.nz/browse_vw.asp?content-set=pal_statutes).

Some ready-to-eat salad producers in New Zealand are known to have registered Food Safety Programmes.

7.1.4 The Fresh Produce Industry's Approved Supplier Programme

In 1999, the New Zealand Vegetable and Potato Growers' Federation (see http://www.vegfed.co.nz) launched an Approved Supplier Programme. They were joined in 2000 by the NZ Fruitgrowers Federation and in 2003 by flower growers. The result is a single industry programme known as the New Zealand Fresh Produce Approved Supplier Programme or ASP. This programme is recognised by the NZFSA as a Code of Practice for the Industry. The programme is based upon the principles of Good Agricultural Practice, HACCP and elements of ISO 9002 and discusses microbial issues and general microbial hazards throughout the programme. The raw in-coming product stage is within the scope of Good Agricultural Practice and therefore the Approved Supplier Programme. The Approved Supplier Programme does not apply to later manufacturing stages (Peter Ensor, personal communication, May 2005).

7.1.5 International context

7.1.5.1 Codex code of hygienic practice for fresh fruit and vegetables

The Codex Committee on Food Hygiene (CCFH) has prepared a Code of hygienic practice for fresh fruits and vegetables (at Step 8 of the procedure, see report from the October 2001 meeting, reported by Codex in 2003 at: ftp://ftp.fao.org/codex/alinorm03/al03_13e.pdf). This Code includes annexes for "Ready-to-eat Fresh Pre-cut Fruits and Vegetables" and "Sprout Production". The Code was developed in response to growing concerns that fruits and vegetables were sources for foodborne pathogens. The Code addresses Good Agricultural Practices (GAP) and Good Manufacturing Practices (GMP). These practices should help to control microbial hazards from primary production to packing. The following areas of importance for microbial control are acknowledged;

- Environmental hygiene,
- Hygienic production;
  - Water
  - Manure
  - Soil
  - Agricultural chemicals
  - Biological control
  - Indoor facilities
  - Personal hygiene,
- Handling,
- Storage,
- Transport,
- Cleaning,
- Maintenance, and
- Sanitation

No microbiological specifications are given in the Code; instead the Code refers to the Codex Recommended International Code of Practice – General Principles of Food Hygiene (http://www.fao.org/DOCREP/005/Y1579E/y1579e02.htm#bm2). These general principles have been further elaborated in the case of *L. monocytogenes* by proposed draft guidelines on the application of general principles of food hygiene to the management of *L. monocytogenes* in foods (ftp://ftp.fao.org/codex/ccfh36/fh0407ae.pdf). This draft Code outlines specific process measures for management of *L. monocytogenes,* including recommendation for an environmental monitoring programme, but has yet to be finalised by the CCFH.

7.1.5.2 International Fresh-cut Produce Association

The International Fresh-Cut Produce Association (IFPA) in association with the Western Growers Association (WGA; US) convened a food safety initiative in 1996 to minimise microbial problems with fresh produce (De Roever, 1998). The initiative identified five main areas of microbial risk:

- Water quality, including irrigation water, postharvest process water, pesticide spray carriers and hand washing water;
- Worker hygiene at preharvest, harvest postharvest cooling, packinghouse and processing levels;
- Manure management for those who use manure, including effective sterilisation, proper storage and proper application intervals;
- Packinghouse and processing plant sanitation, including the facility environment and equipment; and
- Establishment and maintenance of the cold chain from cooling, storage, processing, shipping, retail display and consumer handling.

IFPA/WGA subsequently published "Food Safety Guidelines for the Fresh-cut Produce Industry", now in its fourth edition, see website; (http://www.fresh-cuts.org/Default.aspx?mid=348&tabid=37&ctl=catalogitemdetails&catalogitemid=6&mastercategoryid=3).

7.2 Overseas legislative controls

An important issue for food manufacturers and regulators is whether there should be a zero tolerance for the presence of *L. monocytogenes* in ready-to-eat foods, or whether a low level (usually 100 cfu/g at point of consumption) is tolerable in certain foods where growth of the bacteria is unlikely or where a listericidal step is inappropriate. In the case of ready-to-eat raw salad vegetables, clearly cooking can not be considered as a CCP for the removal of *L. monocytogenes*. This section collates information on the regulatory regimes in place overseas.

7.2.1 Australia

Recall guidelines for Australia only are provided on the FSANZ website under the title 'Recall Guidelines for Packaged Ready-to-eat foods found to contain *L. monocytogenes* at point of sale, April 2001'. For further information on the Australian recall system refer to ; http://www.foodstandards.gov.au/whatsinfood/listeria/listeriarecallguidel1321.cfm).

Table 13 summarises the FSANZ guidelines for action levels for *L. monocytogenes* (applicable to Australia only). Additionally, detection of *L. monocytogenes* in foods prepared specifically for 'at risk' populations should be considered "potentially hazardous".

Table 13: FSANZ Guidelines for *L. monocytogenes* in ready-to-eat foods (applicable in Australia only)

	Microbiological quality (cfu per gram unless other stated)			
Test	**Satisfactory**	**Marginal**	**Unsatisfactory**	**Potentially hazardous**
L. monocytogenes	Not detected in 25g	Detected but $<10^{2*}$		$>10^2$

* Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g
Source: Guidelines for the microbiological examination of ready-to-eat foods (December 2001:6)

This differentiation between ready-to-eat foods for which there is a zero tolerance for *L. monocytogenes*, and ready-to-eat foods which have no listericidal step, is also reflected in the recall guidelines (see Australian recall guide website cited above). Ready-to-eat salad would fall into category 2 below and would necessitate a recall where ≥100 cfu/g were detected (Table 14).

Table 14: Food categories and action levels (applicable in Australia only)

Category of food	Level of *L. monocytogenes*	Action
Category 1 ready-to-eat foods requiring refrigerated storage and able to support the growth of *L. monocytogenes*;* ready-to-eat foods that have been implicated in human listeriosis (e.g. soft & semi soft cheeses, pate, cooked cold chicken, cold-smoked fish**) and/or which may be consumed by at risk groups, especially infants	Detected in 25g[#] (Method: AS/NZS 1766.2.16.1-1998 for the detection of *L. monocytogenes****)	Recall
Category 2 - all other packaged ready-to-eat foods	Equal to or greater than 100 cfu per gram (Method: No AS/NZ enumeration method;)	Recall

* Factors such as freezing, pH, water activity, lactates and organic acids may inhibit the growth of *L. monocytogenes.* When it is difficult to predict whether a given food is supportive of growth for *L. monocytogenes* within the stated shelf-life, the authorities may take a conservative approach and regard growth as possible, unless there is documented evidence provided by the manufacturer that the product does not support growth of *L. monocytogenes.*

** The Joint Australia New Zealand *Food Standards Code* has a sampling plan for cold-smoked fish that allows one out of five samples to contain *L. monocytogenes* up to 100 cfu/g.

*** Equivalent methods may be used AS/NZS 4659.

[#] 10 or >10/g if an enumeration method is used.

In Australia, between 1 January 1990 and 31 December 2003, 205 recalls were notified to FSANZ due to microbial contamination, of these 89 recalls were due to *L. monocytogenes*, but no information is available on the foods involved (see website;

http://www.foodstandards.gov.au/recallssurveillance/foodrecalls/foodrecallstatistics.cfm).

7.2.2 United States of America

The United States of America has a zero tolerance for *L. monocyt*ogenes in ready-to-eat foods, which will include ready-to-eat salads. This means that ready-to-eat foods contaminated at a detectable level with the organism are deemed to be adulterated.

Further to the Joint Risk Assessment carried out by FDA/FSIS (2003) (see section 6.2.4), an update to the *Listeria* action plan in the USA was formulated in November 2003. The interim goal is to reduce *L. monocytogenes* caused illness by 50 percent by 2005. The new action plan can be found at the following FDA website:
(http://www.cfsan.fda.gov/~dms/lmr2plan.html).

The zero tolerance policy adopted in the 1980s makes no distinction between foods contaminated at high or low levels, contamination at a detectable level is enough to deem the food as unfit. This current regulatory approach has been challenged because it concentrates on further reducing prevalence of the organism in ready-to-eat foods and continues zero-tolerance for all ready-to-eat foods. Recently the Food and Drug Administration (FDA) announced (May 24 2004) that a petition had been filed by fifteen US food industry trade associations that requests that the agency establish a regulatory limit of 100 cfu per gram for *L. monocytogenes* in foods that do not support the growth of the microorganism. The agency is requesting comment on the petition.

The US Food and Drug Administration have also published a "Guide to Minimise Microbial Food Safety Hazards for Fresh Fruits and Vegetables";
(http://www.foodsafety.gov/~dms/prodguid.html). The guide identifies eight principles of microbial food safety within the realm of growing, harvesting, packing, and transporting fresh produce:

- Prevention of microbial contamination of fresh produce is favoured over reliance on corrective actions once contamination has occurred;
- To minimise microbial food safety hazards in fresh produce, growers, packers, or shippers should use good agricultural and management practices in those areas over which they have control;
- Fresh produce can become microbiologically contaminated at any point along the farm-to-table food chain. The major source of microbial contamination with fresh produce is associated with human or animal faeces;
- Whenever water comes in contact with produce, its source and quality dictates the potential for contamination. Minimise the potential of microbial contamination from water used with fresh fruits and vegetables;

- Practices using animal manure or municipal biosolid wastes should be managed closely to minimise the potential for microbial contamination of fresh produce;
- Worker hygiene and sanitation practices during production, harvesting, sorting, packing, and transport play a critical role in minimising the potential for microbial contamination of fresh produce;
- Follow all applicable local, state, and Federal laws and regulations, or corresponding or similar laws, regulations, or standards for operators outside the U.S., for agricultural practices; and
- Accountability at all levels of the agricultural environment (farm, packing facility, distribution centre, and transport operation) is important to a successful food safety program. There must be qualified personnel and effective monitoring to ensure that all elements of the program function correctly and to help track produce back through the distribution channels to the producer.

7.2.3 Canada

Canada has implemented a three category system for *L. monocytogenes* in ready-to-eat foods (Farber *et al*., 1996). This categorisation system is summarised in Table 15.

Table 15: The microbiological criteria for *L. monocytogenes* for different categories of food and corresponding action levels in Canada

Category	Foods	Microbiological criteria for *L. monocytogenes*	Action level
1	Foods causally linked to listeriosis, with a shelf-life >10 days.	absence in 50g	>0 cfu/50g Immediate action-Class I recall to retail level.
2	All other ready-to-eat foods capable of supporting growth, refrigerated shelf-life of >10 days.	absence in 25g	>0 cfu/25g Immediate action-Class II recall to retail level.
3 (two types of foods)	• supports growth with refrigerated shelf-life of <10 days	≤100 cfu/g with adequate GMP	Immediate action-allow sale. -follow up at plant level.
	• all other ready-to-eat foods not supporting growth; ➢ pH 5.0 – 5.5 and a_w < 0.95 ➢ pH <5.0 regardless of a_w ➢ a_w ≤0.92 regardless of pH ➢ frozen foods.	≤100 cfu/g with inadequate or no GMP >100 cfu/g	Immediate action-consider class II recall or stop sale.-follow up at plant level. Class II recall or stop sale.-follow up at plant level.

7.2.4 England and Wales

In the United Kingdom, the statute law (Food Safety Act 1990 Sections 7, 8 and 14) provides the legal framework for dealing with the microbial quality of food. Guidelines have been issued by PHLS for the microbiological quality of some ready-to-eat foods sampled at the point of sale (Gilbert *et al*., 2000). The guidelines have no legal standing in their own right. The purpose of the guidelines is to assist food examiners and EHOs to determine the bacteriological quality and indicate the level of contamination that is considered to represent a significant potential risk to health. This information can then be used to assist the enforcement officer in deciding which Section of the Food Safety Act 1990 should be used to initiate a prosecution.

The criteria for *Listeria* spp. has been modified since the 1992 and 1996 revised guidelines. The term *Listeria* spp. (total) is used so that it is fully inclusive of all *Listeria* species. The guidelines state that although *Listeria* spp. other than *L. monocytogenes* are rarely implicated in illness, they are indicators for the likely presence of *L. monocytogenes*.

The quantitative levels given under the 'unacceptable/potentially hazardous' column represent a potential hazard to those who eat such food. This means on the basis of current information, "it is unacceptable that ready-to-eat foods contain any serogroup of *L. monocytogenes* at levels at or above 10^2CFU/g. Some serotypes/phage types of *L. monocytogenes* may rarely be associated with human infection, but their presence represents an inadequate level of hygiene" (Gilbert *et al.,* 2000).

The guidelines for *Listeria* spp.(total) and *L. monocytogenes* are summarised in Table 16.

Table 16: Guidelines for the microbiological quality of *Listeria* spp (total) and *L. monocytogenes* in foods at point of sale in England and Wales

	Microbiological quality (CFU per gram)			
Criterion	**Satisfactory**	**Acceptable**	**Unsatisfactory**	**Unacceptable/ potentially hazardous**
Listeria spp. (total)	<20	20-<100	≥100	N/a*
L. monocytogenes	<20**	20-<100	N/a[#]	≥100

* It is noted that a prosecution based solely on high colony counts and/or indicator organisms (such as *Listeria* spp. (total) in the absence of other criteria of unacceptability is unlikely to be successful therefore quantitative levels in the 'unacceptable/potentially hazardous' column have been made non-applicable.

**Not detected in 25g for certain long shelf-life products under refrigeration.

[#] Not applicable as some quality standards require a zero level at the production stage of a food and 10^2CFU/g at point of sale/consumption would represent a potential risk to health.

Source: (Gilbert *et al.,* 2000).

The ready-to-eat food categories include 'coleslaw' and 'prepared mixed salads and crudités'.

An official guide for industry in England and Wales has been produced by the Department of Health. The 'Fresh Produce Industry Guide' is published by Chadwick House Group in the UK, see website: (http://www.shop.cieh.net/acatalog/The_Fresh_Produce_Guide.html). This guidebook is not legally binding but Food Authorities must give it's contents due consideration when enforcing Regulations.

7.2.5 Denmark

Nørrung *et al.* (1999) describe the control of *L. monocytogenes* in Denmark. The regulatory policy is based on HACCP and a health risk assessment approach. Ready-to-eat foods are categorised into six subsets with the following tolerances (Table 17).

Table 17: Food groups and tolerances for *L. monocytogenes* in Denmark

Category	Food groups	No. of samples (*n*)	Absence in 25g (*c*)	*m*	*M*
I	Foods heat treated in final package	5	0	0	-
II	Heat treated foods, handled after treatment. Shelf life > 1 week, food supports growth	5	0	0	-
III	Lightly preserved, not heat treated, shelf life > 3 weeks	5	0	0	-
IV	Heat-treated foods, handled after treatment. Stabilised against growth within shelf life	5	1	10*	100*
V	Lightly preserved, not heat treated, stabilised against growth during shelf life	5	1	10*	100*
VI	Raw, ready-to-eat foods	5	2	10*	100*

* denotes *L. monocytogenes* per g.

Levels above 100 cfu/g of *L. monocytogenes* are regarded as posing a health risk to consumers (Food Act s.12), control activities include prohibition of sale and recalls (Nørrung *et al*., 1999)

7.3 Adverse Economic Effects from Infection with *L. monocytogenes*

The annual economic cost to New Zealand of cases of invasive listeriosis caused by foodborne transmission has been estimated as $818,000, which represents 1.5% of the estimated total cost of foodborne infectious intestinal disease (Scott *et al*., 2000). The number of cases and outcomes used for this estimate was based on an average of notification and hospitalisation data from 1991 to 1998 (Lake *et al*., 2000). The estimated value includes

direct and indirect medical costs, the value of productive days lost, and the statistical value of mortality, but not the value of lost quality of life.

This estimate was based on several assumptions, the most important of which was that 90% of all cases of listeriosis were caused by foodborne transmission. This proportion was derived from proportions cited in the US. In that country, foodborne transmission of listeriosis has been estimated as 85-95% (Buzby *et al*., 1996) and 99% (Mead *et al*., 1999) of all cases.

This economic estimate covers all potential food vehicles. No data are available on the proportion of transmission by individual foods.

7.4 Risk Management Options

The main risk for foodborne transmission of listeriosis is from foods with high numbers of *L. monocytogenes*, and these are likely to be foods in which *L. monocytogenes* can grow. Targeting these foods for application of zero tolerance, or at least to ensure a count of <100/g when consumed, could be the most effective way to reduce disease. The dose response model indicates that eliminating foods with high levels of *L. monocytogenes* present will have significantly greater effect than eliminating foods with only a few cells present (e.g. preventing one meal containing 10^6 *L. monocytogenes* cells present from being eaten will result in the same reduction in risk as preventing the consumption of a million meals containing 10^o *L. monocytogenes* cells).

Conditions likely to result in large numbers of organisms becoming present in a food will include:

- the presence of the pathogen in the first instance;
- a food that supports the growth of *L. monocytogenes*;
- a suitable storage period to allow growth (this might be either a long period of refrigerated storage or lesser periods of time/temperature abuse); and,
- the absence of a listericidal step prior to consumption.

Risk management steps could be targeted at any of these points.

The USDA FSIS risk assessment concluded that for products that receive a treatment that inactivates *L. monocytogenes*, the risk of listeriosis is determined to a large extent by the potential for recontamination after that treatment. This may occur in production, retail or domestic environments. The risk assessment concluded that new strategies were needed to decrease rates of recontamination during the manufacturing and marketing of ready-to-eat foods. It should be noted that raw, ready-to-eat salads would not fall into this category because they can not receive a treatment that inactivates the organism such as heat treatment.

Occasional contamination of salad raw materials with *L. monocytogenes* appears to be largely unavoidable, due to the prevalence of the organism in the environment. Currently available techniques for microbial decontamination (e.g. chlorine wash) are only partially effective. The processing of vegetables to produce ready-to-eat salads may have a mixture of positive and negative effects on microbial growth, with cutting and slicing of vegetables making nutrients available for microbial growth.

These various factors suggest that risks due to *L. monocytogenes* in ready-to-eat salads will be best managed by a combination of Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) using the principles of Hazard Analysis Critical Control Point (HACCP) to achieve a low prevalence of *L. monocytogenes* on raw product and to control any subsequent sources of contamination within the processing environment.

Education is currently an actively used form of risk management, especially for pregnant women. Direct education campaigns by the NZFSA about the risk of listeriosis to pregnant women are already in place. For example the NZFSA launched a "Food Safety in Pregnancy" leaflet in 2003 which was highlighted in a central page feature in the NZFSA (2004) "FoodFocus" publication. The campaign lists ready made salads and coleslaws from delis and salad bars as a high risk for *Listeria* and advises "Don't eat". Home made salads are listed as a low risk and the advice given is to "wash and dry salad ingredients well just before making and eating salads".

The FSANZ website;
http://www.foodstandards.gov.au/whatsinfood/listeria/listeriapregnancybro738.cfm
advises pregnant women to avoid prepared or stored salads. "It's best not to use salad bars in restaurants, supermarkets or delicatessens". Home made salads are considered safe providing that vegetables are washed thoroughly, the salad is stored in the fridge and used within 12 hours.

8 CONCLUSIONS

8.1 Description of Risks to New Zealand Consumers

8.1.1 Risks associated with ready-to-eat salads

The rate of reported invasive listeriosis in New Zealand is similar to that found in like countries (Table 9). However, there is no epidemiological or surveillance evidence to link cases of *L. monocytogenes* infection in New Zealand with ready-to-eat salads.

Data on the prevalence of *L. monocytogenes* in New Zealand ready-to-eat salads are very limited, and somewhat dated given the apparently dynamic nature of the market. Prevalence data from overseas suggest that *L. monocytogenes* is likely to be a common (up to 10%) contaminant of salads and salad vegetables, albeit generally at very low numbers (<100 cfu/g). Data on the behaviour of *L. monocytogenes* in salads and salad vegetables suggests that under normal conditions of storage (4°C for 7 days) for this type of product, if growth does occur, then a 1-2 $\log_{10}$ increase is the most that could be expected.

The growth of *L. monocytogenes* on ready-to-eat salads will be affected by the interaction of several factors, which include time, temperature, ingredients and possibly the atmosphere. Although direct data on the prevalence of *L. monocytogenes* in ready-to-eat salads in New Zealand are not available, there are no human health surveillance data to suggest that this food/hazard combination currently represents a significant risk to human health. Based on discussions with a small number of companies manufacturing ready-to-eat salads in New Zealand, risk management measures including Food Safety Programmes and testing for *L. monocytogenes* are part of the production process.

Based on overseas risk assessments and outbreak analyses, ready-to-eat salads or vegetables are unlikely vehicles for *L. monocytogenes* infection in New Zealand, and other food vehicles appear to represent a more important route of exposure to this organism.

8.1.2 Risks associated with other foods

Foods appear to be a major vector of human infection with *L. monocytogenes* (ICMSF, 1996). It is likely that ready-to-eat foods contribute to foodborne listeriosis but foods on which it cannot grow, or which have a short shelf life, are less likely to contribute to the disease burden significantly as the organism should not reach high numbers.

The USDA risk assessment listed as high (5 or above) relative risks of listeriosis for the following food groups (Table 12):

1. deli meats,
2. non-reheated frankfurters
3. paté and meat spread
4. fluid unpasteurised milk, and
5. smoked seafood.

In New Zealand, an outbreak of invasive listeriosis linked to smoked mussels has been identified. With regard to non-invasive listeriosis, two outbreaks have been reported (from the same incident) involving cooked ready-to-eat meat products.

8.2.1.1 Other types of salads

Salads of different composition may have a lower relative risk because of inhibitory activities of ingredients such as carrots, lambs lettuce, or acidic dressings. Conversely, the additional ingredients may confer increased risk through greater handling with the potential for contamination. The latter appears to be the case in New Zealand.

A survey of ready-to-eat salads (with cooked ingredients and/or dressings) was conducted by Auckland Healthcare and reported by Consumer magazine (Anonymous, 1997). The survey was of self-serve salads sold in the deli bars of 22 Auckland supermarkets. Salad types were; coleslaw (22 samples), bean, pasta, rice (21 each), egg (16), potato (6), ham (2) and salami (1). Of the 110 samples, 14 (12.7%) were contaminated with *L. monocytogenes*. While data were not presented broken down by salad type, it was reported that "only three coleslaws made the grade [and] over half of the samples that contained listeria (sic) were coleslaw". The reasons for the poor coleslaw results suggested by the authors were; handling, few ingredients (if any) cooked and dressings often not very acidic (13 of the 21 coleslaws tested were pH 4.6 or above). Overall, 41 of the 110 salads tested were pH 4.6 or higher. ESR laboratory records confirm that 8 of 14 salads positive for *L. monocytogenes* were coleslaw, with the remainder being egg (2), pasta (2), rice (1) and bean (1) salads. All isolates were serotype 1/2. The prevalence of *L. monocytogenes* on coleslaw in this survey was 8/22 (36%).

8.1.3 Quantitative risk assessment

A quantitative risk assessment would be feasible for *L. monocytogenes* in ready-to-eat salads, provided sufficient data on the prevalence of the organism in the product at a retail level and better consumption data could be obtained. However, it is difficult to see how the conclusions of such a risk assessment would be markedly different to those derived from the assessment conducted by the US FDA.

8.2 Commentary on Risk Management Options

The risks from *L. monocytogenes* in ready-to-eat salads will be best managed by a combination of Good Agricultural Practice (GAP) and Good Manufacturing Practice (GMP) to achieve a low prevalence of *L. monocytogenes* on raw product and to control any subsequent sources of contamination within the processing environment.

There is currently no international agreement on what is an 'acceptable level' of *L. monocytogenes* contamination in foods. In addition there is no agreement on sample methodologies or sampling plans. For internationally traded foods, harmonisation in microbiological criteria based on risk assessment has been called for by FAO/WHO. It has been estimated by Codex that a 99% reduction in number of illnesses will be obtained by setting a food safety objective at <100 *L. monocytogenes* g^{-1} of food at point of consumption (Codex, 2002).

8.3 Data Gaps

The data gaps identified in this Risk Profile are:

- Current prevalence of *L. monocytogenes* in ready-to-eat salads available in New Zealand;
- Quantitative data on levels of *L. monocytogenes* in ready-to-eat salads when contamination does occur;
- Information on the market size and market structure for ready-to-eat salads, including information on population levels of consumption.

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APPENDIX 1: CATEGORIES FOR RISK PROFILES

The assignment of a category for a food/hazard combination uses two criteria: incidence and severity.

1. Incidence

The incidence is an estimate of the proportion of the foodborne disease rate due to an individual hazard, that is transmitted by a single food or food group.

The overall rate of foodborne disease caused by individual hazards can be derived from information in the published estimate of foodborne disease (Lake *et al*., 2000). This estimate has been updated to reflect more recent notifications rates for the 12 months to June 2001, but still using 1996 census figures (3,681,546 population). Rates include estimates for unreported cases who do not present to a GP.

Disease/organism	Food rate (/100,000 population) Calculated for 12 months to June 2001	Food rate (/100,000 population) Calculated for 12 months to December 1998
Campylobacteriosis	1320	2047
Listeriosis	0.4	0.4
VTEC/STEC	1.9	1.4
Salmonellosis	176	230
Yersiniosis	38	62
Shigellosis	7	7
NLV*	478	478
Toxins*	414	414
Typhoid*	0.3	0.3
Hepatitis A*	0.4	0.4

* not recalculated.

These are **total** foodborne rates, so it is probably safe to assume that in most cases the rates associated with a particular food are likely to be an order of magnitude lower. For instance, a category of ">1000" would only be assigned if it was decided that all campylobacteriosis was due to a single food/food type.

The following categories are proposed for the rates attributable to a single hazard/food (or food group) combination:

Category	Rate range	Comments/examples
1	>100	Significant contributor to foodborne campylobacteriosis Major contributor to foodborne NLV
2	10-100	Major contributor to foodborne salmonellosis Significant contributor to foodborne NLV
3	1-10	Major contributor to foodborne yersiniosis, shigellosis
4	<1	Major contributor to foodborne listeriosis

A further category, of "no evidence for foodborne disease in New Zealand" is desirable, but it was considered more appropriate to make this separate from the others. Also separate is

another category, of "no information to determine level of foodborne disease in New Zealand".

The estimation of the proportion of the total foodborne disease rate contributed by a single food or food group will require information from a variety of sources including:

- exposure estimates
- results from epidemiological studies (case control risk factors)
- overseas estimates

For illnesses where the rate is <1 per 100,000 the ability to assign a proportion is unlikely to be sensible. For such illnesses it may be more useful to consider a Risk Profile across the range of all high risk foods, rather than individual foods or food groups.

2. Severity

Severity is related to the probability of severe outcomes from infection with the hazard.

The outcomes of infectious intestinal disease are defined in the estimate of the incidence (Lake *et al*., 2000) as:

- death
- hospitalised and long term illness (GBS, reactive arthritis, HUS)
- hospitalised and recover
- visit a GP but not hospitalised
- do not visit a GP

The first three categories of cases were classed as severe outcomes. Some hospitalisations will result from dehydration etc. caused by gastrointestinal disease. However, for infections with *Listeria* and STEC hospitalisation will result from more severe illness, even if recovery is achieved.

The proportion of severe outcomes resulting from infection with the hazards can be estimated from the proportion of cases hospitalised and recover, hospitalised and long term illness, and deaths (Lake *et al*., 2000).

Disease/organism	Percentage of outcomes involving death or long term illness from foodborne cases
Campylobacteriosis	0.3
Listeriosis	60.0
VTEC/STEC	10.4
Salmonellosis	1.0
Yersiniosis	0.4
Shigellosis	2.7
NLV	Assumed to be <0.5%
Hepatitis A	15.4
Typhoid	83.3
Toxins	Assumed to be <0.5%

Categories for the probability of severe outcomes are suggested as follows:

Severity Category	Percentage of cases that experience severe outcomes	Examples
1	>5%	listeriosis, STEC, hepatitis A, typhoid
2	0.5 – 5%	salmonellosis, shigellosis
3	<0.5%	campylobacteriosis, yersiniosis, NLV, toxins

There are a number of hazards for which the incidence of foodborne disease is uncertain. These have been assigned to the above severity categories as follows:

Severity category 1:

Bacteria

Clostridium botulinum

Protozoa

Toxoplasma

Severity category 3:

Bacteria

Aeromonas/Plesiomonas
Arcobacter
E. coli (pathogenic, other than STEC)
Pseudomonas
Streptococcus
Vibrio parahaemolyticus

Viruses

Others (e.g. rotavirus)

Protozoa

Giardia
Cryptosporidium
Cyclospora
Others (e.g. *Entamoeba*)

Proposed Category Matrix

Incidence	>100	10-100	1-10	<1
Severity 1				
Severity 2				
Severity 3				

Alternatives:

No evidence for foodborne disease in New Zealand

No information to determine level of foodborne disease in New Zealand